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Microbial Metatranscriptomics Belowground

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Preface

For the compilation of this book, emphasis has been given on the role of functional microbes belowground, i.e., in the rhizosphere to know the response to their metatranscriptomics level and impact on aboveground response. The functional or metatranscriptomics learning provides the detailed acclimation about the functional or transcriptional profiles of discrete microbial populations within a phytomicrobiome that reveals the molecular action of a microbiome and their regulatory mechanisms around the phytosphere.

In the present compendium, main emphasis has been given on the following points:

- Occurrence and distribution of microbial communities
- *In situ* active microbial quorum in the rhizosphere
- Metatranscriptomics for microflora- and fauna
- Functional diversity in the rhizosphere
- Importance of PGPRs in the rhizosphere
- Root endotrophic microbes
- Functional AM fungi in the rhizosphere
- Functional protozoans belowground
- Functional infochemicals
- Location of microbe in plant
- Root epiphytic microbes
- Nitrogen-fixing bacteria
- Functional microbial determinants
- Functional niche under biotic stress
- Functional niche under abiotic stress
- Functional root-derived signals
- Functional microbe-derived signals
- Approaches deployed in metatranscriptomics
- Functional defence signals
- Molecular Tools used in the rhizosphere
- Perspectives of metatranscriptomic in belowground functioning
- Metatranscriptomics for siderophore producing microbes
- Metatranscriptomics for microbe–plant signals

- Metatranscriptomics for Pi-solubilizing producing microbes
- Metatranscriptomics for improving soil fertility
- Metatranscriptomics for pathogenic microbes in the rhizosphere

This book is organized in 31 chapters that deliberate on microbial transcriptomics belowground and their response aboveground wherein structural and functional divergences of microbes rely on the deployment of various phenotypic and molecular approaches incurred.

Chapter 1 seeks to get comprehensive knowledge on soil metatranscriptomics analysis including obtaining biologically important information from transcriptome datasets, comparative information to other transcriptome analysis techniques, bioinformatics tools and technical challenges applied to soil metatranscriptomics.

Chapter 2 concludes with a brief reference to some of the advanced molecular tools available to explore microbial diversity in belowground.

Chapter 3 discusses on the role of the functional rhizosphere in phytomicrobiome wherein extraction and purification of mRNA immediately from plant, decomposition of natural material and soil, accompanied with pooling of expressed genes by using high-throughput sequencing, have spawned metatranscriptomics a new rising area of research.

Chapter 4 defines the utilization of functional infochemicals that provide the pathways for insect management by mating disruption, mass trapping, monitoring of pest infestation, mass annihilation. Thereby, these infochemicals can be an important component of sustainable management of insect pests and also Integrated Pest Management (IPM).

Chapter 5 encompasses the deployment of synthetic biology in the genetics of the nitrogenase enzyme and its engineering in phytomicrobiome responses.

Chapter 6 encompasses the role of functional AM fungi on various fruit crops that considered for useful organic cultivation and also for expanding the fruit crop in low fertility degraded soil with less expenditure and minimum reduction to yield.

Chapter 7 encompasses the role of PGPRs in the rhizosphere and activities performing in that zone with varied potential of PGPRs in crop production for commercial uses.

Chapter 8 seeks to disclose the role of flavonoid infochemicals in the modifier of the rhizospheric ecosystem favouring plant growth and development. These biochemicals play the role of signals to call the beneficial microbes towards plant root and deterring the pathogenic species away from the rhizosphere due to which they are also described as “Infochemicals”.

Chapter 9 discusses various mechanisms adopted by the soil microbes to abrogate the negative effects of abiotic stresses in plants for their better growth and productivity.

Chapter 10 defines the signalling molecules including transcription factors and volatile compounds and their role in plant defence response.

Chapter 11 seeks to get comprehensive knowledge of techniques that are used to study metatranscriptomics and bioinformatics tools to interpret the most valuable knowledge from sequencing data.

Chapter 12 discusses the functional role of rhizobacteria such as biocontrol activity, phytohormone secretion, siderophore production, mineral solubilization, nitrogen fixation and enzyme production and their occurrence, distribution and functions belowground.

Chapter 13 encompasses insights into the biodiversity of psychrotrophic microbes, their adaptation strategies and their potential applications in agriculture, medicine, industry, food and allied sectors.

Chapter 14 defines the functional role of microbial diversity in the rhizosphere zone and its significance to the crop and soil. A diverse population of microbes associated with different activities in the rhizosphere zone is discussed in detail in this chapter.

Chapter 15 seeks to get knowledge of various abiotic stress conditions including temperature, salt, drought, water-logging and metal toxicity stress, and how they influence the structure and diversity of the inhabiting microbial community structure and diversity.

Chapter 16 summarizes the functional behaviour of the microbial communities which may include a group of species trait represented as an individual or species leads to the functional diversity in the rhizosphere.

Chapter 17 provides an insight into the diverse and compact world of root surface associated microbes with their structural and functional divergence.

Chapter 18 reviews the plant root associated endophytes, factor affecting, functionalities and understanding interaction between microbiome associated within plant root.

Chapter 19 focuses on the strategies and methods that are adopted to manipulate the plant–soil microbiome interactions, various mechanisms that are involved in the interactions and the impact of this technology on the plant and soil.

Chapter 20 seeks to get comprehensive knowledge wherein Sundarbans replantation schemes require the microbiome niche to be maintained if successful restoration is to be achieved either in the form of suitable site-specific plantations or microbial consortium based supplementations.

Chapter 21 discusses the major headway in this exponentially proliferating field, comparing the various options used in the computational bioinformatical analysis of data and the challenges associated with them.

Chapter 22 discusses the concepts, tools and techniques used to investigate metatranscriptome and will further highlight its application in understanding the microbial structure and function.

Chapter 23 defines a microbiome wide association study with known disease causing microbial datasets and predict the potential pathogenic microorganisms that are prevalent in ecological niche wherein authors believe that metagenomics can be utilized at a diagnostic scale and using the dataset obtained to predict the pathogenic load of that particular area.

Chapter 24 discusses in detail about the cutting edge high-throughput technologies, *viz*., metagenomic, metatranscriptomics and metaproteomics that are aiding in increasing understanding of the freshwater microbial diversity as well as their functioning.

Chapter 25 encompasses to study about the role of phytohormones in the induction of the defence mechanism in plants. Moreover, it uncovers the defence mechanisms (existing/induced) in the plants against the phytopathogens.

Chapter 26 deals with different metatranscriptomic approaches to explore microbial community transcriptomes in belowground functioning.

Chapter 27 elaborates the role of microbial community transcriptomes using computational metatranscriptomics approaches wherein different available bioinformatics tools are discussed for computational analysis of the data to study the evolutionary processes in a specified pool of microorganisms.

Chapter 28 encompasses the functional role of rhizospheric microbes that not only helps in increased crop production but also enhances soil fertility as well as assists the plant in mitigating the various biotic and abiotic stresses. Thereby, exploring the beneficial properties of these microorganisms one can improve crop growth and productivity in a sustainable way.

Chapter 29 deciphers the molecular network connecting quorum sensing and iron acquisition in case of the rhizosphere associated bacteria wherein display of quorum sensing initiates rhizospheric community formation and its response aboveground.

Chapter 30 signifies the importance of plant growth-promoting rhizobacteria, their mechanism of action, advantage of microbial consortia, aspect of consortia engineering and their various applications.

Chapter 31 seeks to get comprehensive knowledge on plant growth-promoting rhizobacteria (PGPR) that plays a pivotal role in aiding the plants to overcome abiotic stresses and retain their productivity. The basic mechanisms by which PGPR helps plants to cope against abiotic stress include lowering ethylene levels, production and accumulation of compatible solutes such as proline, glycine-betaine; decreasing the production of ROS. Thus the deployment of PGPR is considered a suitable approach for ameliorating the environmental stress encountered by the crop plants and can be considered as an important component of sustainable agricultural practices.

We thank all contributors for their efforts in making this compendium worthy to disseminate complete knowledge for scholarly involvement around the globe.

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Metatranscriptomics in Microbiome Study: A Comprehensive Approach

1

Koushlesh Ranjan, Mahesh Kumar Bharti, R. A. Siddique, and Jitender Singh

Abstract

The soil microbes are an essential component for proper functioning of terrestrial ecosystem. Due to vast diversity of the microbial population in soil microbiome, it is difficult to identify individual microbes, their interactions with neighboring organisms, environment, and plants. The advancement in high throughput sequencing technologies has accelerated the below ground soil metagenomics and metatranscriptomics studies. However, metagenomics study provides only the deep insight of presence of microbial diversity and their genes without providing any knowledge that whether they are active component of the microbiome or not. Therefore, to know the microbial response to their environmental conditions at a specific point of time, metatranscriptomics analysis is highly useful. Metatranscriptomics study provides the detailed knowledge about the transcriptional profiles of discrete microbial populations within a microbiome at the time of sampling which indicates about molecular activities of a microbiome and their regulatory mechanisms. In this chapter, comprehensive knowledge on soil metatranscriptomics analysis including retrieval of biologically important information from transcriptome datasets, comparative information about other transcriptome analysis techniques, bioinformatics tools, and technical challenges applied to soil metatranscriptomics are incorporated.

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Soil · Metatranscriptomics · Bioinformatics · Microbiome

1.1 Introduction

Metatranscriptomics is the study of gene expression of all the microbes present in natural environments at a time. It allows whole gene expression profiling and study of composite nature microbial communities in particular environment (Filiatrault 2011). The microbes are ubiquitous in nature and are highly important for the proper functioning of the ecosystem. Any changes in microbial communities may adversely affect the biological activity of the ecosystem. All the microbial community inhabiting in a specific habitat is called as microbiome. The metatranscriptomics study can be used to identify the genetic diversity of active genes in a composite microbiome. It may provide vital information to quantify microbial gene expression and changes in expression levels at different physiological and pathological conditions. Metatranscriptomics possess specific advantages as it provides accurate information about differential gene expression or active functions of genes in a composite microbiome which otherwise looks similar in metagenomics study. Metagenomics study focuses on the study of the entire genomic content of microbes and specifies the taxonomic position of microbial population, while metatranscriptomics provides information about functional annotations of genes expression in microbial community under specific conditions (Bashiardes et al. 2016). Metatranscriptomics data mining is one of the efficient ways to discover novel genes or gene families in a plant system or soil microbiome. Many of such genes encode specific enzymes essential for various metabolic pathways (Xiao et al. 2013).

In recent years, plant microbiome emerged as an important field of metatranscriptomics study because it influences plant health, animal health and their productivity. The plant microbiome comprises of several types of functional gene pool consisting of viruses, prokaryotes, eukaryotes, etc. associated with plant host. Plants allow inhabiting microbiomes from on the whole plant to specific plant regions such as on roots, shoots, leaves, flowers, seeds, and at the area of interaction between roots and surrounding soil i.e. the rhizosphere. The rhizosphere region of soil remains immediately in touch with plant roots and continuously influenced through rhizo-deposition of mucilages, exudates, and sloughed plant cells. Thus, plant roots influence surrounding soil and inhabiting microbiome and in turn the rhizosphere microbiomes also influence the plant growth and productivity by producing plant growth regulatory compounds (Philippot et al. 2013; Spence et al. 2014). According to some of the researchers rhizospheric microbiomes are considered as the second genome to plant (Berendsen et al. 2012) because they may have the capacity to influence plant growth and productivity.

The rhizospheric microbiomes influence plant growth directly by beneficial or pathogenic microbes and indirectly by nutrient solubilization, nutrient cycling,

antagonism of plant pathogens, induction of the plant immune system, and secretion of plant growth hormones (Mishra et al. 2009; Rudrappa et al. 2010). The activities of soil microbiomes are dependent on several factors including climate changes in that geographical area. Thus, soil microbiomes play an important role in maintenance of soil and plant health and entire ecosystem.

1.2 Techniques Used for Metatranscriptomics Study

The metatranscriptomics study can be carried out by high throughput sequencing techniques such as Next Generation Sequencing (NGS) and Third Generation Single Molecule Long Read Sequencing (Table 1.1) along with Microarray techniques. Before the popularization of high throughput sequencing platforms, microarray technique was one of the methods of choice for quantification of expression of transcripts (mRNA) from known organisms or entire microbial communities (Parro et al. 2007). However, with the application of high throughput NGS technologies, detailed annotation and quantification of known as well as previously unknown transcripts and their variants can be easily done. High throughput machines enable millions of DNA fragments to be sequenced simultaneously and are much faster than conventional sequencer (Minakshi et al. 2014). High throughput sequencing techniques are the preferred method for metatranscriptomics study on soil microbiome, since microarrays techniques can be primarily used for study of gene expression profile for a few specific known model organisms only.

1.2.1 Metatranscriptomics Study by NGS Techniques

Most of the information on soil microbiome such as microbial composition, genome sizes, and relative gene expression of microbes in different environmental conditions usually remains unknown. Many of the high throughput sequencing technologies generate data in the form of short reads which have frequently been used for metatranscriptome studies because of its deep sequencing coverage which is required for differential gene expression studies. However, long read sequencing technologies can generate complete or near to full length mRNAs sequence which can make sequence similarity search easy and can also be helpful in discrimination study among different isoforms. Metatranscriptome sequencing using NGS techniques generates a large volume of sequencing data and provides direct access to both culturable as well as non-culturable microbiomic transcriptome information of a specific environmental condition without any prior sequence knowledge. It allows randomly sequencing of pool of mRNAs from different microorganisms to understand the complex microbial processes in a microbiome. The metatranscriptomics study through NGS techniques allows generating gene expression profiles of the entire microbiome and provides deep insights into several unknown biological systems, which previously remained untouched because of the technical limitations associated with the isolation of individual microbial population in laboratory conditions.

Table 1.1 Comparison of different High throughput sequencing platforms

Manufacturer	Sequencing Principle	Detection	Sequencing platform	Read length (bp)	Number of Reads	Run Time	Data/ run	Accuracy Percentage	Advantage	Disadvantage
Illumina	Reversible terminator sequencing by synthesis	Fluorescence/ optical	HiSeq 2000	100	3 billion	~8 days	200 GB	> 99%	• Very high throughput • Cost effective-instrument • Massive throughput	• Long run time • Shorter read lengths
Roche	Pyrosequencing	Optical	454 GS-FLX+	Up to 1000	1 million	23 hours	0.7 GB	99.97%	• High through-put • Longer read lengths • Short run times	• High reagent costs • High error rate, especially in homopolymer regions
ABI life technologies	Ligation	Fluorescence/ optical	5500xl SOLiD	2X60	800 million	6 days	180 GB	99.94%	• Lowest reagent cost • High throughput • Low error rate	• Long run times • Short read lengths
Life technologies	H^+ detection	pH change detected by ion-sensitive field effect transistors (ISFETs)	Ion-personal genome machine (ion-PGM)	400	12 million	2 hours	2 GB	> 99%	• Short run times • Low cost per sample • Ideal for microbial applications	• High reagent costs • Higher error rate in homopolymer region
Helicos biosciences	Single molecule sequencing	Fluorescence/ optical	Heliscope	>25	~1000 Million	8 days	35 GB	99.99%	• Non-bias representation of templates • Single molecule sequencing technology	• Expensive instrument • Very short read lengths • Higher error rate
Pacific bioscience	Real-time, single molecule	Fluorescence/ optical	PacBio RS	Up to 15,000	Up to 75,000	2 hours	13 GB	85–89%	• Short run times • Low reagent	• Highest error rate • Expensive

	DNA sequencing								costs • Very long read lengths • Easy sample preparation	instrument • Difficulty in installation • No paired reads
Oxford Nano-pore	Nano-pore exonuclease sequencing	Electrical conductivity	GridION	Up to 2 Mb	7–12 million	According to experiment	150 GB	95%	• Extremely long read lengths • Customization; No fluorescent labeling • No optics	• High error rates • Cleaved fragments may be read in wrong order • Difficult to fabricate a multiple parallel pores in a device

1.2.1.1 General Workflow of Metatranscriptomics Sequencing by NGS Technique

The metatranscriptomics sequencing protocols usually vary and are based upon the type and nature of the sample to be analyzed. Although several types of protocols have been standardized for complete metatranscriptome sequencing of microbiome samples, the common steps including microbiome sampling, RNA extraction, mRNA enrichment, cDNA followed by metatranscriptomics libraries preparation, nucleic acid sequencing using NGS techniques, and sequence data analysis remained the same (Fig. 1.1).

After extraction of total RNA from the sample, the qualified RNA is allowed for fragment screening and quality testing. At this juncture of time mRNA enrichment is one of the crucial and trickiest parts. For mRNA enrichment, certain strategies have been used such as (1) removal of rRNA by means of 16S and 23S rRNA probes hybridization or rRNA capture system, (2) degradation of rRNA and tRNA by means of 5′-3′ exonuclease enzyme (Apirion and Miczak 1993), (3) addition of poly(A) to mRNAs by polyA polymerase, and (4) capture of mRNAs by antibodies against specific proteins. However, last two strategies are usually not suggested because of their highly biased nature (Peimbert and Alcaraz 2016).

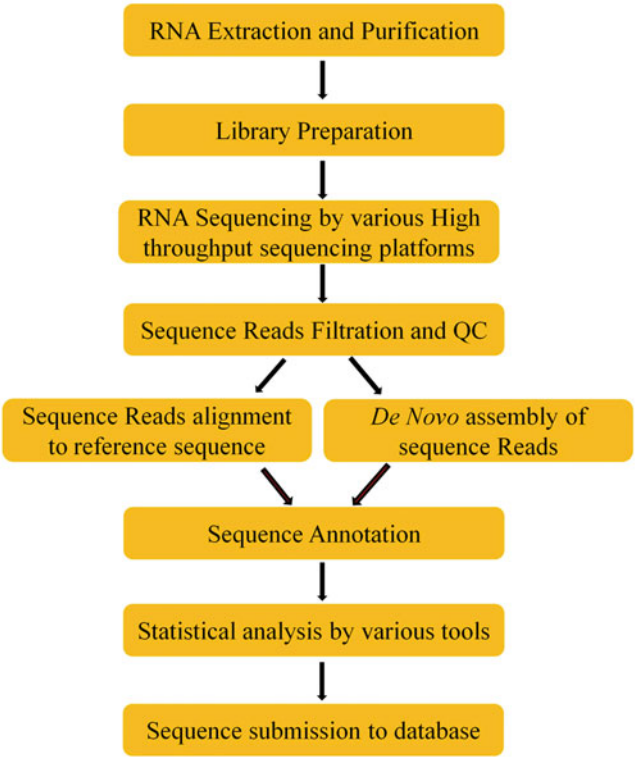

Fig. 1.1 The basic steps of metatranscriptome study by NGS techniques

After mRNA enrichment, the first strand of cDNA is synthesized using reverse transcriptase enzyme and random hexamer primer. However, before preparation of first strand of cDNA, the longer size of mRNA may be fragmented to smaller fragment using ultra-sonication techniques. This fragmentation step is employed to accommodate the read length of sequences according to sequencing platform used. Subsequently, second strand of cDNA is synthesized using DNA polymerase and sequencing adapters are attached to both the ends of double stranded cDNA strands either by PCR or ligation techniques. Thus, sequencing library is prepared which is used for nucleotide sequencing on various NGS platforms such as 454 GS-FLX systems (Roche, USA) or HiSeq (Illumina, USA), etc. followed by transcriptomics analysis (Fig. 1.2). The depth of sequence coverage, the accuracy of sequencing result and cost-effectiveness are the major factors which decide the selection of sequencing platforms. The key consideration taken into account during selection of NGS techniques for metatranscriptomics study of composite soil microbial communities is their depth of sequence coverage which is an essential requirement to identify the expression of a specific gene in question.

1.2.1.2 NGS Platforms for Transcriptome Analysis

High throughput NGS technologies especially with the popularization of RNA sequencing (RNA-seq) techniques have revolutionized the field of transcriptomics. Such technologies may allow generation of RNA sequences of entire microbiome on massive scale with desired sequencing depth. Although, these NGS platforms have their own advantages and disadvantages, the common high throughput platforms used for metatranscriptome studies are discussed below.

454 Genome Sequencer FLX (GS-FLX) System

The 454 Genome Sequencer FLX (GS-FLX) system is based on individual sequencing by synthesis reactions. It facilitates sample multiplexing and optimized the system in such a way that it allows parallel sequencing with several individual sequencing reactions at a time. The term 454 was used as a code name in the project at 454 Corporation, a subsidiary of CuraGen where this system was developed. The GS-FLX system utilizes large scale massive parallel pyrosequencing protocol to sequence approximately 400–600 megabases of DNA per run using GS-FLX Titanium series of reagents (Voelkerding et al. 2009). During sample processing, adapter ligated DNA fragments are fixed onto the DNA capture beads in water in oil emulsion. Subsequently, the fixed DNA to these beads is amplified by PCR. Later on, DNA bound bead are mixed with enzymes ATP sulfurylase, DNA polymerase, and luciferase and placed in 29 μm well of PicoTiter Plate which is then placed into the GS-FLX System for nucleic acid sequencing. This technology is based on the detection of pyrophosphate released after incorporation of new nucleotide in sequence; therefore it is also called as 454 pyrosequencing. In 2008, 454 GS-FLX Titanium platform and its reagents were released which can provide on an average 400–500 base pair read lengths with the capacity to sequence 400–600 million base (Mb) pairs per run. The latest GSFLX+ machine can produce 700 Mb sequence data per run with read length up to 1000 bp (Allseq 2008). Although, GS-FLX is a good

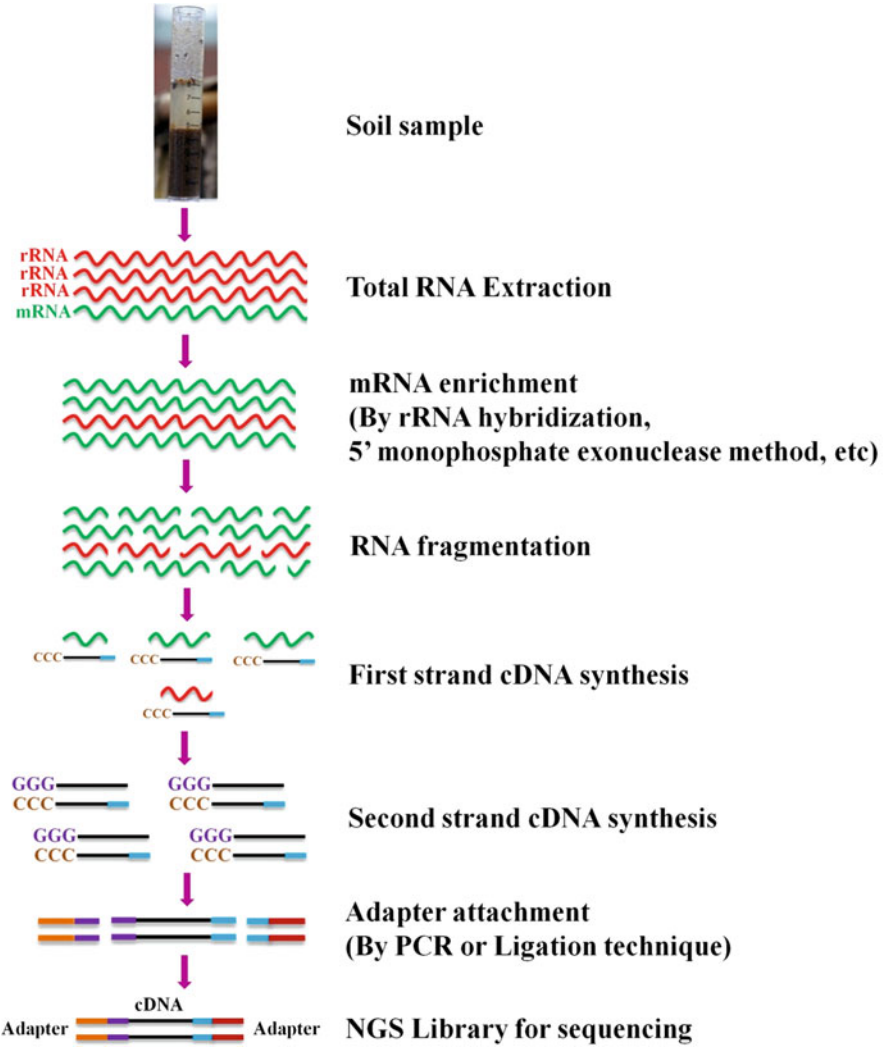

Fig. 1.2 The steps for library preparation for metatranscriptomics study

choice for transcriptome sequencing, its reads are error prone in homopolymer sequencing (Gilles et al. 2011).

Illumina HiSeq Sequencing

Illumina high throughput DNA sequencing technology is based on Solexa technology. The Illumina technology employs bridge amplification step to generate sequence clusters and reversible terminators for determination of actual sequence (Bentley et al. 2008; Balasubramanian 2011). Overall this sequencing system involves important steps such as DNA fragment ligation to chip, primer addition,

incorporation of sequential fluorescent dNTP, and sequence detection. The fragmented DNA ligated with adaptor and corresponding forward and reverse primers are attached to glass surface using a flexible linker. It needs a special type of DNA amplification strategy called as Bridge PCR (Fedurco et al. 2006). The adaptors flanking to DNA fragments are hybridized with forward and reverse primers on glass surface and bridge PCR amplifies the DNA fragment with the help of nucleic acid strand denaturing power of formamide and Bst DNA polymerase. This results in the formation of cluster of clonal amplicons. It is reported that the amplicons of single nucleic acid fragment form a cluster which is located on the array at single location. After generation of amplicon cluster, the sequencing primer hybridizes with flanking region of fragmented DNA of interest. The sequencing reaction proceeds in cyclic manner using modified DNA polymerase and 3′OH end labeled dNTPs with chemically cleavable fluorescent reporter group which allows incorporation of single nucleotide base in each cycle. In each cycle of reaction, single base extension occurs leading to chemical cleavage of fluorescent reporter and identification of incorporated nucleotide.

The entire sequencing reaction occurs within the small sized flow cells which are placed in flow cell compartment. Based on capability of flow cell used, Illumina sequencing is available in different formats such as MiSeq, HiSeq, and NovaSeq. The MiSeq sequencers can generate one million to 30 million reads per run. Similarly, HiSeq and NovaSeq flow cell can generate 3 billion and 13 billion reads per run, respectively. Within flow cell DNA clusters are formed and end of short denatured DNA sample is combined with primers already present in flow cell channel. This is followed by the addition of DNA polymerase and DNA building blocks. This allows synthesis of a new DNA strand in the bottom of the flow cell. Subsequently, the original template is washed out and newly synthesized DNA strand bind to primer present on the surface of flow cell and a new strand is again synthesized. These steps are repeated to generate around 1000 copies in a cluster. The HiSeq 2000 machine produces read length of up to 150 bp and total output of up to 200 Gb per run. The long reads have an additional advantage in the effective sequencing of repetitive regions. Although the read length of HiSeq 2000 is only 150 bp but it is sufficient for transcriptome study because of its capacity to generate large volumes of sequence data (200 Gb) per run which overcomes the difficulty related to short reads length and its quality (Birzele et al. 2010; Camarena et al. 2010).

Ion Torrent

The Ion semiconductor sequencing is sequencing by synthesis method. However, it differs from other sequencing by synthesis methods as it does not utilize either modified nucleotide bases or optics for signal recording. This technology is also known as Ion Torrent sequencing, pH dependent sequencing or ion semiconductor based sequencing (Ambardar et al. 2016). Principally, this technique is based upon identification of hydrogen ions (H^+) which are produced at the time of nucleic acid sequence polymerization. The micro-wells of ion semiconductor chip containing many copies of single-stranded DNA molecule are flooded with DNA polymerase

enzyme and unmodified all the four dNTPs (Pennisi 2010). The incorporation of a dNTP at base complementarity site into growing DNA strand leads to release of a hydrogen ion (H^+) and pyrophosphate (Rusk 2011). The unused dNTPs are washed out before the start of the next cycle of different species of dNTP incorporation (Pennisi 2010). The released hydrogen ion causes pH change in solution, which is identified by an ion-sensitive field effect transistor (ISFET) based sensors. The released hydrogen ion triggers the ISFET base ion sensor which transmit the electrical pulses from the chip to computer where electrical pulse is directly translated into DNA sequence without any intermediate signal conversion (Pennisi 2010). However, if no base complementarity site is found, dNTP incorporation do not occur which lead to the absence of biochemical reaction. In case of presence of homopolymer repeats in template sequence, multiple number of dNTP will be incorporated in a single cycle which leads to release of corresponding number of hydrogen ions and proportionally higher level of signal strength. The ion Torrent produces a read length of 400 base pairs. However, it generates significant error in repeated homopolymer regions (Seneca et al. 2015). Due to availability of other sequencing platforms with longer read length, it is not much suitable for complete genome sequencing of longer genomes. However, it may be suitable for small scale sequencing applications such as targeted sequencing, microbial genome sequencing, microbial transcriptome sequencing, amplicon sequencing, etc. (Chiosea et al. 2015).

SOLiD Technology

It utilizes sequencing by ligation on beads strategy which is based on Multiplex Polony Sequencing technology (Shendure et al. 2005). The adaptors are initially attached to 1 μm paramagnetic beads followed by ligation to flanking region of fragmented template DNA. The PCR amplification is performed in an oil-water emulsion. Later on, beads having attached PCR amplicons are fixed on a solid planar surface and universal PCR primer are allowed to hybridize with adaptor sequence attached to flanking region of fragmented template DNA. The sequencing cycle is initiated by ligation of DNA octamer labeled with fluorescent dye to universal primer according to positional identity of nucleotide sequence. Subsequent chemical cleavage generates pentamer on template DNA and subsequent iteration of this process decodes the actual DNA sequence. This technology platform generates 99.94% accurate sequence since it utilizes a two base coding system which significantly improves the sequence quality.

1.2.2 Metatranscriptomics Study by Third Generation Single Molecule Long Read Sequencing Techniques

Single DNA molecules can be directly sequenced using third generation sequencing technology with low error rates which is generally triggered by amplification associated biasness of PCR, intensity averaging, synchronization related problems, etc.

1.2.2.1 Single-Molecule-Real-Time (SMRT®) Technology

In this technique library of target DNA to be sequence is constructed in such a way that a circular DNA molecule is formed by ligation of a known adaptor to both the ends of the target nucleic acid sequence (Eid et al. 2009). Afterwards, the circular nucleic acid molecule is placed into a SMRT® cell consisting of 150,000 specifically designed zeptolitre wells and immobilized DNA polymerase molecule. The DNA polymerase enzyme binds with hairpin adaptors of circular DNA and initiates replication using fluorescently labeled dNTPs. The incorporation of each of nucleotide generates a specific light pulse which is used for identification of nucleotide base (Rhoads and Au 2015). The major advantage of SMRT® sequencing technique is its specific read length. The first generation C1 machine produced 1500 bp of read length which can be extended up to15 kbp by application of fourth generation C4 chemistry protocols. PacBio RS II system produces 0.5–1.0 billion bases per SMRT cell with comparatively higher error rates of approximately 11–15%.

1.2.2.2 Helicos Genetic Analysis Platform

The Helicos Genetic Analysis platform was the first commercial NGS application used for the single DNA molecule sequencing by synthesis using a sensitive fluorescence detection system (Thompson and Steinmann 2010). The DNA to be sequenced is randomly fragmented and a DNA library is prepared. On fragmented DNA poly A tailing is made which is then hybridized to disposable glass flow cells bounded poly T oligomers to create an array of DNA templates annealed with primer. On the flow cell, the DNA polymerase enzyme adds one by one fluorescent nucleotide until a terminating nucleotide pause the process and an image is captured. Based on the analysis of captured image, the incorporated nucleotide is recognized on growing strand. The reaction cycle is repeated with new species of nucleotide until the DNA fragments is completely sequenced (Thompson and Steinmann 2010).

1.2.2.3 Oxford Nano-pore Technology

Nano-pore detection system is based on the quantification of conductivity difference across a nano-scale pore which eliminates the requirements of optics and DNA amplification (Niedringhaus et al. 2011). In 2014, this technology based MinION model machine was released where nucleic acid molecule is allowed to move through a nano-pore by electrophoresis. The movement of nucleic acid through nano-pore material leads to measurable variation in the pattern of electric current. For library preparation, DNA molecule is fragmented by Covaris g-TUBE (Covaris, USA) system, and resultant template DNA is allowed to repair by a PreCR step. The blunt ended DNA fragments are created by end repair step followed by a poly A tail is created at 3′OH end. Subsequently, two adaptors, namely a Y shaped adapter and a hair pin adaptor are added to DNA molecule. In the next step of reaction, a motor protein is used to open the double stranded DNA and make single stranded at the Y adapter region and single-stranded DNA fragment is passed through the nano-pore. At the nano-pore site nucleotide base calling is performed. This system can generate read length of up to few hundred thousand bases with comparatively poor accuracy of 65 to 88%. It is either 1 dimensional or 2 dimensional system based on either only

one or both the DNA stands is used for base calling process (Lu et al. 2016). The portable size of machine, cost effective performance, and real-time working nature may make this equipment as an essential requirement for real-time diagnostic purpose in hospitals, laboratories, and detection of plant based pathogens (Judge et al. 2015). Other similar type of platform used is GridION system which gives assurance of even short run time, massive throughput with up to 2 Mb read length. The main hurdle with these systems is a low level of sequence accuracy and a lack of sufficient bioinformatics tools to correct specific sequencing errors.

1.2.3 Bioinformatics Analysis of Metatranscriptome Sequencing Data

After metatranscriptome sequencing, the major hurdle is analysis of huge amount of sequence data. The major steps involved in bioinformatics analysis are: filtering the reads, selection of reference sequence and reference based mapping, performing de novo assembly, sequence annotation, statistical analysis, and submission of original assembled and annotated datasets to sequence repository (Fig. 1.3).

1.2.3.1 Filtering and Quality Control of Sequence Reads

After generation of RNA-seq, the sequence data is allowed for Quality Control (QC) analysis to remove wrong reads and minimization of downstream processing errors. Several QC tools such as FastQC (Andrews 2010), fastp (Chen et al. 2018), etc. are available for short read data analysis from Illumina sequencers. During downstream analysis of transcriptome data, ribosomal RNA (rRNA) may cause major problem in differential gene expression study or metabolic pathway characterization. The rRNA transcripts from the microbiome sample should be physically removed using one or other molecular methods prior to sequencing as they may constitute up to 90% of the sequence data if not removed. However, some of the rRNA may still remain in sample and being sequenced. The postsequencing, rRNAs can be removed from downstream analyses by employing tools such as barrnap (Seemann 2014) and SortMeRNA (Kopylova et al. 2012). In specific conditions rRNA reads of a specific organism such as human from human microbiome sample can be removed using faster alignment free methods which search for human specific k-mers in sequence reads, for example, Best Match Tagger (BMTagger) and traditional read mapping methods which map to the human genome and remove rRNA reads (Li et al. 2017).

1.2.3.2 Assembly of Sequence Reads Data

The reference genome sequences for most of the microbiomes are not adequately available in the database. In case of availability, sequence reads are aligned to a reference genome from database using various tools such as Burrows–Wheeler Aligner (BWA) (Li and Durbin 2010), Bowtie2 (Langmead and Salzberg 2012), etc. However, in the case of unavailability of reference genome in database, de novo

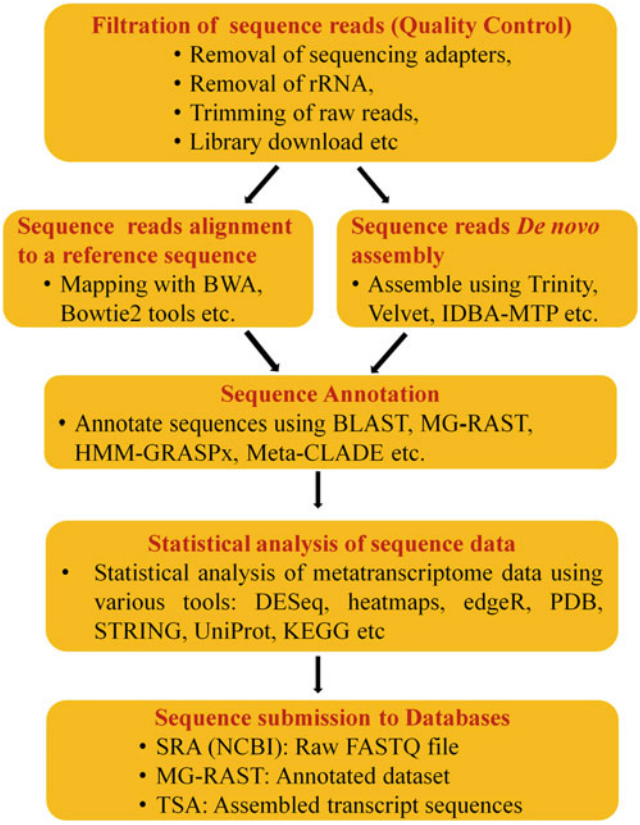

Fig. 1.3 The major steps involved in bioinformatics analysis of metatranscriptomics data

assembler tools are used to generate a reference scaffold from high-quality reads representing the expressed gene sets of a microbial genome. This approach enables the scientists to find sequence homologs easily, mapping for expression analysis, establishment of taxonomic origin, etc. The assembler programs such as Velvet (Zerbino and Birney 2008), Trinity (Grabherr et al. 2011), MEGAHIT (Li et al. 2015), and metaSPAdes (Nurk et al. 2017) used for this purpose were originally made for metagenomics study. They may lead to some erroneous result during metatranscriptome assembly; therefore they should be used with cautions. For De Novo assembly of metatranscriptome, specific tools such as Transcript Assembly Graph (TAG) (Ye and Tang 2016), IDBA-MTP (Leung et al. 2015), etc. are used. The datasets for de novo assembly of metatranscriptomics are still not completely developed. With the advancement in bioinformatics still only a few specific tools have been designed for De Novo assembly for metatranscriptomics. However, the efficacies of these assembler tools on diverse datasets have still not been tested. Moreover, their hardware requirements on complex community and data volume have also not been thoroughly verified.

1.2.3.3 Functional Annotation of Transcriptomes

The metatranscriptomics sequence data are annotated using several bioinformatics tools such as Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990), Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST) (Meyer et al. 2008), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2019) for basic information. However, the metatranscriptomics data is primarily used for the assessment of functional activity of microbiome in study. The functional annotation from microbiome RNA-seq can be done using either assembled contigs or sequence reads. Several read based functional annotation prediction tools such as HMM-GRASPx (Zhong et al. 2016), MetaCLADE (Ugarte et al. 2018), etc. are used for this purpose. However, these tools require predicted open reading frames (ORFs) of sequences as input database which is provided by other tools such as FragGeneScan (Rho et al. 2010). The MetaCLADE tool consists of a database having two million probabilistic models from 15,000 Pfam domains. The MetaCLADE tool represents significant diversity for each domain as database of this tool possesses hundreds of models for a single domain. Thus, sequence search using this database shows large numbers of hits for each sequence read which should be filtered on the basis of sequence probability, redundancy, and bit-scores (Ugarte et al. 2018). Moreover, assembled contigs may also be used for functional annotation study. The gene finding programs such as FragGeneScan (Rho et al. 2010) and Prodigal (Hyatt et al. 2010) are used for this purpose. Apart from these programs, functional assignment of transcriptomes is performed using sequence similarity search tool such as DIAMOND (Buchfink et al. 2015) which search for sequence similarity against functional databases such as NCBI RefSeq, UniProt, etc. After functional annotation of transcriptomes enzymatic functions of transcript may also be mapped to previously well-established metabolic pathways, using specific tools such as iPath (Yamada et al. 2011), MinPath (Ye and Doak 2009), etc.

Apart from direct functional annotations, taxonomic assignments to identify the microorganisms which are involved in active RNA expression and differential expression analyses of metatranscriptome data should also be performed to understand the microbial functional diversity in microbiome in different conditions.

Transcript Taxonomy Study of Metatranscriptome

Transcript taxonomy tools are used to study contig based taxonomic assignments to understand actively RNA expressing organisms. For transcript taxonomy study of metatranscriptomics data several taxonomy classification programs such as MetaPhlan2 (Truong et al. 2015), GOTTCHA (Freitas et al. 2015), etc. are used. Most of these programs work on the basis of nucleotide matches of short sequence reads. Therefore, they are mostly useful for closely related microbiomes in sequence databases. However, sequence reads with nearly full length transcript or longer contigs can be analyzed by several bioinformatics programs such as Kraken 2 (Wood and Salzberg 2014) and centrifuge (Kim et al. 2016) to identify the individual member of microbiome community.

The taxonomic analysis by sequence reads or deduced coding regions suffer from several limitations such as lack of efficient algorithms required to process larger

volume sequence data, accommodation of short sequence reads, and lack of sufficient number of references in reference databases. In microbiome study the differentiation of lower number of sequence hits from false positive hits create a great problem. Moreover, limited knowledge on microbial diversity also severely limits the application of taxonomy classification tools in metatranscriptomics study of microbiome.

Differential Expression Analyses of Metatranscriptomes

The differential analyses of metatranscriptomes discuss about the comparison of differential gene expression in a microbiomes in different environmental conditions and parameters and their effect on microbial biochemical function over the time. Earlier, several bioinformatics programs were developed for use with single microbial genomes only. Later on some of these tools were updated for differential gene expression studies of metatranscriptomics data. Most of such tools require input abundance transcriptome data per gene and per sample for the specific environmental conditions or particular time period. The abundance transcriptome data can be obtained by sequence read alignment or mapping to reference genome or gene set or assembly. Some of the tools such as Limma (Ritchie et al. 2015), DeSeq2 (Love et al. 2014), and edgeR (Robinson et al. 2010) are frequently used to identify differential expression of genes among the microbiome sample at different environmental conditions or time point. Although, several tools are available for differential gene expression for transcriptome data, the differential expression analyses of metatranscriptome of a microbiome are still a challenging task because of availability of transcript sequences from a wide array of organisms. This lead to a special kind of difficulty such as dealing with shared gene problems among closely related organisms along with taxonomic variation of transcripts which may led to incorrect differential gene expression profile assessment.

1.2.3.4 Statistical Analysis of Sequence Data

Statistical analysis of metatranscriptome sequences are performed in several steps including build of count matrix, matrix transformation, similarity search between samples, differential expression analysis of genes, visualization of differentially expressed genes, function prediction of previously known and unknown genes (Peimbert and Alcaraz 2016). The count matrix is built by counting the total mapped reads from all the samples. It is required for analyzing the annotation sequence data in data analysis pipeline. Subsequently, the count matrix is transformed or normalized to avoid dependence of mean values on samples and experiments by employing regularized logarithm transformation (rlog) and DESeq tool (Love et al. 2014). After matrix transformation, the similarity between the samples or experiments can be determined by heatmaps, distance calculation on rlog/log2, and Principal Component Analysis (PCA) tool. Later on, differential expression analysis is performed by calculation of mean log2 fold changes, standard error, test of null hypothesis and p-value calculation between samples or experiments using several bioinformatics tools such as edgeR (Robinson et al. 2010), DESeq (Anders and Huber 2010), baySeq (Hardcastle and Kelly 2010), NOISeq (Tarazona et al.

2011), etc. The significant amount of differentially expressed genes can be visualized by heatmaps or Volcano plots tools. Finally, the functions of annotated and known genes expression are correlated with experimental sample. The data from metatranscriptomics analysis are usually categorized in to two classes, i.e. genes having known functions and genes having unknown functions. It is easy to characterize the genes with previously known functions. For genes with known functions, several data mining tools such as Protein Data Bank (PDB) (Berman et al. 2000), Pfam (Finn et al. 2008), Search Tool for the Retrieval of Interacting Genes (STRING) (Szklarczyk et al. 2017), UniProt (UniProt Consortium 2019), and KEGG (Kanehisa et al. 2019) are utilized to get their appropriate functions. However, a significant amount of metatranscriptome data remained with unknown functions which need further experiments to determine their functions.

1.2.3.5 Sequence Submission to Databases

After the completion of the metatranscriptome data analysis the RNA-seq are submitted to suitable databases or repositories for scientific use by other researchers and comparison with other datasets. The raw FASTQ files are usually deposited to Short Read Archive (SRA) of NCBI with submitter's name and project details. The assembled transcript sequences generated by bioinformatics tools are deposited to Transcriptome Shotgun Assembly (TSA). The annotated dataset is shared through MG-RAST server, because it is vital for genomic studies and addresses the many challenges including curation, exchange, and information dissemination.

1.2.4 Metatranscriptomics Study by Microarray Technique

The microarray is a technique where picomoles of nucleic acid sequences known as probes are deposited on to a microscope glass slide surface and allowed for probe-target hybridization with specific reagents (fluorophore, silver or chemiluminescence) labeled nucleic acids (target) to detect and quantify the nucleic acid (Ranjan et al. 2015). Microarrays have already been used to measure microbial gene expression level of several genes, detection of new transcripts, and structure determination of mRNAs of one or more species simultaneously.

For metatranscriptome analysis a specific variant of microarray called tilling microarray is used. The tiling array is a whole genome based oligonucleotide probe based microarray. It has proved its usefulness in whole genome functional analysis. Since, tiling array is a specific variant of microarray; its basic functionality is similar to regular expression microarray but the difference is within the probe design. The probes of tilling arrays are designed for known contiguous sequences especially the genomic regions whose expression is previously unknown. Thus, resolution limit of tiling arrays is a function of probe design, i.e., whether probes are designed in overlapped or spaced apart manner in entire genomes. The several millions of oligonucleotide probes are required for whole genome tiling array study. For comprehensive identification of entire coding sequences in human genome by tiling arrays 52 million oligoprobes were designed (Bertone et al. 2004).

Tiling microarray is an efficient method to identify gene expression. The traditional methods of gene prediction and transcriptome analysis may not produce an accurate picture of genes and may miss out entire transcript. Similarly, transcriptome analysis by traditional methods using cDNA sequencing may also be proved biased in the detection of genes that expressed themselves only at a specific point of time or in response to specific signals. Moreover, detection of very short or rare RNA molecules is also challenging by cDNA sequencing. Many of such problems can be sort out by tilling array technique. In tilling arrays, millions of copies of specific probes are made within a single array unit called feature and in a microarray chip 10,000–6,000,000 different features are found. Thus, total numbers of probes are much more in tilling arrays in compared to conventional microarray (Mockler et al. 2005). Tiling arrays assisted tremendously in transcriptome mapping, identification of sites for DNA/protein interaction (ChIP-chip), DNA methylation (MeDIP-chip), etc. (Yazaki et al. 2007). It can also be used for metatranscriptome study. Because of higher sensitivity and resolution of tilling arrays rare and small nucleic acid fragments can also be identified. The overlapping probes also permit identification of non-polyadenylated RNA and thus, high resolution image of gene structure can be generated (Bertone et al. 2005).

Besides the advancement in microarray techniques, still it has certain pitfalls such as its low sensitivity, requirement of prior knowledge of gene targets followed by design of specific probes. Many of such limitations of the microarray technique are resolved in NGS based RNA-Seq methods (Table 1.2). By combining the two methods, i.e., high throughput sequencing and microarray, a more comprehensive image of microbial metatranscriptomics can be produced (Filiatrault 2011).

1.3 Metatranscriptomics and Soil Microbiome

Metatranscriptomics is the study of mRNA and rRNA diversity of a microbiome in a particular environment at specific time and space. It permits simultaneous exploration of microbial gene expression (mRNA) as well as its abundance (rRNA). For gene expression study, mRNA is usually preferred over protein because mRNAs delivers a better real-time image of cell functioning in relation to environmental variations. The present era of molecular biology is of transition phase from metagenomics to metatranscriptomics because the latter reveals the functional diversity of the microbiome, rather than only genetic diversity as the former does. Metatranscriptomics studies have disclosed several facts about soil microbiomes and their importance to plants and ecosystems (Table 1.3).

The microbiome composition in plants is dependent on multiple factors such as pH, temperature, chemical constituents of soil, biochemical signals from plants, bacteria, fungi, etc. These factors determine the selective association between functional microbiomes and plants and assists in the identification of phenotypes suitable for increased crop productivity followed by food security (Lakshmanan et al. 2014). However, exact mechanisms by which a functional microbiome improves the plant growth and fitness are still not much clear. In *Oryza sativa* root

Table 1.2 Comparison of High throughput RNA-Seq and Microarray technique

S. n.	Parameters	High throughput RNA-Seq technique	Microarray technique
1	System used	Open system architecture	Closed system architecture
2	Preparation of sample	Easy to prepare from extracted nucleic acid	Relatively complex to prepare microarray slides
3	Higher specificity and sensitivity of result	Ideal for detection of genes especially with low expression level	Difficult to identify rare transcript
4	Cost effect value for multiple samples	More expensive	Less expensive
5	Data analysis	Relatively complex annotation for millions of sequence reads	Signal intensities of microarray are easy to analyze
6	Application in species study at genomic level	Best available method for microbial genome analysis	Best method for DNA–DNA hybridization study
7	Detection of novel transcripts	It can easily detect novel transcripts, single nucleotide variants, gene fusions, indels, etc.	Difficult to identify such variations because it needs specific probe
8	Digital output	NGS reads represent absolute expression of a microbiome and enable identification of low abundance transcripts	Difficult to identify complete expression of a microbiome
9	Wider dynamic range	It produces discrete sequencing reads and quantify expression for larger dynamic range ($>10^5$ for RNA-Seq in comparison to 10^3 for microarrays)	The transcript measurement is limited by background at low end and signal saturation at the high end
10	Specific application	Microbiome diversity study	Study of functional gene diversity

the significant diversity of bacterial species was reported (Hernandez et al. 2015). With the advancement of modern sequencing techniques it is easy to identify and characterize the microbiome in model plants such as *Arabidopsis thaliana* and *Zea mays* (Turner et al. 2013; Gomez-Godínez et al. 2019). The simultaneous approach of time-series sampling, high throughput 16S rRNA gene sequencing, and metatranscriptomics study can disclose the functional diversity of microbiome of biomass degrading and composting ecosystem. The time scale study reveals about the sequence of occurrence of microbes for biomass degradation as beginning from simultaneous destruction of lignocellulose and hemicellulose to cellulose and lignin. Such study also improves the knowledge of microbiome diversity and assists in identification of newer bacterial order such as *Bacillales* (Antunes et al. 2016).

The RNA (16S rRNA gene and mRNA) based research using high throughput sequencing technique may be used for study of effect of soil metal pollution on

Table 1.3 High throughput sequencing based approach to study the soil microbiome

S. n.	Study target	Methodology	High throughput sequencing platform	Conclusions	Reference
1	Bacterial diversity in soil	Bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP) diversity method	GS-FLX system	The bTEFAP technique is a powerful method to study the bacterial diversity of the soil under different managemental and land use conditions.	Acosta-Martínez et al. (2008)
2	Root exudates of *Arabidopsis*	Unidirectional pyrosequencing	GS-FLX titanium system	Salicylic acid revealed positive correlation with bacterial populations from *Streptomycineae, Corynebacterineae,* and *Pseudonocardineae* subfamily and gamma Aminobutyric acid (GABA) with bacteria from *Methylobacterium, Skermanella, Sphingomonas, Micromonosporineae, Frankineae,* and *Variovorax*. The phenolic compounds act as signalling molecules for microbial population in soil.	Badri et al. (2013a)
3	Reverse soil microbiomes from *Arabidopsis thaliana* roots	16S rRNA gene sequencing	GS-FLX system	Correlation analyses were performed to determine the relationship between various factors such as root microbiome treatment, leaf chemical components, plant growth patterns, and *Trichoplusia ni* larvae feeding behavior. The leaf amino acid content was positively correlated with both *Trichoplusia ni* larvae feeding	Badri et al. (2013b)

(continued)

Table 1.3 (continued)

S. n.	Study target	Methodology	High throughput sequencing platform	Conclusions	Reference
				behavior and microbiome composition.	
4	Soil microbiomes of argentine pampas	16S rRNA amplicon sequencing	GS-FLX titanium system	Microbiomes from cultivated fertilized soils with higher nutrient amendment showed tendencies to copiotrophy while that of from non-cultivated homogenous soils showed more oligotrophic type lifestyle.	Carbonetto et al. (2014)
5	Bacterial communities study in heavy metal polluted soil	16S rDNA pyrosequencing	GS-FLX titanium system and GS junior	Bacterial communities were studied from three soil samples contaminated with Pb and Zn followed by with Cr and low level of Zn and Pb. Study revealed that Zn decreased bacterial diversity at species and family level. The core operational taxonomic units (OTUs) of microbes identified were *Flexibacter, Sphingomonas,* and *Candidatus solibacter*.	Gołębiewski et al. (2014)
6	Soil microbiome	High throughput sequencing methods	HiSeq 2000 (Illumina)	The pH, temperature, and chemical signals from plants, bacteria, and nematodes create specific soil environment which influence the organisms residing there and allows the association between different	Lakshmanan et al. (2014)

				microbes and microbes and plant to increase crop productivity.	
7	Rhizosphere microbiome of plant species	16S rRNA sequencing	GS-FLX system	Host plant selectively influences the active bacterial microbiome composition in its vicinity soil	Ofek et al. (2014)
8	Rhizosphere microbiomes of *Zea mays*	16S rRNA gene fragments sequencing of rhizosphere soil	MiSeq (Illumina)	The soil microbiome dynamics can be changed by simple resource amendments in soil near the plant and specific rhizosphere may be developed	Bakker et al. (2015)
9	Soil microorganism *Arabidopsis thaliana* root soil	16 S rRNA gene sequencing	MiSeq (Illumina)	The microbiomes may selectively modify the flowering traits of *Arabidopsis thaliana* plant and may cause changes in the soil resource pools.	Panke-Buisse et al. (2015)
10	Microbial community from artificially polluted soil	Pyrosequencing of 16S rRNA	GA IIx (Illumina)	The aromatic compound pollution to soil causes decrease in microbial diversity and increase in gene pools diversity showing the robustness of such bacterial communities against the against the chemical pollutants	Kato et al. (2015)
11	Microbial biomass and diversity in soil	16S rDNA sequencing	MiSeq (Illumina)	They explore the correlations, intertaxa and between major groups and functional traits was explored to gain a more integrated understanding of microbiome and ecological rules guiding soil community fostered by fertilization regimes	Ling et al. (2016)
12	Anoxic bulk soil containing mercury and methyl mercury from rice roots	16S rRNA sequencing	MiSeq (Illumina)	In furrow irrigated area, lower number of genera having mercury methylators were found suggested an association	Rothenberg et al. (2016)

(continued)

Table 1.3 (continued)

S. n.	Study target	Methodology	High throughput sequencing platform	Conclusions	Reference
				between rice methyl mercury and soil microbiome	
13	Bulk soil samples from high and low productivity area	Shotgun sequencing	HiSeq2000 (Illumina)	The crop productivity variations were found associated with bulk soil microbiome diversity and nitrogen utilization related microbial taxa	Chang et al. (2017)
14	Microbiome from soil, Rhizosphere, and roots of perennial grass	16S rRNA gene sequencing	HiSeq 2500 (Illumina)	The considerable variations were identified in microbiome of soil, rhizosphere, and roots from low diversity grass mixture (*Bouteloua curtipendula*, *Sorghastrum nutans,* and *A. gerardii*) and warm season grasses (*Andropogon gerardii* and *Panicum virgatum*).	McPherson et al. (2018)
15	Washed *Salix purpurea* plant roots from petroleum hydrocarbon contaminated soil	Differential gene expression study	HiSeq 2500 (Illumina)	In petroleum hydrocarbon contaminated soil plant root, fungus, and bacteria genes are expressed to form the necessary apparatus for biofilm formation and reduction of stress to plant from petroleum hydrocarbon contamination.	Gonzalez et al. (2018)
16	Microbiomes from soils, plants and herbivorous insects	16S rRNA sequencing	MiSeq (Illumina)	Plants influence the soil microbiomes and microbiomes of insects are dependent on soil microbiomes. The effects of plants on soil microbiomes are transferred to aboveground insect	Hannula et al. (2019)

				populations by insect microbiomes and subsequently transferred to other plant by insect feeding.	
17	Effect of swine manure on soil microbiome through mobile element and antibiotic resistance genes on agricultural soil	16 s rRNA sequencing	MiSeq (Illumina)	The addition of manure from swine feeding on concentrated animal feeding operations (CAFO) to soil revealed that manure contains major microbes as *Firmicutes* and *Bacteroidetes* the soil samples possess microbial communities *Verrucomicrobia*, *Actinobacteria, Acidobacteria, Proteobacteria* and unclassified bacteria. The study also suggested that manure bacteria do not survive well in soil and antibiotic resistance gene dynamics of soil due to swine manure vary by resistance genes.	Lopatto et al. (2019)
18	Rhizomicrobiome from genetically modified maize	16S rRNA gene sequencing	MiSeq (Illumina)	The genetic modifications of maize plant significantly affect the microbial community of rhizosphere as detected in *nirK* denitrifiers.	Szoboszlay et al. (2019)
19	Soil sample from wild oat (*Avena fatua*) root	Metatranscriptome of RNA viruses of eukaryotes	HiSeq2000 (Illumina) MiSeq (Illumina)	RNA viruses from *Narnaviridae* and *Leviviridae* family infect fungi and Proteobacteria, respectively, lead to cell death and mobilization of cell carbon in soil for soil carbon cycling	Starr et al. (2019)
20	Rhizobacterial communities from tomato cultivars	16S rRNA gene sequencing	MiSeq (Illumina)	Soil is established as a major factor for the microbial species diversity in tomato rhizosphere in comparison to plant genotype	Cheng et al. (2020)

microbial composition and its activity. It is reported that overall soil Copper pollution inhibits the microbial activity. However, despite adverse conditions caused by Copper pollution, still some of the soil microbial activities may be observed as evidenced by increase in phage mRNA signatures (Jacquiod et al. 2018). The metatranscriptome analysis of artic soil may give an indication about the climate warming by greenhouse gas emissions from soil. The metatranscriptomics study of Arctic cryosols sample revealed the presence of active form of denitrifying and nitrogen fixing bacteria such as *Cyanothecaceae, Azotobacteraceae, Rhizobiaceae, Burkholderiaceae, Ectothiorhodospiracea, Chloroflexaceae, Nostocaceae,* and *Rhodobacteraceae*. These microbial populations can be correlated with elevated N_2O (potent greenhouse gas) flux from wetter trough soils in comparison to drier interior soils which indicate about the climate warming since Arctic is expected to develop wetter and warmer conditions (Altshuler et al. 2019).

The metatranscriptome based approach is a useful technique to detect the changes in microbiome which would have been otherwise remains undetected by traditional assays such as PCR (Shakya et al. 2019). In one of such studies, the non-fungal eukaryotic species were detected from mutant oat plants which were not detected from its wild relatives (Turner et al. 2013). This technique is also helpful in the detection of genes which determines the mutualistic relationship between microbiome and seagrass plant (Crump et al. 2018). Moreover, functionally active microbiome and pathways showing its significance in maturation of ripe fruits can also be studied using metatranscriptomics (Saminathan et al. 2018). In one of the studies, researcher has revealed that metatranscriptomics analysis can be used to understand the suppressive and non-suppressive mechanisms of associated genes from *Rhizoctonia solani* fungal infection in wheat plants which can be used as a potential molecular target for overall enhancement of plant productivity (Hayden et al. 2018). Thus, soil metatranscriptomics study provides a comprehensive knowledge about the overall functioning of diverse form of ecosystems from crop land to polluted lands and arctic soil which have direct influence on global warming, plant growth, diseases, and productivity.

1.4 Limitations and Challenges in Metatranscriptomics

Despite the advancement in high throughput sequencing technologies, advanced algorithms and high speed computing facilities metatranscriptomics analyses are still facing several challenges.

1.4.1 Problems Associated with Total RNA Extraction from Soil Sample

The average half-lives of mRNA of a species are within the range of few seconds to few minutes (Deutscher 2006). However, the stability of mRNA is also dependent on the diversity of microbial species and nutritional status of the cell (Redon et al.

2005). Therefore, for proper study of metatranscriptional profiles of a microbiome it is essential to immediately store the samples in liquid nitrogen or transfer to RNA preservation solution. The RNA isolation from soil sample is especially tricky because of insufficient cell lysis, adsorption of RNA molecules to soil particle, and presence of inhibitory enzymes such as RNases in soil. The RNA extraction using conventional buffers also facilitate the adsorption of mRNA molecules to soil particle (Chomczynski and Sacchi 1987). Most of the methods used for soil mRNA extraction utilize an initial bead-beating step (Lakay et al. 2007). The other common methods for RNA extraction from soil samples are liquid nitrogen grinding (Volossiouk et al. 1995), enzymatic lysis (Zhou et al. 1996), and microwave-based rupture (Orsini and Romano-Spica 2001) which are less efficient than bead-beating method. Moreover, during mRNA extraction contamination with genomic DNA (gDNA) is a common problem which may lead to over estimation of RNA concentration in UV spectrophotometry based quantification of nucleic acid. To minimize the gDNA, the DNaseI treatment to RNA extract may be employed (Marchetti et al. 2012).

During mRNA extraction from soil samples several PCR inhibitory substances such as fulvic acids and humic are also co-precipitate (Opel et al. 2010). Therefore, several strategies have been employed to eliminate the fulvic and humic acids from extracted RNA such as precipitation of cells using aluminum sulfate prior to cell lysis (Persoh et al. 2008), adsorption by activated charcoal (Desai and Madamwar 2007), incorporation of polyvinyl polypyrolidone (PVPP) during mRNA extraction (Rajendhran and Gunasekaran 2008), $CaCO_3$ pretreatment of soils (Sagova-Mareckova et al. 2008), RNA extraction at pH 5.0, and subsequently purification of mRNA using Q-Sepharose columns (Mettel et al. 2010). Recently, several commercial kits have also been developed for total RNA extraction from soil samples followed by selective removal of rRNA (i.e. mRNA enrichment) from total RNA extract for downstream metatranscriptome analysis (Table 1.4).

1.4.2 Problems Associated with mRNA Enrichment

The major component of total RNA extract from an environmental sample is rRNA and tRNA (Karpinets et al. 2006). However, the total RNA also possesses 1–5% of mRNA constituent (He et al. 2010). Therefore, mRNA enrichment from total RNA extract is an essential step for metatranscriptomic study. To recover or enrich mRNA from soil total RNA several approaches have been utilized (Table 1.4).

1.4.2.1 Subtractive Hybridization of mRNA

This technique is used for identification, characterization, and differentiation of nucleic acid populations. It allows the differentiation between different RNA species from several origins (cells, tissues or organisms, etc.) at different phases of growth in normal or diseased conditions. It relies on subtraction of dsDNA molecules formed by hybridization of control and test sample. Therefore, the subtractive hybridization technique allows specific elimination of cDNAs and retains the desired transcripts or

Table 1.4 Commercially available soil total RNA extraction and mRNA enrichment kits

S. n.	Name of the Kit	Kit Manufacturer	Basic principal of kit	Application
1	FastRNA Pro Soil-Direct Kit	MP Biomedicals (USA)	Adsorption using binding matrix	Total RNA Extraction
2	FastRNA Pro Soil-Indirect kit	(MP Biomedicals, USA)	Adsorption using binding matrix	Total RNA Extraction
3	ISOIL for RNA	NIPPON GENE (Japan)	By precipitation	Total RNA Extraction
4	RNA Power Soil™ Total RNA Isolation kit	MO BIO (USA)	Adsorption in single gravity flow column	Total RNA extraction
5	ZR soil/fecal RNA MicroPrep	Zymo research (USA)	Adsorption or gel filtration in multiple spin columns	Total RNA extraction
6	EZNA soil RNA kit	Omega BioTek (USA)	Adsorption in single spin column	Total RNA extraction
7	IT 1–2-3 platinum path™ sample purification kit	Idaho technology (USA)	By magnetic beads	Total RNA extraction
8	Soil Total RNA purification kit	Norgen (Canada)	Adsorption in single spin column	Total RNA extraction
9	Ribo-zero rRNA removal kit	EPICENTRE biotechnologies (USA)	Subtractive hybridization	rRNA removal
10	MICROExpress bacterial mRNA purification kit	Ambion (USA)	Subtractive hybridization	rRNA removal
11	mRNA ONLY prokaryotic mRNA isolation kit	EPICENTRE biotechnologies (USA)	Exonuclease digestion	rRNA removal
12	RiboMinus Transcriptome isolation kit	Invitrogen (USA)	Subtractive hybridization	rRNA removal

genomic sequences. It involves the removal of specific set of rRNA from total RNA reference sample using probes complementary to rRNA, i.e. the sample to be subtracted (Pang et al. 2004). There are several commercial kits have been developed for subtractive hybridization of mRNA (Table 1.4). However, due to insufficient base complementarity of capture probes, the kit may not be effective enough to remove all the rRNA from total RNA sample. For complex environmental samples, subtractive hybridization technique is modified by using sample specific rRNA probes for mRNA enrichment (Stewart et al. 2010).

1.4.2.2 Exonuclease Treatment to Degrade rRNA from Total RNA

This method utilizes a 5'phosphate dependent exonuclease to degrade RNA molecules containing 5'monophosphate (Table 1.4). The presence of

5'monophosphate moiety in most of the bacterial rRNA makes it suitable target to remove using this method. However, soil derived total RNA contains significant amount of humic acids, which may inhibit most of the enzymes such as exonuclease and may subsequently inhibit the rRNA degradation. The study also showed that the subtractive hybridization method is more efficient for mRNA enrichment from soil samples in comparison to exonuclease method (Mettel et al. 2010). Therefore, to target unprocessed mRNA both the methods, i.e. exonuclease treatment followed by subtractive hybridization should be used (Mettel et al. 2010). The fidelity and effectiveness testing of relative transcript abundances by high throughput metatranscriptome sequencing suggested that the least biasness was observed when only subtractive hybridization method was used followed by only exonuclease method and in combination of these two methods (He et al. 2010).

1.4.2.3 Size Separation by Gel Electrophoresis

The size separation method is unique from other method because it involves least sample processing post-RNA extraction. However, it needs slightly large amount of total RNA for processing (McGrath et al. 2008). In this approach, total RNA is analyzed on 1.5% agarose gel in tris acetate EDTA (TAE) buffer and allow for electrophoresis at 100 V for 45 minute. Upon completion of electrophoresis the mRNA regions in lanes between the rRNAs (23S, 16S, and 5S) bands are visualized on UV transilluminator, excised away and purified using commercially available suitable gel elution kit. However, there is enough possibility that mRNA having the same size as that of rRNA (23S, 16S, and 5S) bands might be missed out because, such mRNA will be remain present in the excised regions of the gel.

1.4.2.4 Duplex Specific Nuclease (DSN) Treatment of Total RNA Extracts

DSN is an enzyme system which has property to preferentially degrade the dsDNA at high temperature. DSN treatment has successfully been utilized to normalize the relative transcript abundance in eukaryotic as well as prokaryotic organisms (Yi et al. 2011). For preservation of relative abundance of the mRNA among different samples, DSN treatment based mRNA enrichment method revealed higher relative efficiency of rRNA removal from total RNA in comparison to subtractive hybridization method (Yi et al. 2011). The thermodynamic principle of DSN treatment method also allows its use in bacterial mRNA enrichment and metatranscriptomics study. The comparative study between subtractive hybridization and DSN treatment revealed that the DSN treatment has better efficiency of mRNA enrichment from total RNA (Yi et al. 2011).

Apart from the contaminating microbial rRNA, total RNA from soil samples also contain eukaryotic RNA of plants and fungi origin. The eukaryotic mRNA removal approaches are based on the fact that non-eukaryotic RNA molecules either do not possess 3′ polyA tail or if it is present, then such RNA molecules are rapidly degraded (Belasco 2010). The eukaryotic mRNA from soil total RNA pool can be selectively removed using several strategies such as application of surface coated poly(dT) probes to capture eukaryotic RNAs having 3′ poly A tail, cDNA synthesis

using anchored oligo dT primers to remove mRNA, poly(dT) oligonucleotide coated magnetic bead based affinity capture of eukaryotic mRNAs (Bailly et al. 2007).

1.4.3 Problems Associated with cDNA Synthesis and Amplification

The total RNA extraction from the soil sample usually contains small amounts of microbial mRNA which needs an additional step to amplify the microbial mRNA to obtain sufficient starting material for metatranscriptome study. This process is performed by linear amplification approach. The microbial RNA is allowed to poly adenylation using *E. coli* poly A polymerase prior to reverse transcription. Later on, the polyadenylated RNA is transformed to cDNA using an oligo (dT) primer, T7 RNA polymerase promoter sequence along with a recognition site for a restriction endonuclease enzyme (Frias-Lopez et al. 2008). The similar type of procedure may also be applied for eukaryotic RNA, but without polyadenylation step of mRNA as it already contains poly A tail. Subsequently, random primers are used to synthesize double stranded cDNA by reverse transcription. Finally, the poly A tails are removed by enzymatic cleavage at restriction sites inserted to the oligo (dT) primers (Frias-Lopez et al. 2008).

Most of the high throughput sequencing platforms utilize cDNA as input template. However, during cDNA synthesis the reverse transcriptase enzyme may introduce errors in template strand (Roberts et al. 1989). Study also suggested that short transcripts (mRNA) possess better efficiency to reverse transcribed than long transcripts (Stewart et al. 2010). It has also been reported that sometimes non-target RNA molecules may act as primer and non-specific cDNA molecules may be generated (Haddad et al. 2007). However, such non-specific cDNA synthesis can be reduced by performing reverse transcription reaction at higher temperatures in the presence of RNase H+ enzyme (Haddad et al. 2007). Many of the cDNA synthesis and amplification related problems may be avoided by direct sequencing of RNA (Mamanova et al. 2010). However, direct sequencing of RNA approach needs further validation for wider applications (Ozsolak and Milos 2011).

1.4.4 Problems Associated with Identification of Transcripts from Fewer Microbial Populations

In soil microbiome, many of the microbial species are present in very less numbers. Therefore, despite the advancement in high throughput sequencing, the diverse groups of microbes with very less in number are still difficult to study. In metatranscriptomics analysis large population of microbial cells from a microbiome are used for study at a time which leads to difficulty in the identification of specific variances among the individual subpopulations of microbes. The variance study in microbial subpopulation is important because many of such microbes are responsible for production of specific biomolecules which may be either useful or may causes diseases too. To overcome such problem specific strategies are employed such as

isolation of specific microbial populations from microbiome and reduction of complexity of metatranscriptomics dataset by focusing on single microbial cell. The lesser number of microbial populations generates transcriptome sequences with greater coverage. The stable isotope probing (SIP) is a technique to identify the specific microbial functional groups or compounds in their natural environmental condition (Whiteley et al. 2007). The specific modification of SIP technique, i.e., RNA-SIP can be used in advance study of metatranscriptomic analysis for mRNA identification from specific microorganisms. In SIP study specific radio isotope materials such as 13C, 15 N, 18O, etc. are supplied in microbial growth medium which lead to synthesis of radio labeled microbial anabolic products such as nucleic acid and other product. Later on radio isotope labeled and unlabeled nucleic acids are separated by density gradient centrifugation techniques and subsequently specific nucleic acid fractions are isolated and analyzed using modern techniques such as metagenomics and metatranscriptomics. However, in cases of low yields of RNA from SIP a specific technique called multiple displacement amplification (MDA) is used for downstream metatranscriptomics analyses. MDA is used for amplification of very small amounts of DNA using non-PCR type of DNA amplification approach (Panelli et al. 2006).

1.4.5 Problems Associated with Bioinformatics Tools

For metatranscriptomics study many of the commercially available bioinformatics tools have been used. However, sometimes it became difficult to select an appropriate bioinformatics tool for metatranscriptome data analysis under given situation because many of these tools are specific for sequencing platforms and their accuracy also varies. Thus, it is essential to select a bioinformatics tools with optimum accuracy and performance for metatranscriptomics data. The microbiome genomes are highly complex and diverse in nature. Thus, the complexity of microbiomes and inadequate information of microbial species or their genome sequences create huge challenges for bioinformatics tools to provide appropriate results. Researchers are working on to create advance bioinformatics algorithm that may create simulated sequence data from previously known genomes and simulations of metatranscriptomics datasets which can be used for validations and parameter settings of bioinformatics tools (Shakya et al. 2019). The further advancement in sequencing technologies such as from next generation to third generation technologies may provide acceleration in bioinformatics algorithm development. We may assume that advance metatranscriptomics tools of coming generation will help us to improve our understanding of the biologically functional component of microbiome.

1.5 Conclusion and Future Prospective

Metatranscriptomics provide an accurate insight of microbial transcript in a microbiome at a specific moment of time under specific environmental conditions. In contrast to metagenomics, metatranscriptomics reveals the actual microbial gene expression status rather than its potential. The metatranscriptomics study may open a new research area for understanding the molecular mechanisms of gene expression regulation and characterization of functional changes at microbe–microbe, microbe–environment, and microbe–host interactions level. The metatranscriptomics analysis enhances our knowledge about the complex microbiome behavior. Soil microbiome constitutes of several microbial communities which require combination of several existing technical and analytical approaches to extract useful information. However, several challenges starting from sample selection, RNA extraction to bioinformatics data analysis appear during soil metatranscriptomics analysis which may adversely affect the reproducibility, accuracy, and general applicability of metatranscriptomics results. Despite the several challenges, metatranscriptomics study of soil microbiome may provide valuable information for deeper understanding of role of microbes in soil fertility, plant productivity, and disease resistance or susceptibility. The detailed knowledge of soil metatranscriptomics may be used in understanding the soil nutrient enrichment and mobilization, pathogenic microbe suppression, plant growth enhancement, and recycling of organic waste materials and pollutants. Thus, a sound knowledge of microbial metatranscriptomics may assists in development of effective strategies to deal with terrestrial ecosystems.

Although, metatranscriptomics provides deeper insight about the microbial functioning and diversity inside a microbiome but still it have certain limitations. Therefore, integrative approaches of various techniques related with microbiome analysis such as metagenomics, shotgun metatranscriptomics, metaproteomics, metabolomics, and 16S rRNA characterization may be applied, especially where budget is not a prohibitive factor. Individually, each one of these techniques contributes only single piece of useful information of a complex and large puzzle like problem. Moreover, reference dataset for metatranscriptome of a specific microbiome, large scale computing facilities, and advance algorithms for bioinformatics data analysis for metatranscriptome data are need of time.

References

Acosta-Martínez V, Dowd S, Sun Y et al (2008) Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biol Biochem 40:2762–2770

AllSeq (2008) The sequencing marketplace. https://allseq.com/knowledge-bank/ngs-necropolis/454-roche/. Accessed 10 June 2020

Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215 (3):403–410

Altshuler I, Ronholm J, Layton A et al (2019) Denitrifiers, nitrogen-fixing bacteria and N2O soil gas flux in high Arctic ice-wedge polygon cryosols. FEMS Microbiol Ecol 95(5):fiz049

Ambardar S, Gupta R, Trakroo D et al (2016) High throughput sequencing: an overview of sequencing chemistry. Indian J Microbiol 56(4):394–404
Anders S, Huber W (2010) Differential expression analysis for sequence count data. Genome Biol 11(10):R106
Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. Available: http://www.bioinformatics.babraham.ac.uk?/projects/fastqc/. Accessed 25 May 2020
Antunes LP, Martins LF, Pereira RV et al (2016) Microbial community structure and dynamics in thermophilic composting viewed through metagenomics and metatranscriptomics. Sci Rep 6:38915
Apirion D, Miczak A (1993) RNA processing in prokaryotic cells. BioEssays 15(2):113–120
Badri DV, Chaparro JM, Zhang R et al (2013a) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. J Biol Chem 288(7):4502–4512
Badri DV, Zolla G, Bakker MG et al (2013b) Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. New Phytol 198(1):264–273
Bailly J, Fraissinet-Tachet L, Verner MC et al (2007) Soil eukaryotic functional diversity, a metatranscriptomic approach. ISME J 1:632–642
Bakker MG, Chaparro JM, Manter DK et al (2015) Impacts of bulk soil microbial community structure on rhizosphere microbiomes of Zea mays. Plant Soil 392:115–126
Balasubramanian S (2011) Sequencing nucleic acids: from chemistry to medicine. Chem Commun (Camb) 47(26):7281–7286
Bashiardes S, Zilberman-Schapira G, Elinav E (2016) Use of Metatranscriptomics in microbiome research. Bioinform Biol Insights 10:19–25
Belasco JG (2010) All things must pass: contrasts and commonalities in eukaryotic and bacterial mRNA decay. Nat Rev Mol Cell Biol 11:467–478
Bentley DR, Balasubramanian S, Swerdlow HP et al (2008) Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456(7218):53–59
Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486
Berman HM, Battistuz T, Bhat TN et al (2000) The Protein Data Bank. Acta Crystallogr D Biol Crystallogr 58:899–907. (Pt 6 No 1)
Bertone P, Gerstein M, Snyder M (2005) Applications of DNA tiling arrays to experimental genome annotation and regulatory pathway discovery. Chromosom Res 13(3):259–274
Bertone P, Stolc V, Royce TE et al (2004) Global identification of human transcribed sequences with genome tiling arrays. Science 306(5705):2242–2246
Birzele F, Schaub J, Rust W et al (2010) Into the unknown: expression profiling without genome sequence information in CHO by next generation sequencing. Nucleic Acids Res 38:3999–4010
Buchfink B, Xie C, Huson D (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60
Camarena L, Bruno V, Euskirchen G et al (2010) Molecular mechanisms of ethanol-induced pathogenesis revealed by RNA sequencing. PLoS Pathog 6:e1000834
Carbonetto B, Rascovan N, Álvarez R et al (2014) Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and tillage systems in argentine pampas. PLoS One 9(6):e99949
Chang HX, Haudenshield JS, Bowen CR et al (2017) Metagenome-wide association study and machine learning prediction of bulk soil microbiome and crop productivity. Front Microbiol 8:519
Chen S, Zhou Y, Chen Y et al (2018) Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890
Cheng Z, Lei S, Li Y et al (2020) Revealing the variation and stability of bacterial communities in tomato Rhizosphere microbiota. Microorganisms 8(2):170
Chiosea SI, Williams L, Griffith CC et al (2015) Molecular characterization of apocrine salivary duct carcinoma. Am J Surg Pathol 39(6):744–752

Chomczynski P, Sacchi N (1987) Single step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. Anal Biochem 162:156–159
Crump BC, Wojahn JM, Tomas F et al (2018) Metatranscriptomics and amplicon sequencing reveal mutualisms in Seagrass microbiomes. Front Microbiol 9:388
Desai C, Madamwar D (2007) Extraction of inhibitor-free metagenomic DNA from polluted sediments, compatible with molecular diversity analysis using adsorption and ion-exchange treatments. Bioresour Technol 98:761–768
Deutscher MP (2006) Degradation of RNA in bacteria: comparison of mRNA and stable RNA. Nucleic Acids Res 34:659–666
Eid J, Fehr A, Gray J et al (2009) Real-time DNA sequencing from single polymerase molecules. Science 323(5910):133–138
Fedurco M, Romieu A, Williams S et al (2006) BTA, a novel reagent for DNA attachment on glass and efficient generation of solid-phase amplified DNA colonies. Nucleic Acids Res 34:e22
Filiatrault MJ (2011) Progress in prokaryotic transcriptomics. Curr Opin Microbiol 14(5):579–586
Finn RD, Tate J, Mistry J et al (2008) The Pfam protein families database. Nucleic Acids Res 36 (Database issue):D281–D288
Freitas TAK, Li PE, Scholz MB et al (2015) Accurate readbased metagenome characterization using a hierarchical suite of unique signatures. Nucleic Acids Res 43:e69
Frias-Lopez J, Shi Y, Tyson GW et al (2008) Microbial community gene expression in ocean surface waters. Proc Natl Acad Sci U S A 105:3805–3810
Gilles A, Meglécz E, Pech N et al (2011) Accuracy and quality assessment of 454 GS-FLX titanium pyrosequencing. BMC Genomics 12:245
Gołębiewski M, Deja-Sikora E, Cichosz M et al (2014) 16S rDNA pyrosequencing analysis of bacterial community in heavy metals polluted soils. Microb Ecol 67(3):635–647
Gomez-Godínez LJ, Fernandez-Valverde SL, Martinez Romero JC et al (2019) Metatranscriptomics and nitrogen fixation from the rhizoplane of maize plantlets inoculated with a group of PGPRs. Syst Appl Microbiol 42(4):517–525
Gonzalez E, Pitre FE, Pagé AP et al (2018) Trees, fungi and bacteria: tripartite metatranscriptomics of a root microbiome responding to soil contamination. Microbiome 6(1):53
Grabherr MG, Haas BJ, Yassour M et al (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29(7):644–652
Haddad F, Qin AQX, Giger JM et al (2007) Potential pitfalls in the accuracy of analysis of natural sense-antisense RNA pairs by reverse transcription-PCR. BMC Biotechnol 7:21
Hannula SE, Zhu F, Heinen R et al (2019) Foliar-feeding insects acquire microbiomes from the soil rather than the host plant. Nat Commun 10(1):1254
Hardcastle TJ, Kelly KA (2010) baySeq: empirical Bayesian methods for identifying differential expression in sequence count data. BMC Bioinformatics 11(1):422
Hayden HL, Savin KW, Wadeson J (2018) Comparative Metatranscriptomics of wheat Rhizosphere microbiomes in disease suppressive and non-suppressive soils for Rhizoctonia solani AG8. Front Microbiol 9:859
He S, Wurtzel O, Singh K et al (2010) Validation of two ribosomal RNA removal methods for microbial metatranscriptomics. Nat Methods 7(10):807–812
Hernandez M, Dumont MG, Yuan (2015) Different bacterial populations associated with the roots and rhizosphere of rice incorporate plant-derived carbon. Appl Environ Microbiol 81 (6):2244–2253
Hyatt D, Chen GL, Locascio PF et al (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinf 11:119
Jacquiod S, Nunes I, Brejnrod A et al (2018) Long-term soil metal exposure impaired temporal variation in microbial metatranscriptomes and enriched active phages. Microbiome 6(1):223
Judge K, Harris SR, Reuter S et al (2015) Early insights into the potential of the Oxford Nanopore MinION for the detection of antimicrobial resistance genes. J Antimicrob Chemother 70:2775–2778

Kanehisa M, Sato Y, Furumichi M et al (2019) New approach for understanding genome variations in KEGG. Nucleic Acids Res 47(D1):D590–D595

Karpinets TV, Greenwood DJ, Sams CE et al (2006) RNA: protein ratio of the unicellular organism as a characteristic of phosphorous and nitrogen stoichiometry and of the cellular requirement of ribosomes for protein synthesis. BMC Biol 4:30

Kato H, Mori H, Maruyama F et al (2015) Time-series metagenomic analysis reveals robustness of soil microbiome against chemical disturbance. DNA Res 22(6):413–424

Kim D, Song L, Breitwieser FP et al (2016) Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Res 26:1721–1729

Kopylova E, Noe L, Touzet H (2012) SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28:3211–3217

Lakay FM, Botha A, Prior BA (2007) Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. J Appl Microbiol 102:265–273

Lakshmanan V, Selvaraj G, Bais HP (2014) Functional soil microbiome: belowground solutions to an above ground problem. Plant Physio 166:689–700

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with bowtie 2. Nat Methods 9 (4):357–359

Leung HC, Yiu SM, Chin FY (2015) IDBA-MTP: a hybrid Metatranscriptomic assembler based on protein information. J Comput Biol 22(5):367–376

Li D, Liu CM, Luo R et al (2015) MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31:1674–1676

Li H, Durbin R (2010) Fast and accurate long-read alignment with burrows-wheeler transform. Bioinformatics 26(5):589–595

Li PE, Lo CC, Anderson JJ et al (2017) Enabling the democratization of the genomics revolution with a fully integrated web-based bioinformatics platform. Nucleic Acids Res 45(1):67–80

Ling N, Zhu C, Xu C et al (2016) Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. Soil Biol Biochem 99:137–149

Lopatto E, Choi J, Colina A et al (2019) Characterizing the soil microbiome and quantifying antibiotic resistance gene dynamics in agricultural soil following swine CAFO manure application. PLoS One 14(8):e0220770

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550

Lu H, Giordano F, Ning Z (2016) Oxford Nanopore MinION sequencing and genome assembly. Genomics Proteomics Bioinformatics 14(5):265–279

Mamanova L, Andrews RM, James KD et al (2010) FRT-seq: amplification-free, strand-specific transcriptome sequencing. Nat Methods 7:130–163

Marchetti A, Schruth DM, Durkin CA et al (2012) Comparative metatranscriptomics identifies molecular bases for the physiological responses of phytoplankton to varying iron availability. Proc Natl Acad Sci U S A 109:E317–E325

McGrath KC, Thomas-Hall SR, Cheng CT et al (2008) Isolation and analysis of mRNA from environmental microbial communities. J Microbiol Methods 75:172–176

McPherson MR, Wang P, Marsh EL et al (2018) Isolation and analysis of microbial communities in soil, Rhizosphere, and roots in perennial grass experiments. J Vis Exp 137:57932

Mettel C, Kim Y, Shrestha PM et al (2010) Extraction of mRNA from soil. Appl Environ Microbiol 76(17):5995–6000

Meyer F, Paarmann D, D'Souza M et al (2008) The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9:386

Minakshi P, Ranjan K, Brar B et al (2014) New approaches for diagnosis of viral diseases in animals. Adv Anim Vet Sci 2(4S):55–63

Mishra PK, Mishra S, Selvakumar G et al (2009) Enhanced soybean (Glycine max L.) plant growth and nodulation by Bradyrhizobium japonicum-SB1 in presence of Bacillus thuringiensis-KR1. Acta Agric Scand B soil. Plant Sci 59:189–196
Mockler TC, Chan S, Sundaresan A et al (2005) Applications of DNA tiling arrays for whole-genome analysis. Genomics 85(1):1–15
Niedringhaus TP, Milanova D, Kerby MB et al (2011) Landscape of next-generation sequencing technologies. Anal Chem 83:4327–4341
Nurk S, Meleshko D, Korobeynikov A et al (2017) metaSPAdes: a new versatile metagenomic assembler. Genome Res 27:824–834
Ofek M, Voronov-Goldman M, Hadar Y et al (2014) Host signature effect on plant root-associated microbiomes revealed through analyses of resident vs. active communities. Environ Microbiol 16(7):2157–2167
Opel KL, Chung D, McCord BR (2010) A study of PCR inhibition mechanisms using real-time PCR. J Forensic Sci 55:25–33
Orsini M, Romano-Spica V (2001) A microwave-based method for nucleic acid isolation from environmental samples. Lett Appl Microbiol 33:17–20
Ozsolak F, Milos PM (2011) RNA sequencing: advances, challenges and opportunities. Nat Rev Genet 12:87–98
Panelli S, Damiani G, Espen L et al (2006) Towards the analysis of the genomes of single cells: further characterisation of the multiple displacement amplification. Gene 372:1–7
Pang X, Zhou DS, Song YJ et al (2004) Bacterial mRNA purification by magnetic capture-hybridization method. Microbiol Immunol 48:91–96
Panke-Buisse K, Poole AC, Goodrich JK et al (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. ISME J 9:980–989
Parro V, Moreno-Paz M, González-Toril E (2007) Analysis of environmental transcriptomes by DNA microarrays. Environ Microbiol 9:453–464
Peimbert M, Alcaraz LD (2016) A Hitchhiker's guide to Metatranscriptomics. In: Field guidelines for genetic experimental designs in high-throughput sequencing. Springer, Cham, pp 313–342
Pennisi E (2010) Semiconductors inspire new sequencing technologies. Science 327(5970):1190
Persoh D, Theuerl S, Buscot F et al (2008) Towards a universally adaptable method for quantitative extraction of high-purity nucleic acids from soil. J Microbiol Methods 75:19–24
Philippot L, Raaijmakers JM, Lemanceau P et al (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799
Rajendhran J, Gunasekaran P (2008) Strategies for accessing soil metagenome for desired applications. Biotechnol Adv 26:576–590
Ranjan K, Minakshi P, Prasad G (2015) Application of molecular and serological diagnostics in veterinary parasitology. J Adv Parasitol 2(4):80–99
Redon E, Loubière P, Cocaign-Bousquet M (2005) Role of mRNA stability during genome-wide adaptation of Lactococcus lactis to carbon starvation. J Biol Chem 280:36380–36385
Rho M, Tang H, Ye Y (2010) FragGeneScan: predicting genes in short and error-prone reads. Nucleic Acids Res 38:e191
Rhoads A, Au KF (2015) PacBio sequencing and its applications. Genomics Proteomics Bioinformatics 13:278–289
Ritchie ME, Phipson B, Wu D et al (2015) Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43(7):e47
Roberts JD, Preston BD, Johnston LA et al (1989) Fidelity of two retroviral reverse transcriptases during DNA-dependent DNA synthesis in vitro. Mol Cell Biol 9:469–476
Robinson MD, Mccarthy DJ, Smyth GK (2010) edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140
Rothenberg SE, Anders M, Ajami NJ et al (2016) Water management impacts rice methylmercury and the soil microbiome. Sci Total Environ 572:608–617
Rudrappa T, Biedrzycki ML, Kunjeti SG et al (2010) The rhizobacterial elicitor acetoin induces systemic resistance in Arabidopsis thaliana. Commun Integr Biol 3:130–138

Rusk N (2011) Torrents of sequence. Nat Methods 8:44
Sagova-Mareckova M, Cermak L, Novotna J et al (2008) Innovative methods for soil DNA purification tested in soils with widely differing characteristics. Appl Environ Microbiol 74:2902–2907
Saminathan T, García M, Ghimire B et al (2018) Metagenomic and Metatranscriptomic analyses of diverse watermelon cultivars reveal the role of fruit associated microbiome in carbohydrate metabolism and ripening of mature fruits. Front Plant Sci 9:4
Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069
Seneca S, Vancampenhout K, Van Coster R et al (2015) Analysis of the whole mitochondrial genome: translation of the ion torrent personal genome machine system to the diagnostic bench? Eur J Hum Genet 23(1):41–48
Shakya M, Lo CC, Chain PSG (2019) Advances and challenges in Metatranscriptomic analysis. Front Genet 10:904
Shendure J, Porreca GJ, Reppas NB et al (2005) Accurate multiplex polony sequencing of an evolved bacterial genome. Science 309(5741):1728–1732
Spence C, Alff E, Johnson C et al (2014) Natural rice rhizospheric microbes suppress rice blast infections. BMC Plant Biol 14:130
Starr EP, Nuccio EE, Pett-Ridge J et al (2019) Metatranscriptomic reconstruction reveals RNA viruses with the potential to shape carbon cycling in soil. Proc Natl Acad Sci U S A 116 (51):25900–25908
Stewart FJ, Ottesen EA, DeLong EF (2010) Development and quantitative analyses of a universal rRNA-subtraction protocol for microbial metatranscriptomics. ISME J 4(7):896–907
Szklarczyk D, Morris JH, Cook H et al (2017) The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 45(D1): D362–D368
Szoboszlay M, Natther A, Mullins E et al (2019) Annual replication is essential in evaluating the response of the soil microbiome to the genetic modification of maize in different biogeographical regions. PLoS One 14(12):e0222737
Tarazona S, García-Alcalde F, Dopazo J et al (2011) Differential expression in RNA-seq: a matter of depth. Genome Res 21(12):2213–2223
Thompson JF, Steinmann KE (2010). Single molecule sequencing with a HeliScope genetic analysis system. Curr Protoc Mol Biol. Chapter 7:Unit7.10. doi: https://doi.org/10.1002/0471142727.mb0710s92
Truong DT, Franzosa EA, Tickle TL et al (2015) MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods 12(10):902–903
Turner TR, Ramakrishnan K, Walshaw J et al (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME J 7(12):2248–2258
Ugarte A, Vicedomini R, Bernardes J et al (2018) A multi-source domain annotation pipeline for quantitative metagenomic and metatranscriptomics functional profiling. Microbiome 6:149
UniProt Consortium (2019) UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47 (D1):D506–D515
Voelkerding KV, Dames SA, Durtschi JD (2009) Next-generation sequencing: from basic research to diagnostics. Clin Chem 55(4):641–658
Volossiouk T, Robb EJ, Nazar RN (1995) Direct DNA extraction for PCR-mediated assays of soil organisms. Appl Environ Microbiol 61:3972–3976
Whiteley AS, Thomson B, Lueders T et al (2007) RNA stable-isotope probing. Nat Protoc 2:838–844
Wood DE, Salzberg SL (2014) Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:R46
Xiao M, Zhang Y, Chen X et al (2013) Transcriptome analysis based on next-generation sequencing of non-model plants producing specialized metabolites of biotechnological interest. J Biotechnol 166(3):122–134

Yamada T, Letunic I, Okuda S et al (2011) iPath2.0:interactive pathway explorer. Nucleic Acids Res 39:W412–W415

Yazaki J, Gregory BD, Ecker JR (2007) Mapping the genome landscape using tiling array technology. Curr Opin Plant Biol 10(5):534–542

Ye Y, Doak TG (2009) A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. PLoS Comput Biol 5:e1000465

Ye Y, Tang H (2016) Utilizing de Bruijn graph of metagenome assembly for metatranscriptome analysis. Bioinformatics 32:1001–1008

Yi H, Cho YJ, Won S et al (2011) Duplex-specific nuclease efficiently removes rRNA for prokaryotic RNA-seq. Nucleic Acids Res 39(20):e140

Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18(5):821–829

Zhong C, Edlund A, Yang Y et al (2016) Metagenome and metatranscriptome analyses using protein family profiles. PLoS Comput Biol 12:e1004991

Zhou JZ, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microbiol 62:316–322

Molecular Tools to Explore Rhizosphere Microbiome

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Abstract

Rhizosphere microbial diversity plays an important role in plant health and agricultural sustainability. Several scientific groups have developed a wide range of methodologies for analyzing the structure, diversity, and functions of microbial populations to better understand rhizosphere biology and rhizosphere–microbe interactions. In this chapter we will discuss some of the advanced molecular tools available to explore microbial diversity of rhizosphere.

Keywords

Rhizosphere · Microbiome · Omics technology · Bacteria

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2.1 Introduction

Rhizosphere microbiomes play an important role in plant health and sustainability. Several scientific groups have developed a wide range of methodologies for analyzing the structure, diversity, and functions of microbial populations to better understand rhizosphere biology and rhizosphere–microbe interactions. It has been suggested that microbial inoculants are promising components for integrated solutions to agro-climatic issues because inoculants possess the capacity to influence the plant growth (Compant et al. 2010; Lugtenberg and Kamilova 2009), enhance nutrient availability, and uptake and improve plant health (Adesemoye et al. 2009; Yang e al. 2009; Berendsen et al. 2012; Packialakshmi et al. 2020). Further, plants have evolved to adjust with biotic and abiotic stresses in association with rhizosphere microbiome (Lemanceau et al. 2017). Some recent findings have shown that soil microbiome can directly and indirectly interact with the plants, improving their fitness and health (Sapkota et al. 2015).

2.2 Rhizosphere Microbiome

Soil is the mother and media for all the biological processes on the earth. Soil nurtures numerous flora and fauna in it. It also provides basic habitat for crop plants and the rhizosphere soil which is the most active part of soil provides a balanced atmosphere for many biological processes which directly or indirectly influences plant growth and development. Soil also contains billions of microorganisms which influences various biological processes (McNear Jr 2013). Microorganisms like fungi, bacteria, nematodes, actinomycetyes, archaea present in soil at different proportions. The number and activities are more in rhizosphere soil when compared to outside the rhizosphere zone. These microbiomes are involved in the various biological processes which can regulate plant growth and development positively and negatively. As plant growth promoters they help in better crop growth and development. Species of *Trichoderma*, mychorhizal fungi helps in performing these functions. On the other hand, they also cause numerous diseases like wilts, root rots, damping off, etc. which serious hamper crop growth and development. The native microbial communities play an important role in biogeochemical cycles of essential elements such as nitrogen, carbon, phosphorous. Apart from this, they also help in organic matter decomposition and remineralization of the elements (Pierre-Alain et al. 2007). A better understanding of these biological processes is critical for maintaining plant health thereby feeding ever-growing population of the planet earth (Morrisseey et al. 2004). Before this it is important to understand the diversity of different microbiomes in soil which gives an idea of exploiting their role for beneficial functions.

2.2.1 Bacterial Diversity

The bacterial community found in the rhizosphere is known for its colonization around the roots due to availability of nutrients, and composition, and it affects the plant growth directly or indirectly (Alawiye and Babalola 2019). The plant is able to specifically select microorganisms for rhizosphere colonization from the large pool of microbes living in the surrounding soil (Rosier et al. 2016). It was reported that rhizosphere habitats large number of bacterial population and the population densities in the rhizoplane range from 10^5–10^7 CFU g/1 of fresh weight (Bais et al. 2006). The rhizosphere microbiome has a strong effect on plant health by facilitating nutrient acquisition and helping plants to tolerate abiotic stresses (Pérez-Jaramillo et al. 2015). Several beneficial microorganisms (bacterial and fungal) have plant growth promotion activities or strengthen the defenses of the plant against pathogens and insects (Mendes et al. 2011; Pieterse et al. 2014; Goel et al. 2017; Kumar et al. 2019a, b).

To characterize the bacterial diversity and composition, molecular techniques have successful been applied. These methods facilitate characterization of representative microorganisms on the basis of biomolecules which includes Nucleic acid based either DNA or RNA based Fingerprinting techniques, restriction fragment length polymorphism (RFLP), denaturing/temperature gradient gel electrophoresis (DGGE/TGGE)ARDRA, RISA, DNA Microarray, Real Time PCR (Q-PCR), fluorescent in situ hybridization, Dot blot, Clone library sequencing), Protein based (Protein microarray), fatty acid/lipid based characterization includes (Microbial lipid analysis). Generally, 16S rRNA is used as a phylogenetic marker gene for microbial diversity analysis because this gene is remarkably well conserved through billions of years of evolution (Hugenholtz and Tyson, 2008; Soni and Goel 2010). This conservation allows amplification and analysis from bacteria and archaea, revealing the taxonomic distribution and evolutionary relationships among microorganisms.

In the advancement of genomic technologies, high-throughput sequencing techniques have allowed to characterize the genome without culturing them known as culture independent methods. In addition, community level analysis of microbial diversity is also performed using advanced genomics tools using DNA, RNA or protein as initial sample. These techniques allow the identification of entire bacterial diversity of any sample, tissue includes metagenomics, metaproteomics, proteogenomics, metatranscriptomics, metabolomics, whole genome sequencing, G+ C fractionation.

Rhizospheric bacterial diversity has been characterized in several crops species including bacterial communities associated with arabidopsis (Lundberg et al. 2012; Bulgarelli et al. 2012), barley (Bulgarelli et al. 2015), wheat and maize (Mazzola et al. 1995; Peiffer et al. 2013). Bacterial diversity in maize rhizoplane showed the abundance of genera *Bacillus, Arthrobacter, Listeria,* and *Sporolactobacillus* followed by *Azotobacter*, *Micrococcus,* and *Pseudomonas* genera (Cavaglieri et al. 2009). PCR-RFLP techniques used to explore the seasonal variation of the microbial community and the microbial succession of rice rhizoplane and identified the microbial diversity (Ikenaga et al. 2002). Knief et al. (2012) applied

metaproteogenomic analysis of microbial communities in the rhizosphere of rice. The diversity of bacterial endophytes from rice roots were analyzed using 16S RNA amplicon sequencing and identified microbes having plant growth promoting and antagonistic activities against bacterial and fungal pathogens (Kumar et al. 2020).

Culture dependent and independent bacterial diversity in Duckweed (*Spirodela polyrhiza*) an aquatic plant and identified the number of bacterial lineages includes *Alphaproteobacteria, Betaproteobacteria, Bacteroidetes,* and *Verrucomicrobia* (Matsuzawa et al. 2010). The rhizoplane-associated bacterial diversity was also analyzed using the high-throughput 16S rRNA amplicon sequencing strategy (Knief 2014). Furthermore, the application of next-generation sequencing (NGS) techniques may be more powerful tool that possibly helpful in the detection and identification of microbial communities in plants. NGS enables rapid analysis of the composition and diversity of microbial communities using culture independent amplicon or shotgun based sequencing in several habitats including rhizosphere (Trujillo et al. 2015; Soni et al. 2017; Goel et al. 2018). The 16S ribosomal RNA (rRNA) gene multiplex amplicon sequencing by PacBio sequencer targeting target the V1–V9 regions was performed. The community-based culture collection (CBC) recovered 399 unique bacteria representing 15.9% of the rhizosphere core microbiome and 61.6–65.3% of the endophytic core microbiomes of sugarcane stalks (Armanhi et al. 2018). Rhizospheric microbiome of *Lathurus sativa* was analyzed using illumina based NGS approach (Kumar et al. 2018a, b). By using paired-end sequencing on an Illumina sequencer identified a number of OTUs ($n = 637$) in rhizosphere samples of apple trees with the higher abundance of proteobacterial class of bacteria (Singh et al. 2019), diversity and composition of bacterial communities in rhizosphere soils of *Panax ginseng,* bacterial genera, namely *Asticcacaulis, Actinomadura, Knoellia, Rhodomicrobium, and Nakamurella* were detected from the soil of rusty root-affected (Wei et al. 2020). Rhizospheric bacterial communities of *Adenium obesum, Aloe dhufarensis* and *Cleome austroarabica* were explored using next-generation sequencing approaches (Khan et al. 2020).

2.2.2 Fungal Diversity

The soil has many species of fungi, and so far 80,000 or more species have been taxonomically named and described based on their distinguishing characters. These fungi function both active and inactive roles. Our current knowledge on soil fungal biodiversity is largely based on their morphological features like fruiting bodies in the environment, or, characters of mycelia on artificial/selective isolation media under laboratory conditions. Both these methods have certain limitations which are the obstacles for their detection, and diversity analysis. Fungi are the successful soil inhabitants due to their high capacity to withstand fluctuating environmental conditions (Sun et al. 2005). Fungi can be found in almost all the environmental conditions (Frac et al. 2018). The fungal species, their diversity and numbers are controlled by numerous biotic (presence of plants and other microbes) and abiotic

(soil texture, structure, temperature, soil pH, moisture, salinity, and alkalinity) conditions (Lopez-Bucio et al. 2015; Rouphael et al. 2015). Fungi perform both beneficial and harmful functions in plants. As beneficial microbes, they got the capability to produce a number of extracellular enzymes helps in different functions like break down of organic matter, decomposing soil components, and provide various nutrients for metabolic functions of plants (Zifcakova et al. 2016).

Several researchers have carried out experiments to analyze the fungal population in different cropping systems and their effect on growth and development of crop plants. Qin et al. (2017) determined the impact of various mulching techniques (furrow-ridge) rhizosphere fungal diversity of potato under continuous cropping, and found that, furrow planting with half mulch have highest population of fungi (89%). They also found that the rhizosphere soil was dominated by Zygomycota, Chytridiomycota, Ascomycota, Basidiomycota, and unidentified fungal communities. Similarly Tan et al. (2017) analyzed rhizosphere soil and root endogenous fungal diversity and composition in response to continuous cropping of *Panax notoginseng*, Chinese ginseng. They found that, continuous cropping becomes vulnerable to fungal pathogen attack. Ascomycota, Zygomycota, Basidiomycota, and Chytridiomycota were the dominant phyla observed during continuous cropping of *Panax notoginseng*. Fungal diversity was less in diseased plant's rhizosphere than healthy plants. This study clearly indicated that, diseased rhizosphere soil will have less biodiversity of fungal species than healthy. The present study also found that, soil organic matter and pH play greatest impact on microbial community composition in different cropping systems. Twenty soil samples collected from crop fields of Nanjangud Taluk, Karnataka were analyzed for fungal diversity; it was found that, ten species of fungi belonging to seven genera were prominent. The predominant genus was *Aspergillus, Penicillium,* and *Mucor* species (Chandrashekar et al. 2014). The study suggested that, the microclimate and soil properties greatly influence fungal communities and biodiversity. The abondance, composition, activity, and diversity in rhizosphere also influenced by alteration in soil micro environment (Cycon and Seget 2009). Many investigations also concluded that, soil moisture and temperature play significant role in soil microbial communities (De Curtis et al. 2012). Soil moisture plays a significant role in increasing the activities of soil fungi (Zou et al. 2010). Molecular techniques were followed to analyze the fungal diversity in the wheat rhizosphere by Smit et al. (2010). They followed sequencing of cloned PCR-amplified genes encoding 18 s rRNA and temperature gradient gel electrophoresis (TGGE), and found that, Ascomycota, Basidiomycota, Zygomycota, and chytridiomycota were the predominant species in wheat rhizosphere. Study conducted in China to estimate fungal biodiversity in a forest soil ecosystem using ITS sequence reads indicated that, Basidiomycota (47.8%), Ascomycota (32.4%), and zygomycota (13.4%) were the major fungal communities observed, but, basidiomycetes fungi found to be dominant among the three phyla (Hanif et al. 2019). Studies have also indicated that, the soil fungal diversity also influenced by root released compounds in different cropping systems (Garbeva et al. 2004). Rhizopshere soils of potato, eggplant, and peanut shown decreased level of bacteria and actinomycetes as the population of fungi increased over continuous cropping

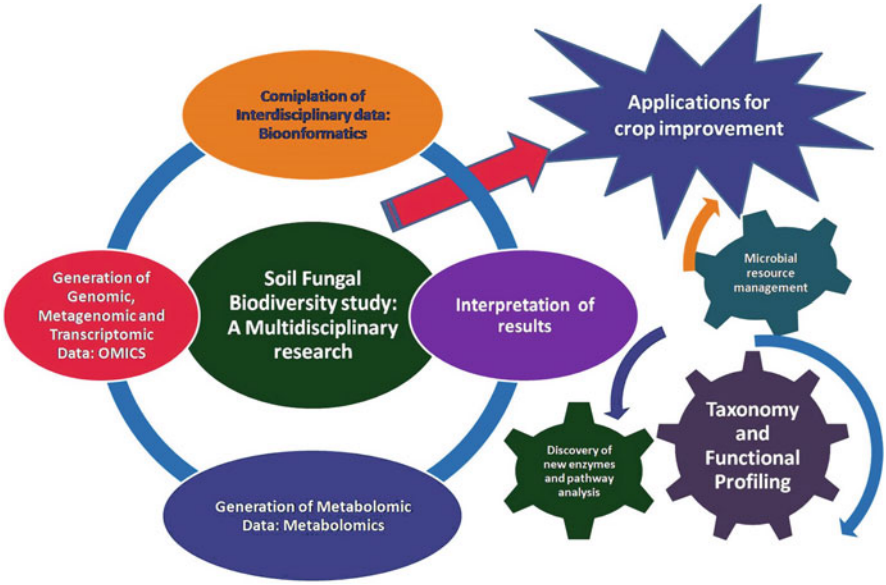

Fig. 2.1 Application of multidisciplinary research for exploring soil fungal diversity for crop improvement

system (Li and Guo 2014). Finally, it can be concluded that, the fungal diversity in rhizosphere soil greatly influenced by type of crop plants grown, cropping system, soil ecology, soil physical and chemical properties. Crop rotation, tillage will produce a number of changes on fungal diversity with a particular ecological importance (Lupwayi et al. 2010). Exploring soil fungal diversity is a multidisciplinary subject and, Fig. 2.1, gives a complete picture of utilization of soil fungal diversity for crop production and other areas.

2.3 Omics Technology

2.3.1 Genomics

Genomics is the branch of genetics that deals with the analysis of the genomes. The genome represents the haploid set of genes or chromosomes within an organism. Its mapping, sequencing, or any other analysis is known as genomics that comes under the branch of genetics. It may be classified as structural and functional genomics (Wang et al. 2020). Structural genomics involves the location, sequence, and physical characterization of the genes within a genome (Fig. 2.2). While, functional genomics refers the analysis of gene functions and regulation (Forouhar et al. 2007). Nowadays, genomics becomes very popular to sequence and analyze the whole genome of an organism. It has been expanded to the functional aspect of the entire

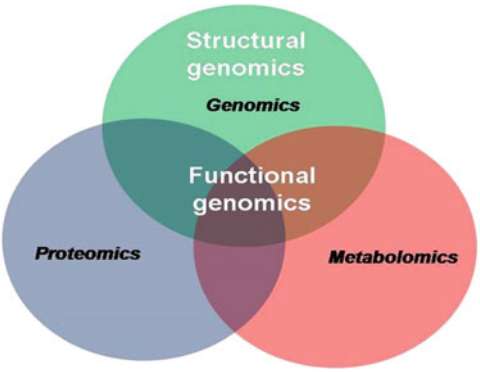

Fig. 2.2 Mutual relationship among the omics approaches

genome, i.e. transcriptomics (the study of RNA), proteomics (the study of proteins), and metabolomics (the study of metabolites) (Soni et al. 2015; Suyal et al. 2017, 2018, 2019b). Moreover, the combinations of various "meta-" and "-omics" technologies have made it beneficial to humankind, especially in medical, industrial, and agricultural fields (Rawat et al. 2019; Suyal et al. 2014b, 2019c). Although several genomics tools and techniques are emerging day by day, here, the basic technologies are being discussed briefly. These methods and techniques are the basis of genomics and lie in the heart of advanced technologies.

2.3.1.1 Polymerase Chain Reaction (PCR)

This technique was originally isolated by Kary Banks Mullis in 1983, for which he got Nobel Prize in 1993. This technique has revolutionized the whole molecular biology field and is relevant till today. It allows the amplification of target DNA fragments extracted from any source. Moreover, in combination with gel electrophoresis techniques, viz. agarose gel electrophoresis, denaturing gradient gel electrophoresis (DGGE); temperature gradient gel electrophoresis, etc. it offers several benefits to the researchers and has increased our understanding in microbial community analysis (Kumar et al. 2018a, b; Rajwar et al. 2018; Joshi et al. 2019).

2.3.1.2 Restriction Fragment Length Polymorphism

Restriction enzymes are endonucleases that can cleave DNA at specific sites. They are also called molecular scissors. These enzymes are widely used to map the genomes (O'Donnell et al. 2020). Restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), plasmid fingerprinting, etc. are some techniques that explore the principle of restriction endonucleases.

2.3.1.3 DNA Sequencing

DNA sequencing is the most significant advancement in genomics (Kumar et al. 2014; Suyal et al. 2014a; Shukla et al. 2015). It allows the identification of the nucleotide sequences in a given genome. Nowadays, high-throughput, automated, efficient, and reliable next-generation sequencing technologies are available which made it easier to sequence and analyze the whole genome. In recent years,

Microfluidics and Fluorescent Activated Cell Sorting (FACS) becomes popular to sequence single cells. This technique involves tagging, isolation, and sequencing of fluorescent cells (O'Donnell et al. 2020).

2.3.1.4 DNA Cloning

This technique involves the transfer of a DNA segment from one cell to another to make its identical copies in vivo (O'Donnell et al. 2020). In recent years, several vector systems have been developed that can accommodate various types and sizes of DNA fragments, viz. plasmids, hybrid vectors (cosmid, phagemid), phages, artificial chromosomes (Yeast artificial chromosome, bacterial artificial chromosome).

2.3.1.5 Hybridization Techniques

This technique measures the level of genetic similarity between two different nucleic acid molecules by allowing their complementary sequence to combine and separate. DNA dissociation/re-association kinetic analysis and fluorescent in situ hybridization (FISH) are basic methods that employ this principle. Furthermore, DNA microarray analysis is an advanced technique that is based on it. It involves hybridization between a probe and DNA fragment on a chip known as DNA chip. In most cases, DNA chips involve a single genome; however, multiple genomes can also be analyzed. A technique "representational difference analysis (RDA)" analyzes the variations among the strains of a species concerning previously sequenced representative. This method involves the combination of PCR, DNA sequencing, and DNA–DNA re-association kinetics. It is a very popular method to analyze the prokaryotic genomes because they can vary significantly in their genome size (Barcellos et al. 2009).

The combination of genomics with other omics technologies is frequently being used in the study of rhizospheric microorganisms (Giri et al. 2015; Suyal et al. 2015a; Goel et al. 2017). Moreover, blending the bioinformatics tools with these technologies has opened newer insights into microbial ecology research and development.

2.3.2 Metagenomics

Genomic methods limit analysis of those microorganisms that can be cultured. It is widely accepted that only 0.1–1% (depending upon the environmental sample) of microorganisms can be grown on synthetic growth media. This leaves more than 99% of the microbial diversity unexploited (Suyal et al. 2015b, c, 2019a). Moreover, various environmental stresses force bacteria to enter under viable but nonculturable state that again reduces their accessibility through genomics approach. Thus, cultivation dependent microbial identification can underestimate the microbial diversity. To overcome the difficulties and limitations associated with cultivation technologies, metagenomics has emerged as a potential tool (Soni and Goel 2011; Soni et al. 2016; Soni et al. 2017; Joshi et al. 2017; Kumar et al. 2019a, b). It involves the direct

extraction of nucleic acids from the environment. However, when isolating metagenomic DNA from the environment samples, three major issues are important that need to be taken into consideration. The first one is the DNA should be extracted from such source that has a broad a range of microorganisms. Secondly, during the DNA extraction steps, DNA shearing must be avoided. Thirdly, the DNA must be free from contaminating substances which interfere with downstream DNA processing such as restriction and ligation because the bacterial community composition is significantly influenced by the efficiency of DNA extraction method (Lai et al. 2006).

In general the analysis of DNA provides information on structural diversity of environmental sample and does not allow conclusion on metabolic activity or gene expression of members of that community. This information can be attained by isolating mRNA from the environmental samples followed by cDNA synthesis through Reverse Transcriptase PCR and targeting this cDNA for the downstream processes. However, there are several technological challenges regarding quality and stability of the RNA because of short half-life of mRNA and presence of RNases (Jensen et al. 2017).

2.3.3 Transcriptomics

Recent advances in DNA sequencing technologies have revolutionized the rhizosphere biology by elucidating the microbial composition with deeper coverage through metagenomics. However, metagenomics could not provide the functional insight, thus rendering the functional role of active rhizospheric microbiome elusive. Transcriptomics and metatranscriptomics are therefore sought as they can elucidate both structure and function of the active rhizospheric microbiome thus complementing metagenomics data. Transcriptomics is the study of total RNA complement expressed under certain environmental condition. However, metatranscriptomics refer to the high-throughput sequencing of total RNA isolated from the environmental sample. The two most popular metatranscriptomics tools to study rhizosphere are RNA sequencing and gene expression microarray.

2.3.3.1 RNA Sequencing

RNA sequencing (RNA-seq) is a technique to sequence and quantify RNA molecules in the sample with NGS technology. RNA-seq reveals the complete transcriptome with qualitative and quantitative insight of mRNA, rRNA, and tRNA and currently considered gold standard for gene expression analysis. The first step in this technique involves isolation of high quality RNA from the rhizospheric soil followed by conversion into cDNA fragments (a cDNA library) which are subsequently sequenced by NGS. Urich et al. (2008) first time used "Double-RNA approach" to characterize function and structure of the soil microbial communities by sequencing both rRNA and mRNA in a single metatranscriptome. Previous studies highlighted the active rhizospheres microbiome of wheat (*Triticum*

aestivum), oat (*Avena strigosa*), pea (*Pisum sativum*), and grapevine (*Vitis vinifera*) through metatranscriptomics (Turner et al. 2013; Berlanas et al. 2019).

2.3.3.2 Gene Expression Microarray

Microarray is a collection of microscope probes attached on solid surface used for high-throughput expression analysis and comparative genomic hybridization studies (Martínez et al. 2015). It has also been used to monitor gene expression and bacterial identification in different environment samples. Mendes et al. (2011) used a microarray based approach to characterize the rhizosphere microbiome and detected 33,000 bacterial and archaeal species. Previously, metabolic capabilities of maize, pea, and alfalfa rhizosphere have been documented with functional gene microarray (Li et al. 2014). Effect of *Rhizobium leguminosarum* biovar *viciae* inoculation on gene expression of pea, alfalfa, and sugar beet rhizosphere was studied in the past using microarray which revealed the presence of conserved factors for plant colonization (Ramachandran et al. 2011) (Fig. 2.3).

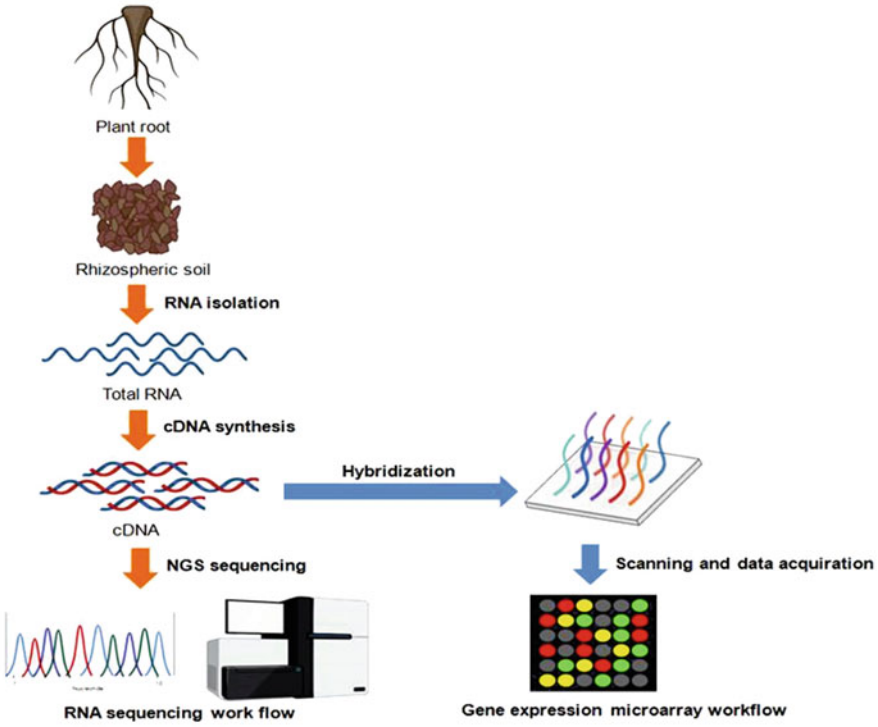

Fig. 2.3 Workflow for the rhizospheric metatranscriptomics

2.3.4 Proteomic Approaches

Recent advances in microbial ecology research taking researchers for studying microorganisms in their ecologies without cultivating in laboratory media. This is helping us to get access to large number of uncultivable microbes which may have tremendous potential in solving many problems of basic science. In this view, proteomics has provided new opportunities in assessing soil microbial diversity and functions. It is the most appropriate and alternate approach to metagenomics where useful information on key biological players which carryout frontline metabolic activities to solve mystery of adoption capabilities of soil microbes in a given ecology (Ploetze et al. 2015).

Further, proteomics is a system biology approach and considered as a logical choice for investigating the plant–microbe interactions. Here the investigations were based upon two-dimensional gel electrophoresis (2-D) which is a good choice for rapid identification of major proteome differences in microbes and their interactive ecologies (Rampitsch and Bykova 2012). This is a functional genomics or system biology approach allowing the study of protein expression of an organism or to obtain protein map of all the proteins expressed. Proteomics is complementary to genome sequencing, gives information on the non-model microorganisms and their activities in a given environment (Muller et al. 2007; Weiss et al. 2009). While the expression profiling of gene provides information at the level of transcript accumulation, proteomics provides information on all the expressed proteins. Information such as location and time in which each functional protein accumulates its level of accumulation and posttranslational modifications to the proteins can be obtained through proteomic approach. Moreover, proteomics also provide more precise information on gene expression since the functional product of most of the genes are not the RNA but the protein. In this approach, most separations for proteins analysis were done with 2-dimentional gel electrophoresis (2-D). The identification of the separated proteins has been aided by sequence library available in the database. Initially the protein spot is from agarose gel is eluted and subjected for electrolytic cleavage where peptide fragments are formed. Matrix-assisted laser desorption/ionization time of light (MALDI-TOF) mass spectrometric analysis (MS) will be performed to analyze the cleaved peptide fragments. MALDI-TOF analysis generates a list of peptide masses for the cleaved fragments. The size of the fragment is the specific characteristic of the protein which can be predicted from gene sequence. The sequence results for a protein can be compared with database of a calculated/submitted peptide masses for each open reading frame (ORF) in the genome. If there is no match found in sequence database, the proteins can be analyzed by peptide sequencing.

Furthermore, proteomics has a wide range of applications. One such application is detection and diagnosis of plant diseases, their management. Diversity analysis of microbiomes associated with soil, water, and other ecologies. In addition, study of plant diseases, resistance or immunity is benefiting tremendously from proteomics. A fruitful approach in plant pathology has been to perform proteomic experiments on pathogens grown in vitro, or, where their artificial culture is not possible (rusts,

mildews) on a partially purified fungal structure (Bindschedler et al. 2009; Song et al. 2011). The utilization of proteomics to explore biological control agents and their mechanisms is gaining much more attention. The interactions between a potential biological control agent, a phytopathogen, and a plant (tripartite interaction) bring significant changes to the plants proteome and metabolome (Chinnasamy 2005). Microarray technology will be adopted for use in proteomics. Here array of antibodies for a large number of proteins on a chip to analyze for the changes at protein level in an analogous way to how mRNA changes are currently measured. Proteomics has wider application in characterization of intercellular proteins which gives insights in to microbial functions in rhizosphere soil. Biological control of rice brown spot disease, caused by a deadly pathogen *Helminthosporium oryzae* was studied. Tripartite interaction between pathogen-biocontrol agent (Bacillus)—rice was investigated using proteomic approach. Nine proteins including ribulose 1,5 bisphosphate carboxylase, ATP synthase, serine/threonine protein kinases, 2-cys-peroxiredoxin, trehalase-phosphatase, and 50S ribosomal proteins were detected using 2-D PAGE analysis followed by differential expression using MALDI-TOF mass spectrometry (Prabhukarthikeyan et al. 2019). These proteins may help in plant metabolism and defense response against brown spot pathogen. A complex process governing the interactions between host plants with symbiotic microorganisms and vice versa in case of mycorrhizae has been studied through proteomic approach (Bona et al. 2011). Recently, Wang et al. (2011) have applied proteomic approach to study the expression of proteins in rhizosphere soil during the interactions between crop and soil microbes. 2-D polyacrylamide gel electrophoresis, 2D-differential gel electrophoresis, and mass sprectrometry were employed to study expression of gene involved in interaction between plant pathogen, nitrogen fixing bacteria in legumes through bacterial proteomic analysis (Cheng et al. 2010). Thus, proteomics is an appropriate and most useful approach in solving the complex problems of plant–microbe interactions.

2.3.5 Metaproteomic Approaches

Metaproteomics is the most recent and new approach within the "Omics" umbrella is gaining significant importance. This approach investigates the expression pattern of proteins from a complex biological system and gives a direct evidence of physiological and metabolic activities of microbiome. Metaproteome characterization from a biological system will enhance the knowledge of understanding of microbial world and liking microbial communities to ecological functions (Wang et al. 2014). Metaproteomics otherwise is a technology of harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities (Hettich et al. 2013). In recent years, the availability of extensive metagenomic sequences from various microbial communities has extended post genomic era to a new exiting area of research.

Metaproteomics even though in earlier stage has shown its potential with regard to functional gene expression within microbial habitats in relation to ecologies. The

interaction of these microbial communities with surrounding environment is also assessed through metaproteomic analysis (Wang et al. 2011). This approach is one of the best approaches in soil microbial community analysis. Metaproteomics can be performed in four major steps, 1. Rhizosphere soil Sample collection, 2. Protein extraction, 3. Purification and fractionation; MS analysis, and finally 4. Protein interpretation and bioinformatics analysis (Wang et al. 2014). Two major work flows for metaproteomic analysis have been developed: (1) Sodium dodecyl sulfate polyacrylamide gel electrophoreses (SDS-PAGE) coupled either with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF-TOF) mass spectrometry (MS) analysis or with electrospray ionization source tandem MS (ESI-MS/MS) analysis. (2) Liquid chromatography coupled with electrospray ionization source tandem MS (LC-ESI-MS/MS).

Metaproteomic analysis of rhizosphere soil is very useful and powerful scientific to solve the mystery of interactions between plants and microorganisms in the soil ecosystem. Role of these microbial communities will help us in utilizing this information in enhancement of yield. Wang et al. (2011) standardized method for extraction of protein from different soil samples and identified 1000 separate spots with high reproducibility stained in 2-DE gels. 189 spots represented 122 proteins on a 2-DE gel of rice samples identified by MALDI-TOF/TOF-MS successfully. These identified proteins mainly originated from rice and microorganisms which were involved in various metabolic activities like protein, energy, nucleotide, and secondary metabolism as well as signal transduction and stress resistance. Similarly in Sugarcane, metaproteomic analysis combined with community level physiological profiles analysis (CLPP) of rhizosphere soil was carried out to understand the reason for sugarcane yield decline. Significant results were found that, sugarcane rationing induced significant changes in soil enzyme activities, the catabolic microbial community, and, the expression level of soil proteins. They influences biochemical processes in the rhizosphere ecosystem and mediated sugarcane and microbial interactions (Lin et al. 2013). Comparative metaproteomic analysis identified that, 38 proteins were differentially expressed in ratoon sugarcane soil which were responsible for yield decline. Knief et al. (2012) carried out the metaproteomic analysis of microbial communities (bacteria and archaea) in the phyllopshere and rhizosphere soil of rice. Total of 4600 identified proteins obtained from metaproteomic database, they indicated one carbon conversion process in the rhizosphere and phyllopshere. Rhizosphere was dominated by proteins involved in methanogenesis and methanotrophy and phyllosphere by Methylobacterium. These proteins were mainly involved in transport process and stress responses in phyllosphere. Dinitrogenase, reductase were exclusively found in rhizosphere despite the presence of *nifH* genes (Knief et al. 2012).

2.3.6 Metabolomics

Metabolomics is the qualitative and quantitative study of low molecular weight metabolites (<1KDa). Metabolomics serves as a powerful tool for detection,

quantification, and elucidation of molecular interactions in the rhizosphere. In rhizospheric niche, majority of plant-to-microbe and microbe-to-microbe communication is mediated by small metabolites. Exploring these metabolites in rhizosphere explicate different molecular interactions operating at the plant microbe interface which further reveals several critical signaling pathways involved in plant growth promotion, plant disease, defense priming and induces systemic resistance. Thus metabolomics enhances our understanding of molecular and cellular pathways operating in rhizosphere.

Typical mass spectrometry (MS) based metabolomics has three major steps. First step is sample preparation which involves extraction of metabolites through organic solvents or solid phase extraction. Second step is the separation and detection, where metabolites are separated through different chromatographic methods based on the nature of metabolites and then detected by the mass analyzers. Gas chromatography-mass spectrometry (GC-MS) is preferred for volatile and thermally stable compounds which separate metabolite through gas chromatography and detect through quadrupole, qTOF (Quadrupole Time-of-Flight) or QqQ (triple-quadrupole) mass analyzers (van Dam and Bouwmeester 2016). On the other hand, liquid chromatography-mass spectrometry (LC-MS) mostly use normal phase (NP) or reverse phase (RP) chromatography to separate metabolites based on their polarity. With LC-MS, soft ionization like electron spray ionization (ESI) is the most preferred ionization method as it provides accurate mass determination. Capillary electrophoreses (CE) coupled with TOF mass analyzer is used for intermediate primary metabolic pathways (glycolysis, tricarboxylic acid cycle, and pentose phosphate pathway) (Mhlongo et al. 2018). Finally data is analyzed with freely available software like MarVis1, Mzine, MAVEN, Metaboanalyst, and MetAlign or commercial software like Markerlynx, Profiling solutions, and Mass profiler pro.

Several primary and secondary metabolites (non-volatile and volatile) have been documented as major messengers between plant roots and PGPR establishing mutualistic relationship (van Dam and Bouwmeester 2016). Metabolomics of rhizosphere was previously used to study various plant growth modulatory compounds like ACC deaminase, auxins, abscisic acid, cytokinins, gibberellins, jasmonic acid, salicylic acid, and siderophores produced by microorganisms (Mhlongo et al. 2018). Similarly, metabolomics has been the ideal tool to study signaling molecules of root nodule symbiosis like flavonoids (bacterial nod gene induces) and lipochitooligosaccharides (product of nod genes). Rothballer et al. 2018 studied the role of AHLs (acyl homoserine lactones) and its degradation products in quorum sensing of rhizospheric bacteria. Metabolomics has also demonstrated the change is microbial community in grass (*Avena barbata*) rhizosphere with bacterial succession dynamics with respect to substrate preference in changing root exudates over the course of development (Zhalnina et al. 2018). However, the cost of equipment, limited public reference database, and lack of proper expertise make metabolomics more challenging than the DNA sequencing based approaches.

2.3.7 Phenomics

Phenomics is defined as the systematic study of phenotypes on a genome-wide scale. In other words, phenomics is set of multidimensional approaches to study how genome of an organism translates into the full set of phenotypic traits. However, prediction of the phenotype from the genotype is not state forward because large number of genes interact with themselves and the environment to produce the phenotype. Metagenomics has provided an access to complete genotype of rhizospheric microorganisms to genus, species, and subspecies level. However, large fractions of the genes in metagenomics have no assigned function and even the ascribed functions for most of the genes are based on DNA sequence homology.

Apart from the traditional techniques of phenotypic characterization, transcriptomics, proteomics and metabolomics are the widely used tools which provide enormous phenomic data, thus elucidating the phenomics of rhizospheric microorganisms (Houle et al. 2010). Phenotypic features (phenome) of the rhizobia have been studied for their classification and placing them into different cross inoculation groups. Phenomics have also been very useful to study the plant pathogen interactions and host-pathogen co-evolution at molecular level.

Complexity of biological processes at cellular and developmental level needs to be addressed with high quality digital phenotypic data. Recently, global *Escherichia coli* promoter activity was accessed using PFIboxes to obtain high quality phenome data of gene expression under the 15 different antibiotics stress (French et al. 2018). Growth measurement is the key phenotype for accessing microbial fitness in any ecosystem. Automated microbial phenomics framework was developed to records and analyzes over 100,000 growth curves in parallel (Zackrisson et al. 2016). However, limited high-throughput tools are available to study the phenomics of rhizospheric bacteria. Therefore, development of high-throughput and high-resolution phenotyping tools are required to address the detailed phenomics of rhizospheric microorganisms.

2.4 Conclusion

The rhizospheric microbial diversity from Himalayan agro-ecosystems has been revealed by author group extensively. It has been observed that the microbial diversity from *Phaseolus vulgaris* rhizosphere was varied significantly in Kumaun and Grahwal Himalayan regions of Uttarakhand. Contrary to the Kumaun where, genus *Pseudomonas* was predominant; Garhwal Himalayan *P. vulgaris* rhizosphere was inhabited primarily by *Sphingomonas* (Suyal et al. 2019c). However, among the diazotrophs, genus *Rhizobium* was predominant throughout the Uttarakhand Himalayas (Suyal et al. 2015b). Here we can conclude our discussion in a short statement that is the use of more advanced molecular tools may help us to reveal several unexplored microbiomes which ultimately benefitted to sustainable agriculture.

References

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Alawiye TT, Babalola OO (2019) Bacterial diversity and community structure in typical plant Rhizosphere. Diversity 11:179

Armanhi JSL, de Souza RSC, Damasceno N d B, de Araújo LM, Imperial J, Arruda P (2018) A community-based culture collection for targeting novel plant growth-promoting Bacteria from the sugarcane microbiome. Front Plant Sci 8:1–17. https://doi.org/10.3389/fpls.2017.02191

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233e266

Barcellos FG, Batista JSS, Menna P et al (2009) Genetic differences between Bradyrhizobium japonicum variant strains contrasting in N2-fixation efficiency revealed by representational difference analysis. Arch Microbiol 191:113–122

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486. https://doi.org/10.1016/j.tplants.2012.04.001. Epub 2012 May 5. PMID 22564542

Berlanas C, Berbegal M, Elena G, Laidani M, Cibriain JF, Sagües-Sarasa A, Gramaje D (2019) The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. Front Microbiol 10:1142

Bindschedler LV, Burgis TA, Mills DJ, Ho J et al (2009) In Planta proteomics and Proteogenomics of the biotrophic barley fungal pathogen *Blumeria graminis* f. sp. Hordei. Mol Cell Proteomics 8:2368–2381

Bona E, Marsano F, Massa N et al (2011) Proteomic analysis as a tool for investigating arsenic stress in Pteris vittata roots colonized or not by arbuscular mycorrhizal symbiosis. J Proteome 74:1338–1350. https://doi.org/10.1016/j.jprot.2011.03.027

Bulgarelli D et al (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488:91–95

Bulgarelli D et al (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe 17:392–403

Cavaglieri L, Orlando J, Etcheverry M (2009) Rhizosphere microbial community structure at different maize plant growth stages and root locations. Microbiol Res 164:391–399

Chandrashekar MA, Soumya Pai K, Raju NS (2014) Fungal diversity of Rhizosphere soils in different agricultural fields of Nanjangud Taluk of Mysore District, Karnataka. India Int J Curr Microbiol AppSci 3(5):559–566

Cheng Z, McConkey BJ, Glick BR (2010) Proteomic studies of plant–bacterial interactions. Soil Biol Biochem 42:1673–1684

Chinnasamy G (2005) A proteomics perspective on biocontrol and plant defense mechanism. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht. https://doi.org/10.1007/1-4020-4152-7_9

Compant S, Van Der Heijden MG, Sessitsch A (2010) Climate change effects on beneficial plant–microorganism interactions. FEMS Microbiol Ecol 73:197–214. https://doi.org/10.1111/j.1574-6941.2010.00900.x

Cycon M, Seget ZP (2009) Changes in bacterial diversity and community structure following pesticides addition to soil estimated by cultivation technique. Ecotoxicology 18(5):632–642. https://doi.org/10.1007/s10646-009-0321-6

De Curtis F, de Felice DV, Ianiri G, De Cicco V, Castoria R (2012) Environmental factors affect the activity of biocontrol agents against ochratoxigenic *Aspergillus carbonarius* on wine grape. Int J Food Microbiol 159:17–24. https://doi.org/10.1016/j.ijfoodmicro.2012.07.023

Forouhar F, Kuzin A, Seetharaman J et al (2007) Functional insights from structural genomics. J Struct Funct Genom 8:37–44

Frac M, Hannula SE, Belka M, Jedryczka M (2018) Fungal biodiversity and their role in soil health. Front. Microbiol 9:707. https://doi.org/10.3389/fmicb.2018.00707

French S, Coutts BE, Brown ED (2018) Open-source high-throughput phenomics of bacterial promoter-reporter strains. Cell Syst 7(3):339–346

Garbeva P, van Veen JA, van Elsas JD (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270. https://doi.org/10.1146/annurev.phyto.42.012604.135455

Giri K, Paliwal R, Suyal DC, Mishra G, Pandey S, Rai JPN, Verma PK (2015) Potential application of plant-microbe interaction for restoration of degraded ecosystems. In: Singh S, Srivastava K (eds) Handbook of research on uncovering new methods for ecosystem management through bioremediation. IGI Global, Hershey, pp 255–285

Goel R, Kumar V, Suyal DK, Dash B, Kumar P, Soni R (2017) Root-associated bacteria: rhizoplane and endosphere. In: Singh D, Singh H, Prabha R (eds) Plant-microbe interactions in agro-ecological perspectives. Springer, Singapore

Goel R, Kumar V, Suyal DC, Narayan SR (2018) Toward the unculturable microbes for sustainable agricultural production. In: Meena V (ed) Role of Rhizospheric microbes in soil. Springer, Singapore. https://doi.org/10.1007/978-981-10-8402-7_4

Hanif MA, Guo Z, Moniruzzaman M, He D, Yu Q, Rao X, Liu S, Tan X, Shen W (2019) Plant taxonomic diversity better explains soil fungal and bacterial diversity than functional diversity in restored Forest ecosystems. Plan Theory 8:479

Hettich RL, Pan C, Chourey K, Giannone RJ (2013) Metaproteomics: harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities. Anal Chem 85:4203–4214

Houle D, Govindaraju DR, Omholt S (2010) Phenomics: the next challenge. Nat Rev Genet 11 (12):855–866

Hugenholtz P, Tyson GW (2008) Microbiology: metagenomics. Nature 455(7212):481–483. https://doi.org/10.1038/455481a

Ikenaga M, Muraoka Y, Toyota K, Kimura M (2002) Community structure of the microbiota associated with nodal roots of rice plants along with the growth stages: estimation by PCR-RFLP analysis. Biol Fertil Soils 36:397–340

Jensen S, Fortunato SAV, Hoffmann F et al (2017) The relative abundance and transcriptional activity of marine sponge-associated microorganisms emphasizing groups involved in sulfur cycle. Microb Ecol 73:668–676

Joshi D, Chandra R, Suyal DC, Kumar S, Goel R (2019) Impact of bioinoculants *Pseudomonas jesenii* MP1 and *Rhodococcus qingshengii* S10107 on Cicer arietinum yield and soil nitrogen status. Pedosphere 29(3):388–399

Joshi D, Kumar S, Suyal DC, Goel R (2017) The microbiome of the Himalayan ecosystem. In: Kalia V, Shouche Y, Purohit H, Rahi P (eds) Mining of microbial wealth and meta genomics. Springer, Singapore, pp 101–116

Khan AL, Asaf S, Abed RMM, Chai YN, Al-Rawahi AN, Mohanta TK et al (2020) Rhizosphere microbiome of arid land medicinal plants and extra cellular enzymes contribute to their abundance. Microorganisms 8. https://doi.org/10.3390/microorganisms8020213

Knief C (2014) Analysis of plant microbe interactions in the era of next generation sequencing technologies. Front Plant Sci 5:216

Knief C, Delmotte N, Chaffron S, Stark M, Innerebner G, Wassmann R, von Mering C, Vorholt JA (2012) Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME J 6:1378–1390

Kumar S, Suyal DC, Dhauni N, Bhoriyal M, Goel R (2014) Relative plant growth promoting potential of Himalayan psychrotolerant Pseudomonas jesenii strain MP1 against native Cicer arietinum L., Vigna mungo (L.) Hepper; Vigna radiata (L.) Wilczek., Cajanus cajan (L.) Millsp. and Eleusine coracana (L.) Gaertn. Afri. J Microbiol 8(50):3931–3943

Kumar S, Suyal DC, Bhoriyal M, Goel R (2018a) Plant growth promoting potential of psychrotolerant *Dyadobacter* sp. for pulses and finger millet and impact of inoculation on soil chemical properties and diazotrophic abundance. J Plant Nutr 41(8):1035–1046

Kumar V, Jain L, Jain SK, Kaushal P (2018b) Metagenomic analysis of Endophytic and Rhizospheric bacterial diversity of Lathyrus sativus using illumina based sequencing. In: Published in the proceedings of international conference on microbiome research 2018, Pune with abstract ID 2018EVM0152

Kumar S, Suyal DC, Yadav A, Shouche Y, Goel R (2019a) Microbial diversity and soil physiochemical characteristic of higher altitude. PLoS One 14(3):e0213844

Kumar V, Soni R, Jain L, Dash B, Goel R (2019b) Endophytic Fungi: recent advances in identification and explorations. In: Singh B (ed) Advances in Endophytic fungal research. Fungal biology. Springer, Cham. https://doi.org/10.1007/978-3-030-03589-1_13

Kumar V, Jain L, Jain SK, Chaturvedi S, Kaushal P (2020) Bacterial endophytes of rice (Oryza sativa L.) and their potential for plant growth promotion and antagonistic activities. S Afr J Bot. https://doi.org/10.1016/j.sajb.2020.02.017

Lai XZX, Fang S, Huang Y, Cao L, Zhou S (2006) Denaturing gradient gel electrophoresis (DGGE) analysis of bacterial community composition in deep-sea sediments of the South China Sea. World J Microbiol Biotechnol 22:1337–1345

Lemanceau P, Blouin M, Muller D, Moënne-Loccoz Y (2017) Let the core microbiota be functional. Trends Plant Sci 22:583–595

Li GQ, Guo HC (2014) Effect of potato continuous cropping on the rhizosphere soil bacteria community structure. Mol Plant Breed 12:914–928

Li X, Rui J, Xiong J, Li J, He Z, Zhou J et al (2014) Functional potential of soil microbial communities in the maize rhizosphere. PLoS One 9(11):e112609

Lin W, Wu L, Lin S, Zhang A, Zhou M, Lin R et al (2013) Metaproteomic analysis of ratoon sugarcane rhizospheric soil. BMC Microbiol 13:135. https://doi.org/10.1186/1471-2180-13-135

Lopez-Bucio J, Pelagio-Flores R, Herrera-Estrell A (2015) *Trichoderma* as biostimulant: exploiting the multilevel properties of a plant beneficial fungus. Sci Hortic 196:109–123. https://doi.org/10.1016/j.scienta.2015.08.043

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918. PMID 19575558

Lundberg DS et al (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90

Lupwayi ZN, Grant CA, Soon YK, Clayton GW, Bittman S, Malhi SS, Zebarth BJ (2010) Soil microbial community response to controlled-release urea fertilizer under zero tillage and conventional tillage. Appl Soil Ecol 45:254–261. https://doi.org/10.1016/j.apsoil.2010.04.013

Martínez MA, de los Dolores Soto-del M, Gutiérrez RM, Chiu CY, Greninger AL, Contreras JF et al (2015) DNA microarray for detection of gastrointestinal viruses. J Clin Microbiol 53 (1):136–145

Matsuzawa H, Tanaka Y, Tamaki H, Kamagata Y, Mori K (2010) Culture-dependent and independent analyses of the microbial communities inhabiting the giant duckweed (Spirodela polyrhiza) rhizoplane and isolation of a variety of rarely cultivated organisms within the phylum Verrucomicrobia. Microb Environ JSME 25(4):302–308. https://doi.org/10.1264/jsme2.ME10144

Mazzola M et al (1995) Variation in sensitivity of gaeumanno- mycesgraminis to antibiotics produced by fluorescent pseudomonas spp and effect on biological-control of take-all of wheat. Appl Environ Microbiol 61:2554–2559

McNear DH Jr (2013) The Rhizosphere - roots, soil and everything in between. Nature Education 4 (3):1

Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH et al (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332 (6033):1097–1100

Mhlongo MI, Piater LA, Madala NE, Labuschagne N, Dubery IA (2018) The chemistry of plant–microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front Plant Sci 9:112

Morrisseey J, Dow J, Mark G, O'Gara F (2004) Are microbes at the root of a solution to world food production? EMBO Rept 5(10):922–926

Muller D, Médigue C, Koechler S et al (2007) A tale of two oxidation states: bacterial colonization of arsenic-rich environments. PLoS Genet 3:e53. https://doi.org/10.1371/journal.pgen.0030053

O'Donnell ST, Ross RP, Stanton C (2020) The Progress of multi-Omics technologies: determining function in lactic acid Bacteria using a systems level approach. Front Microbiol 10:3084

Packialakshmi JS, Dash B, Singh AK, Suyal DC, Soni R (2020) Nutrient cycling at higher altitudes. In: Goel R et al (eds) Microbiological advancements for higher Altitude Agroecosystems & Sustainability, Rhizosphere biology. Springer Nature Singapore Pte Ltd, Singapore. https://doi.org/10.1007/978-981-15-1902-4_18

Peiffer JA et al (2013) Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc Natl Acad Sci U S A 110:6548–6553

Pérez-Jaramillo JE et al (2015) Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol. https://doi.org/10.1007/s11103-015-0337-7

Pierre-Alain M, Christophe M, Séverine S, Houria A, Philippe L, Lionel R (2007) Protein extraction and fingerprinting optimization of bacterial communities in natural environment. Microb Ecol 53:426–434

Pieterse CMJ et al (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375

Ploetze FA, Bertin PN, Carapito C (2015) Proteomic tools to decipher microbial community structure and functioning. Environ Sci Pollut Res 22:13566–13612. https://doi.org/10.1007/s11356-014-3898-0

Prabhukarthikeyan SR, Yadav MK, Anandan A, Aravindan S, Keerthana U, Raghu S, Baite MS, Parameswaran C, Panneerselvam P, Rath PC (2019) Bio-protection of brown spot disease of rice and insight into the molecular basis of interaction between *Oryza sativa*, *Bipolaris oryzae* and *Bacillus amyloliquefaciens*. Biol Control 137:104018

Qin S, Yeboah S, Xu X, Liu Y, Yu B (2017) Analysis on fungal diversity in Rhizosphere soil of continuous cropping potato subjected to different furrow-ridge mulching managements. Front Microbiol 8:845. https://doi.org/10.3389/fmicb.2017.00845

Rajwar J, Chandra R, Suyal DC, Tomer S, Kumar S, Goel R (2018) Comparative phosphate solubilizing efficiency of psychrotolerant *Pseudomonas jesenii* MP1 and *Acinetobacter* sp. ST02 against chickpea for sustainable hill agriculture. Biologia 73(8):793–802

Ramachandran VK, East AK, Karunakaran R, Downie JA, Poole PS (2011) Adaptation of rhizobium leguminosarum to pea, alfalfa and sugar beet rhizospheres investigated by comparative transcriptomics. Genome Biol 12(10):R106

Rampitsch C, Bykova NV (2012) Proteomics and plant disease: advances in combating a major threat to the global food supply. Proteomics 12:673–690. https://doi.org/10.1002/pmic.201100359

Rawat J, Saxena J, Sanwal P (2019) Biochar: a sustainable approach for improving plant growth and soil properties. In: Biochar-An imperative amendment for soil and the environment. IntechOpen, London

Rosier A, Bishnoi U, Lakshmanan V (2016) A perspective on inter-kingdom signaling in plant – beneficial microbe interactions. Plant Mol Biol 90:537–548. https://doi.org/10.1007/s11103-016-0433-3

Rothballer M, Uhl J, Kunze J, Schmitt-Kopplin P, Hartmann A (2018) Detection of the bacterial quorum-sensing signaling molecules N-acyl-homoserine lactones (HSL) and N-acyl-homoserine (HS) with an enzyme-linked immunosorbent assay (ELISA) and via ultrahigh-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS). In: Quorum Sensing. Humana Press, New York, pp 61–72

Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, Pascale SD, Bonini P, Colla G (2015) Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci Hortic 196:91–108. https://doi.org/10.1016/j.scienta.2015.09.002

Sapkota R, Knorr K, Jørgensen LN, O'Hanlon KA, Nicolaisen M (2015) Host genotype is an important determinant of the cereal phyllosphere mycobiome. New Phytol 207:1134–1144

Shukla A, Dhauni N, Suyal DC, Kumar S, Goel R (2015) Comparative plant growth promoting potential of psychrotolerant diazotrophs *Pseudomonas* sp. JJS2 and *Enterobacter* sp. AAB8 against native *Cajanus cajan* (L.) and *Eleusine coracana* (L.). Afri J Microbiol 9 (20):1371–1375

Singh J, Silva KJP, Fuchs M, Khan A (2019) Potential role of weather, soil and plant microbial communities in rapid decline of apple trees. PLoS One 14:1–19. https://doi.org/10.1371/journal.pone.0213293

Smit E, Leeflang P, Glandorf B, van Elsas JD, Wernars K (2010) Analysis of fungal diversity in the wheat Rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. Appl Environ Microbiol 65(5):2614–2621

Song X, Rampitsch C, Soltani B, Mauthe W et al (2011) Proteome analysis of the wheat leaf rust fungus, *Puccinia triticina*, infection structures enriched for haustoria. Proteomics 1:944–963

Soni R, Goel R (2010) Triphasic approach for assessment of bacterial population in different soil systems. Ekologija 56(3&4):99–104

Soni R, Goel R (2011) nifH homologs from soil Metagenome. Ekologija 57(3):87–95

Soni R, Suyal DC, Agrawal K, Yadav A, Souche Y, Goel R (2015) Differential proteomic analysis of Himalayan psychrotolerant diazotroph Pseudomonas palleroniana N26 strain under low temperature diazotrophic conditions. CryoLetters 36(2):74–82

Soni R, Kumar V, Suyal DC, Jain L, Goel R (2017) Metagenomics of plant Rhizosphere microbiome. In: Singh R, Kothari R, Koringa P, Singh S (eds) Understanding host-microbiome interactions - an Omics approach. Springer, Singapore. https://doi.org/10.1007/978-981-10-5050-3_12

Soni R, Suyal DC, Sai S, Goel R (2016) Exploration of nifH gene through soil metagenomes of the western Indian Himalayas. 3Biotech 6(1):1–4

Sun JM, Irzykowski W, Jedryczka M, Han FX (2005) Analysis of the genetic structure of *Sclerotinia sclerotiorum* (lib.) de Bary populations from different regions and host plants by random amplified polymorphic DNA markers. Jintegr Plant Biol 47:385–395. https://doi.org/10.1111/j.1744-7909.2005.00077.x

Suyal DC, Kumar S, Yadav A, Shouche Y, Goel R (2017) Cold stress and nitrogen deficiency affected protein expression of psychrotrophic Dyadobacter psychrophilus B2 and Pseudomonas jessenii MP1. Front Microbiol 8:430. https://doi.org/10.3389/fmicb.2017.00430

Suyal DC, Kumar S, Joshi D, Soni R, Goel R (2018) Quantitative proteomics of psychotrophic diazotroph in response to nitrogen deficiency and cold stress. J Proteome 187:235–242

Suyal DC, Joshi D, Debbarma P, Soni R, Dash B, Goel R (2019a) Soil Metagenomics: Unculturable microbial diversity and its function. In: Varma A, Choudhary D (eds) Mycorrhizosphere and Pedogenesis. Springer, Singapore, pp 355–362

Suyal DC, Joshi D, Kumar S, Soni R, Goel R (2019b) Differential protein profiling of soil diazotroph Rhodococcus qingshengii S10107 towards low-temperature and nitrogen deficiency. Sci Rep 9(20378):1–9

Suyal DC, Kumar S, Joshi D, Yadav A, Shouche Y, Goel R (2019c) Comparative overview of red kidney bean (Phaseolus valgaris) rhizospheric bacterial diversity in perspective of altitudinal variations. Biologia 74(10):1405–1413

Suyal DC, Shukla A, Goel R (2014a) Growth promotory potential of the psychrophilic diazotroph Pseudmonas migulae S10724 against native Vigna radiata (L.) Wilczek. 3Biotech 4(6):665–668

Suyal DC, Yadav A, Shouche Y, Goel R (2014b) Differential proteomics in response to low temperature diazotrophy of Himalayan psychrophilic nitrogen fixing Pseudomonas migulae S10724 strain. Curr Microbiol 68(4):543–550. https://doi.org/10.1007/s00284-013-0508-1

Suyal DC, Soni R, Sai S, Goel R (2015a) Microbial inoculants as biofertilizer. In: Singh DP et al (eds) Microbial inoculants in sustainable agricultural productivity. Springer-Verlag, Berlin, pp 311–318

Suyal DC, Yadav A, Shouche Y, Goel R (2015b) Diversified diazotrophs associated with the rhizosphere of Western Indian Himalayan native red kidney beans (Phaseolus vulgaris L.). 3Biotech 5(4):433–441

Suyal DC, Yadav A, Shouche Y, Goel R (2015c) Bacterial diversity and community structure of Western Indian Himalayan red kidney bean (Phaseolus vulgaris L.) rhizosphere as revealed by 16S rRNA gene sequences. Biologia 70(3):305–313

Tan Y, Cui Y, Li H, kuang A, Li X, Wei Y, Ji X (2017) Rhizospheric soil and root endogenous fungal diversity andcomposition in response to continuous *Panax notoginseng* cropping practices. Microbiol Res 194:10–19

Trujillo ME, Riesco R, Benito Pand Carro L (2015) Endophytic Actinobacteria and the interaction of Micromonospora and nitrogen nixing plants. Front Microbiol 6:1341

Turner TR, Ramakrishnan K, Walshaw J, Heavens D, Alston M, Swarbreck D et al (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME J 7(12):2248–2258

Urich T, Lanzén A, Qi J, Huson DH, Schleper C, Schuster SC (2008) Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 3(6)

van Dam NM, Bouwmeester HJ (2016) Metabolomics in the rhizosphere: tapping into belowground chemical communication. Trends Plant Sci 21(3):256–265

Wang D, Li F, Cao S et al (2020) Genomic and functional genomics analyses of gluten proteins and prospect for simultaneous improvement of end-use and health-related traits in wheat. Theor Appl Genet 133:1521–1539

Wang DZ, Xie ZX, Zhang SF (2014) Marine metaproteomics: current status and future directions. J Proteome 97:27–35. https://doi.org/10.1016/j.jprot.2013.08.024

Wang HB, Zhang ZX, Li H, He HB, Fang CX, Zhang AJ, Li QS, Chen RS, Guo XK, Lin HF, Wu LK, Lin S, Chen T, Lin RY, Peng XX, Lin WX (2011) Characterization of Metaproteomics in crop Rhizospheric soil. J Proteome Res 10(3):932–940. https://doi.org/10.1021/pr100981r

Wei X, Wang X, Cao P, Gao Z, Chen AJ, Han J (2020) Microbial community changes in the Rhizosphere soil of healthy and rusty Panax ginseng and discovery of pivotal fungal genera associated with rusty roots. Biomed Res Int 2020. https://doi.org/10.1155/2020/8018525

Weiss S, Carapito C, Cleiss J et al (2009) Enhanced structural and functional genome elucidation of the arsenite-oxidizing strain *Herminiimonas arsenicoxydans* by proteomics data. Biochimie 91:192–203. https://doi.org/10.1016/j.biochi.2008.07.013

Zackrisson M, Hallin J, Ottosson LG, Dahl P, Fernandez-Parada E, Ländström E et al (2016) Scan-o-matic: high-resolution microbial phenomics at a massive scale. G3: Genes Genomes Genetics 6(9):3003–3014

Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, Cho H, Karaoz U, Loqué D, Bowen BP et al (2018) Dynamic root exudates chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol 3:470–480

Zifcakova L, Vetrovsky T, Howe A, Baldrian P (2016) Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environ Microbiol 18:288–301. https://doi.org/10.1111/1462-2920.13026

Zou L, Tang QM, Wang Y (2010) Ecological distribution of soil microorganism in pure and mixed forests of *Pinus sylvestris* var. *mongolica* and *Larix gmelini*. J Northeast For Univ 138:63–69

Relevance of Metatranscriptomics in Symbiotic Associations Between Plants and Rhizosphere Microorganisms

3

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Abstract

Interaction between plant and microbes in the rhizosphere, the place of soil influenced with the aid of plant roots, are fundamental to biogeochemical cycling, plant immunity, and productivity. These interactions are properly understood, however, exceedingly little is about the plant microbiome. The study of the interactions between plants and their microbial communities in the rhizosphere is important for developing sustainable management practices and agricultural products such as biofertilizers and biopesticides. Plant roots release a broad variety of chemical compounds to attract and select microorganisms in the rhizosphere. Rhizosphere symbiosis is arguably the most ecologically important eukaryotic symbiosis, yet it is poorly understood at the molecular level. Understanding this symbiotic relationship at a molecular level provides important contributions to the understanding of forest ecosystems and global carbon cycling. Metatranscriptomics allowed the profiling of different microorganism communities and their evaluation of relative and quantitative profusion and metabolism from large number of samples. Extraction and purification of mRNA immediately from plant, decomposition of natural material and soil, accompanied with pooling of expressed genes by using high throughput sequencing, have spawned metatranscriptomics a new rising area of research. Every metatranscriptome offers a view of relative abundance and composition of genes which are actively transcribed and consequently provides the evaluation about the interaction between plant and soil microbes Metatranscriptomics can also evaluate the collective metabolism pathways of microorganism in different environments.

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3.1 Introduction

Bacteria and fungi associated with plant roots (rhizosphere microbiome) can have positive impacts on the health of terrestrial plants. Microorganism in their surrounding environment along with plants can survive quietly or compete with each other for their existence. In their surrounding, plants are connected with various types of microbes; these microbes either may be pathogenic and can be the reason of many types of plant disorder or are worthwhile and have the capacity to stimulate plant innate immune system. These interaction between plant and microbes may hold complex communication structures, which are thousand years old in case of symbiotic relationship such as with arbuscular mycorrhiza. Rhizosphere competence is an essential method has been blended to apprehend a molecular label foundation of bacterial characteristics worried between plant and microbes interaction.

Microbes have key roles in ecosystems and influence a large number of important ecosystem processes, including plant nutrient acquisition, nitrogen and carbon cycling, and soil formation (Wardle et al. 2004; Van der Heijden et al. 2008; Van Elsas et al. 2012; Wagg et al. 2014). Phylogenetically and functionally, microbes are very different organism on the earth. They play a very crucial role to protect the lifecycle on the earth, but still we confine a very little information about the microorganism present in the environment such as soil, water, and atmosphere, which are not culture able. Culture dependent strategies are lengthly and allowed to learn about of microbial isolates under laboratory condition, while the microbes which are culture independent, the strategies at molecular level are permitting to study the whole microbe community in their herbal environment. Profiling of neighborhood microbes in their environment has emerged as frequent region by using high throughput sequencing strategies such as 16S ribosomal RNA (rRNA) gene sequencing. Various associations have been completed between unique microbe corporations and plant features such as ailment (Greenblum et al. 2012), food plan (Martinez et al. 2012), and genetics (Spor et al. 2011) and human microbiome exploitation has proven efficiency in the treatment of illnesses (Brandt 2013).

Similarly the microorganisms associated with plant are a very important factor of plant fitness and productiveness (Berendsen et al. 2012) and various efforts to make bigger appreciation of it have done (Bulgarelli et al. 2013). A diverse and again exclusive, metabolic efficiencies of microorganism, specifically the archaea and bacteria, their ability to concerned in different plant cycle such as Nitrogen cycle, Phosphorous cycle and Sulphur Cycle etc. Microorganism associated with plant plays a very crucial role in biogeochemical cycles. The plant parts and plant surfaces that can carry a microbial region can be divided into three different parts:

Rhizosphere, Phyllosphere, and Endosphere. Rhizosphere is the region where interface between roots and soil takes place. Microorganism in these niches can have useful, neutral or destructive relations with their host plant.

Significance of specific plant and microbe interactions has been recognized for many extra years. Mainly the Rhizobium and legume symbiotic relationship, which strongly contributed in the improvement of extended agricultural production. In legumes root nodule symbiosis involves host specific recognition and post-embryonic development of a nitrogen-fixing organ, the root nodule. This type of model structures was nicely analyzed (Oldroyd et al.), however, ordinary microbiome of the plant, regarded as host plant prolonged phenotype, is no longer as but properly explained. Plant microbiome management has the conceivable to limit occurrence of plant diseases (Bloemberg and Lugtenberg 2001), by minimizing chemical inputs (Adesemoye et al. 2009) and greenhouse gasses emissions (Singh et al. 2010), ensuing for additional sustainable agriculture. This purpose can be considered as essential for supporting the developing population of the word.

3.2 Rhizosphere

In rhizosphere roots of plants are surrounded by using a slight area of soil. This rhizosphere is influenced by way of plant roots and it has an excessive range of microorganism. Its surrounding organization is projected to be one of a kind than the determined one in the soil bulk (Reinhold et al. 2015). The composition of rhizosphere microbiome is affected by means of unique factors such as soil properties, ambient conditions, and tradition composition of microorganism (Qiao et al. 2017). The rhizosphere is the area of soil influenced with the aid of roots through rhizodeposition of mucilage and exudates. Root exudates are concerned as major determinants of rhizosphere microbiome (Shi et al. 2011; Badri et al. 2013). Composition of root exudates in Arabidopsis thaliana plant has proven variant throughout unique accessions ensuing in correspondingly unique microbial community of rhizosphere (Micallef et al. 2009). Plant root exudates contain a wide range of compounds mainly sugars, natural lipids, fatty acid, amino acids, vitamins, hormones, and antimicrobial compounds (Bertin et al. 2003). Root exudates composition differs spatially and temporally with a large range of abiotic and biotic factors. These are made up of plant species and cultivar (Micallef et al. 2009), as nicely as age and stage of plant development (Chaparro et al. 2013; Cavaglieri et al. 2009). In the areas along with wild oat root, 8% of total bacterial communities have been located for root zones enrichment in compare to soil and greater numbers of stay cells had been remoted from developing root recommendations and hairs compare to mature root (De Angelis et al. 2009). Generally, tries are constituted to outline the complete rhizosphere, nevertheless microorganism enriched predominantly at different root zones may also be diluted with the help of this approach, by providing the customary concern that they are no longer enriched at all. This may be the essential consideration during the sampling of rhizosphere soil.

Plants which grow anexically have distinctly one of a kind exudate compositions from these influenced by using microbes. Rhizosphere of different plants such as alfalfa and pea are trigger gluconeogenesis, which can be introverted via sugar presence in R. leguminosaram (Ramachandran et al. 2011). These results and the authenticity that how carbon is allotted to roots is determined by plant dietary fame (da Dakora and Phillips 2002) create the extraction of root exudates an exceptional challenge.

Additionally different carbon sources such as glucose, citrate, and glycine to unique soil were used in attempts to reconstruct the effect of rhizosphere has concluded in improvement of beta and gammaproteobacteria as appropriately as Actinobacteria (Eilers et al. 2010). In rhizosphere these carbon sources are enriched in compare to bulk soil. Even though root exudates can integrate a wide range of carbon source. This taxa enrichment is the use of carbon make available and suggests some colonizers may in addition be opportunistic fast growers. Nevertheless, the decision of taxonomy to know about the species and genera counter to the source of carbon to be acknowledged. These degrees of taxonomy are the place actual variations in metabolic skills concept to be obligatory for the colonization of rhizosphere.

Even though, essential exudates are not only the thing for rhizodeposition and here is a confirmation to recommend this might also be solely vital at root pointers development (Dennis et al. 2010). The root cells sloughing and the launching of mucilage drops into the rhizosphere, a massive amount of fabric along with plant cellular wall of plant polymers such as pectin and cellulose. The degradation of cellulose is a huge characteristic surrounded by microorganism of excessive natural count number soils (Stursova et al. 2012). Pectin decomposition releases methanol (Galbally and Kirstine 2002) which can be used as carbon source by microorganism. In the rhizosphere, metabolism of C1 compounds has been analyzed (Ramachandran et al. 2011; Knief et al. 2012).

3.2.1 Rhizosphere Colonization

Rhizosphere colonization is one of the first steps in the pathogenesis of soil-borne microorganisms. It can also be crucial for the action of microbial inoculants used as biofertilizers, biopesticides, phytostimulators, and bioremediators. Pseudomonas is used as one of the best root colonizers as a model root colonizer. To utilize the plant derived carbon is not plenty useful until, if the microorganism is not able to discover a plant in the soil. So, it is a belief that each motility and chemotaxis is the core capability for the microorganism of rhizosphere. Nonetheless it is complex truth that plant root base attachment entails a swap from motile to sedentary survival. The genes involved in flagellar meeting and chemotaxis had been up regulated in the maize rhizosphere for P. putida, though have been down regulated in the pea, alfalfa, and sugar beet rhizosphere for R. leguminosarum (Ramachandran et al. 2011). Ralstonia solanacearum, the plant pathogenic bacterium chemotatactically retort to

root exudates of tomato of host plants, particularly for amino acid and natural components.

Any defect in the main chemotaxis regulators, cheA and cheW, concluded in traces with wild type motility though decrease in virulence. But, when they were inoculate in to the plant stem without delay they had been found in the condition to motive ailment (Yao and Allen 2006). Pseudomonas fluorescens PGPR, WCS 365 in tomato rhizosphere in addition required the cheA gene for chemotaxis, where it responds to citrate and malate. Mutation in cheA gene established decreased antagonism in colonization of rhizosphere (de Weert et al. 2002). It may be helpful to review the expression of genes associated with chemotaxis in enormous metatranscriptomic information units of rhizosphere with that is recently observed from model system. It may permit that the chemical derived from plant alerts are magnetize one of a kind corporations of microorganism in the rhizosphere and how they are dispersed throughout distinctive plants. Otherwise, PGPR might be genetically modified to reply to a molecule produced by the host plant.

At the rhizosphere the direct contact with the plant roots should be regarded top of the line for the achievement of carbon derived by plant and it is the requirement for the colonization of inside tissues with the help of endophytes. This may additionally be regarded the destructive reason of all the microbes responsible for rhizosphere colonization, though the results of the defense response of plant would possibly be felt better powerfully at the rhizosphere, including in addition resolution pressure. A full size overlap (around 40% of operational taxonomic devices (OTUs)) used to be viewed in these microbes attachment to the plant roots and to an immobile timber shape (Bulgarelli et al. 2013), signifying that the impact of rhizosphere is in the phase due to evolution to a immobile survival. Cellular partitions of plants consist of proteoglycans, which are very crucial for the attachment of bacteria and formation of biofilm. The arabinogalactan protein can set off in the formation of biofilm in R. leguminosarum from root exudates of pea (Xie et al. 2012) and a Arabidopsis thaliana mutant, which was deprived in the development of a lysine rich protein is opposed to transformation via Agrobacterium tumefaciens (Gasper et al. 2004).

3.3 Immune System of Plant

The plant immune system has evolved with microbiome of plant and that is why play a crucial role in identifying its composition. Innate immunity of plant is induced via exposure to microorganism with the help of molecular patterns related with microorganisms (Bittel and Robatzek 2007). These aspects of microorganism are prevalent and gradually evolving and diverse microorganism such as peptidoglycan bacterial flagellin, components related with elongation Tu (EF-Tu) and fungal chitin. A flagellin factor can be used to induce the immune system of plant in particular through LLR receptor kinase FLS2. Likewise, EF-Tu is diagnosed by different LRR kinases, which is known as EFR. Fascinatingly responses to these molecules set off approximately identical plant transcriptional responses (Jones and Dangl 2006).

In plant pathogenic microorganism studied, Microbe Associated Molecular Patterns are termed as pathogen related molecular patterns. The response of plant to MAMPs or PAMPs concerned about plant immunity. It consists with the development of reactive oxygen species (ROS), callose deposition is responsible to strengthen the cell wall and induction of genes related with defense. These responses may be affected by plant pathogens through secretion of effectors molecules (Dou and Zhou 2012), which additionally set off a response from plant, considered as immunity effectors (Spoel and Dong 2012). Each MAMP cognizance and effector triggered immunity activates systemic received resistance (SAR). This is plant response involving in the congregation of antimicrobials spectrum for the benefit of tissue, therefore restraining the unfold of contamination (Ryals et al. 1996). The comparable key response is brought on systemic resistance, which shows in similar responses to SAR, though is caused by means of exceptional stimulus. The signaling of plant defense is synchronized with the help of plant hormones depending on the type of plant pathogen (Bari and Jones 2009). Production of ethylene hormone is taken in response to necrotrophic and herbivores pathogens, developmental and environmental factors. Additionally it can amend the signaling cycles of salicylic acid and jasmonic acid. Microorganisms accumulate signals from the mechanism of plant immune system and these signals create the problem of plant itself. Members of microbiome community might be in addition having the capacity to suppress or change the immune system of plant through degradation and production of plant hormones or by manipulating the signaling pathways. Degradation mainly happens due to the effecter molecules, these effecter molecules are renowned by plant receptors as nucleotide binding-leucine rich repeat proteins due to the reason they can integrate nucleotide binding and leucine rich repeat domains. Plants grown in the soil are previously in position to elicity a response in pathogen resistance because of the occurrence of different microorganisms. Additionally these types of large responses are destructing to other beneficial microorganism. The quantity of plant associated microorganism such as Xanthomonas, Pseudomonas, and Sinorhizobium species contain very superior methods for this type of adaptation. Effecter components of Pseudomonas syringae can restrain the immune response elements (Jones and Dangl 2006). Various interactions between microorganism of microbiome and immune system of plant are very complicated, attractive, and very vibrant.

Effects of different elements of immune system of plant on plant microbiota have been analyzed. Arabidopsis mutants which are poor in systemic received resistance (SAR) have already verified elite rhizosphere microorganism communities in compare to wild type (Hein et al. 2008), even as the activation of ISR and SAR chemically did no longer end result in the shifting of locality of rhizosphere microbes (Doornbos et al. 2011).

3.4 Plant and Microbes Interaction in Rhizosphere

In rhizosphere the plant and microbe have interaction fantastically coordinated cellular methods that decide the ultimate consequence of the relationship and decide whether or not interplay will be nasty. Plant and microbes interactions in the rhizosphere are very responsible for various types of intrinsic strategies such as nutrient cycle, carbon sequestration and for the function of ecosystem (Singh et al. 2004). Microorganism available in the soil may utilize the surrounding plant as the source of carbon, in this manner involving the selective secretion of plant of exclusive components can also motivate suitable shielding and symbiotic relationship, while the secretion of various elements can inhibit the pathogenic associations (Bais et al. 2005). Symbiosis is long-term relationship between two or more superior diverse species. In this relationship one organism resides on another one. Out of various plant and microorganism interactions, two symbiotic relationships have been studied very nicely. First one is arbuscular mycorrhizal symbiosis and second one is root nodule symbiosis. Arbuscular mycorrhizal symbiosis is the major studied relationship between microorganism and plants. Microbes associated with plant roots such as arbuscular mycorrhizal fungi (AMF) and PGPR can play crucial role in the enhancement of plant immune system and plant health.

3.4.1 Types of Plant and Microbes Interactions

The association of microorganism associated with plants is specifically very complex. It is made up of different pathogenic, commensal, and various beneficial microorganisms (Vandenkoornhuyse et al. 2015). Different studies recommended that the main symbionts are arbuscular mycorrhizal fungi (AMF), Ectomycorrhizal fungi (EMF), Frankia, and Rhizobia. Though additionally, there is a developing physique of lookup regarding the microorganism and endophytic fungi that can expand inside and outside of the leaves (Van der Heijden et al. 2008; Shaffer et al. 2017). Development of coevolution is very complicated and does not take place solely between plant and symbiont though in addition between symbiont and symbiont even though between symbiont-plant-symbiont. On the basis of interaction between plant and microbes there are four various types of interactions as follows:

3.4.1.1 Arbuscular Mycorrhizal Fungi (AMF)

Among the plant–fungi symbiotic relationship, Arbuscular mycorrhiza (AM) has the largest sharing in the nature. Arbuscular mycorrhiza (AM) fungi live in the diversity of ecosystems such as grasslands, forests, agriculture land, and many more strained environment and has the capability to colonize the most of the plant roots. These mycorrhizas are generally divided into two types; ectomycorrhiza and endomycorrhiza. The presence of endomycorrhiza is erratic. They can be further divided as arbuscular mycorrhiza, monotropoid mycorrhiza, ericoid mycorrhiza, arbutoid mycorrhiza, and orchid mycorrhiza. Arbuscular mycorrhiza fungi are obligate biotrops that come under the Glomeromycotina subphylum of

Mucoromycota phylum (Spatafora et al. 2016). This markedly tiny crew of fungi form obligate endosymbiosis through a range of plant species over 200 species and generally has low specificity for the host plant (Johnson and Jansa 2017). Symbiotic relationship of Arbuscular mycorrhiza (AM) fungi confirms to comprise emerged round the identical moment to bryophytes deviated as of a single lineage of single cell algal charophytes (Lenton et al. 2016; Martin et al. 2017; Lutzoni et al. 2018; Strullu et al. 2018).

Attainment of a root making nutrient lacks in bryophytes. A hypothesis was given that in the beginning bryophytes plants overcome this problem with attaining the symbiosis with Arbuscular mycorrhiza (AM) fungi. In the Arbuscular mycorrhiza (AM) fungi symbiosis, a fungus enters in to the cell wall of plant cell and creates an complicated branched structure known as "arbascule" inside the cell cortex. Early fossil data clarify this assumption as fossils from 407 Ma reveal creations that are like arbuscule (Pressel et al. 2014; Strullu et al. 2014). Additionally experimental statistics confirm that in the formation of mutualistic symbiosis, Glomeromycota and some ancient Mucoromycota are involved successfully with existing liverworts underside environmental extensive CO2 prerequisites analogous to the concentration to the mid palaeozoic atmosphere (Field et al. 2012).

Colonization of Arbuscular mycorrhiza (AM) fungi begins with understanding of fungus for plant derived indicators such as flavonoids and strigolactones that aggravate hyphal branching, germination of spore and developing of indicators by fungal symbiosis (Akiyama et al. 2005; Besserer et al. 2006). Symbiotic alerts of Arbuscular mycorrhiza (AM) fungi are made up of a mixture of various effecter proteins and chitin derived compounds such as short chain chitin oligosaccharides (CO) and Lipo-chitin oligosaccharides (LCO) (Maillet et al. 2011). Lipo-chitin oligosaccharides (LCO) understanding of plant prompts calcium oscillations inside the nucleus of plant cell through a good conserved set of genes of that is known as "Common Symbiosis Signaling Pathway (CSSP)" (Delaux et al. 2013). Common Symbiosis Signaling Pathway (CSSP) activation suppresses the immunity and permits the attachment of hyphae to the plant root accompanied by plant cortical cells penetration by using pre-penetration tools.

Then the formation of specialized arbuscule formation responsible for nutrient cycle takes place. After the formation of arbuscule, the Common Symbiosis Signaling Pathway (CSSP) is no longer lively and the conversation between plant and fungus is performed by using signaling choice and nutrient cycles.

3.4.1.2 Ectomycorrhizal Fungi (EMF)

Ectomycorrhizal Fungi (EMF) play a very crucial role in the functioning of forest ecosystem, and the root colonization of most tree species (Tedersoo et al. 2010). The host plant provides the carbon to EMF in swap for providing copious settlements such as water and nutrients access, defense against pathogenic microbes or heavy metal acceptance (Smith and Read 2008). Various Ectomycorrhizal Fungi (EMF) species and individuals with different useful characteristics fight to plant root colonization. Many analyses have analyzed negative effects of Ectomycorrhizal Fungi (EMF) competition in the plant biomass and root colonization (Kennedy

et al. 2007; Hortal et al. 2008). Just like AMF, Ectomycorrhizal Fungi (EMF) create a symbiotic relationship with plant root and also help the host plant by getting vitamins (mainly nitrogen, phosphorus, and water) and preclude contamination through undesirable pathogenic organisms. Formation of ectomycorrhizae takes place through over 20 different species from the fungal phylum Zygomycota, Basidiomycota, and Ascomycota (Brundrett 2009) and mainly associate with woody plants. Through phylogenetic analysis it reveals that Ectomycorrhizal Fungi (EMF) developed a pair of instances from number saprotrophic lineages as a long way lower back as ~180 Ma (Kohler et al. 2015). Like to their saprotropic ancestors, Ectomycorrhizal Fungi (EMF) have an abundant array of enzyme degradation that are involved in the breakdown of natural molecules into smaller components such as phosphorus and nitrogen; though, many have omitted the majority of enzyme related with cell wall degradation (Kohler et al. 2015). This loss in cell wall degrading enzymes essential for saprotrophy looks to be requisite for the formation of suggested Ectomycorrhizal Fungi (EMF) plant symbiosis, such as approximately all the enzymes of cellulose degradation have been misplaced in the sequenced Ectomycorrhizal Fungi (EMF) (Martin et al. 2016).

Even though the symbiotic constructions which formed by variety of Ectomycorrhizal Fungi (EMF) are unusually similar. Primarily Ectomycorrhizal Fungi (EMF) make hyphal attachment with lateral root of plants analyzed through the initiate of effecters proteins and aquaporins that provide the facility for the creation of a fungal hyphae community in between plant cells inside the cell apoplast (Navarro et al. 2015). Eventually a layer of hyphae is formed on plant root surface to construct the mantle. Ectomycorrhizal Fungi (EMF) symbiosis seems to have arisen more than one instances over the path of an extended length between of a hundred and forty and 300 Ma from various saprotrophic lineages (Kohler et al. 2015; Martin et al. 2017; Lutzoni et al. 2018; Strullu et al. 2018).

3.4.1.3 Rhizobia

The rhizobia are bacteria able to establish a mutualistic nitrogen-fixing endosymbiosis with specific legumes forming root nodules on the host plant. From the genus Parasponia, one nonlegume and more than 70% of legumes develop symbiosis with nitrogen-fixing bacteria. These are widely extend throughout the α- and β-subdivision (classes) of Proteobacteria, which often are united by the multipartite genome structure, consisting in a chromosome and additional plasmid, acquired later, and enriched in dispensable genes that play a key role in the determination of bacterium fitness in different ecological niches. Rhizobia belongs to a polyphyletic group which is associated with 15 genera in 8 specific households (Remigi et al. 2016). Concerning the evolution of plant rhizobia symbiosis, two types of hypothesis have been given. First hypothesis assumes that more than one genes are there for this type of symbiotic relation given the phylogenetic space between parasponia and legumes (Behm et al. 2014). Even though, an extra current speculation recommends that symbiosis related with nitrogen fixation superior from a single angiosperm

lineage that used to be previously in affiliation with Arbuscular mycorrhiza (AM) fungi (Werner et al. 2014); this type of mysterious adaptation used to be recognized in the rosids I clade creating -100 Ma and It is assumed to have in a position to structure protected nodule formation with rhizobia. This secure nitrogen fixing symbiosis with rhizobia is assumed to have because of this mislaid and regained a couple of times, resulting specifically in the host symbiont typically situated (Oldroyd et al. 2011a, b).

Rhizobial detonation is motivated by plants with the help of flavonoids excretion as the ability of engaging suitable nitrogen-fixing symbints. While in response, various effectors proteins are launched by rhizobia, which are commonly referred as nodulation (Nod) factors and a mixture of LCOs. Effecter proteins and Nod elements set off the similar Common Symbiosis Signaling Pathway (CSSP) in plants requisite for the establishment of Arbuscular mycorrhiza fungi (AMF) symbiosis. Activation of plant instigates plant root detonation and the younger nodules formation. Then rhizobia enter in the nodules through a transitory, committed shape recognized as the "Symbiosome" that converts into a nutrient interchange boundary between plant and rhizobia.

3.4.1.4 Actinobacteria (Frankia)

The actinorhizal symbiosis is an endosymbiotic nitrogen-fixing association between members of Frankia, a genus of actinobacteria, and a variety of angiosperms. Inside the genus Frankia, filament like nitrogen-fixing microorganisms develop nodules with an enormous range of actinorhizal plants. Two hundred and sixty species of the Cucurbitales, Rosales and Fagales command as adversarial with rhizobia which exclusively connect with Parasponia and legumes (Dawson 2007). Usually, Frankia operate analogous signaling mechanisms with rhizobia to set off symbiosis with clusters I and III exceptionally, which lack the main nodulation genes nod ABC. The authenticity is still not known though clusters I and III species have been verified to produce chitinase resistant compounds that results in Ca spiking features of activation of Common Symbiosis Signaling Pathway (Chabaud et al. 2015). This analysis recommends that Frankia with clusters I and III secret molecules that can act as Nod elements. These elements are structure wise remarkable and are chitin based Lipochitooligosaccharides (LCOs). The mechanism projected for symbiotic signaling pathways of cluster I and cluster III in all likelihood includes the Common Symbiosis Signaling Pathway (CSSP) given that Lysine-M receptor like kinases (LysM-RLK). The Common Symbiosis Signaling Pathway (CSSP) are stimulated through a wide range of molecules which are unusual than Lipochitooligosaccharides (LCOs) such as polysaccharides and peptidoglycans (Willmann et al. 2011).

The life of Actinorhizal plants correlate with Fabales clade and additionally intensify the assumption that a single predisposed angiosperm lineage is responsible for the life of the nodule forming plant (Doyle 1998). Nodule formation is taken place in the same way as the nodulation in rhizobia. Although, thread like structure of actinobacteria, termed as a 'Fixation Thread' and it is similar as a symbiosome in

order to dermis and transit penetration of nodule. Distinct from symbiosomes, this fixation thread remains integral as soon as interior with the plant cell; as a opening substitute. The plant cell wall becomes thin to facilitate the switch of vitamins between the symbiont and the host plant (Holmer et al. 2017). The fixation threads situated in the Rhizobia and Parasponia shows mutualisms and are a young relationship as a result indicate a superior inherited mechanism of contamination than symbiosomes (Behm et al. 2014).

3.5 Signaling of Plant Hormones

The mechanism of plant and microorganisms derived alerts and have been renowned (Venturi and Keel 2016). Even though a wide variety of indicators participate in the signaling between plant and microbes such as flavonoids. Strigolactones analyzed some high quality plant based indicators, while some effecter proteins, Cos and LCOs are the majority of most implicit microorganism derived compounds (Table 3.1). Most of the symbiotic relationships involving Frankia, AMF, and Rhizobia have sophisticated to involve the Common Symbiosis Signaling Pathway (CSSP) pathway in the plants to select these microorganisms based compounds and modify the signaling of plant hormone in order to develop the wonderful endosymbiotic relationship (Delaux et al. 2013). As discussed in the subsequent part, plants utilize this pathway to differentiate between bacterial and fungal symbionts that cause the development of absolutely unique endosymbiotic relationship. This system at the back of how particular symbionts are perceived with the aid of the CSSP stays one of the principal awesome questions (Rinku et al. 2020).

3.6 Approaches Used to Study the Plant Microbiome Interaction

3.6.1 Culture Dependent Approaches

The standard microbiology techniques involve background separately and culture of microorganisms from their surrounding area by using one kind of nutrient media and boost rudiments relying on the target microbes. Whereas attaining an uncontaminated existence of a microbe is mandatory for scrupulous research of microbe physiology and genetics, culture dependent techniques exclude the great mass of microorganisms variety in an particular environment. Mini metagenome (Mclean et al. 2013) and single cell sequencing techniques (Hutchison and Venter 2006) are the connection between established impartial and subculture methods, though these techniques are nevertheless in their immaturity. It is predictable from diversity of DNA found in the soil that only very small percentage of microorganism species available in the soil are culturable (Torsvik and Ovreas 2002).

The most important obstruction during the culture of intangible microorganisms from the soil shows the availability of rapid developing microorganisms. By giving a

Table 3.1 Major biomolecules and their role in the formation of plant-microbe symbioses

Sl. No.	Process	Name of biomolecules	Functions	Reference
1.	Nitrogen fixation	Flavanols, flavanones, exopolysaccharides	Nod gene expression	Coronado et al. (1995)
		Lectins, Isoflavonoids	Stimulate mitotic division essential for nodule formation	Mathesius and Watt (2010)
2.	AMF symbiosis	Strigolactone, flavonoids (glyceollin, coumestrol, and daidzein)	AMF root colonization	Morandi et al. (1984)
		Myc factors	Mycorrhization	Zhuang et al. (2013)
3.	Metal uptake	Siderophores (Pyoverdine, pyochelin, etc.), Gluconic acid, 5-ketogluconic acid, Glutathione, metallothioneins, etc.	Solubilization of unavailable form of heavy metal to available form	Schalk et al. (2011), Saravanan et al. (2007), Fasim et al. (2002)
4.	PGPR	Jasmonate, Salicylic acid, phytohormones, PGRs, mineralization, cyanogens, siderophores, and phytoalexins	Suppress plant pathogens	Compant et al. (2010), Saharan and Nehra (2011), Mukerji et al. (2006), Nadarajah (2016)
5.	Defense	Glucanases, Chitinases, myrosinases, Arabinogalactan protein (AGP),	Activation of defense reactions	De la Pena et al. (2010), Nguema et al. (2013)
6.	Quorum sensing	Acylated homo-Ser lactones (AHLs), GABA	Cell-to-cell communication regulates virulence factors	Zhuang et al. (2013), Chevrot et al. (2006)
7.	Antimicrobial	Rosmarinic acid (RA), Bacteriocin, Polyketides	Defend plants against negative interactions	Bais et al. (2002), Raaijmakers et al. (2010)

rich culture media, these microbes will compete the greater part of diverse species of microbes. Due to their extreme increase level these microbes are fewer difficult to separate and detail analysis. Even though it is assumed that the ample majorities of microorganisms develops very gradually and are barely ever developing at leading cost in their natural surroundings. By using the negative nutrient culture media and very prolonged incubation periods the culturing of new traces of soil microorganisms has permitted, dazzling these microbes that are identified by using the molecular techniques in the plant rhizospheres and soil, such as Verrucomicrobia (Da Rocha et al. 2010) and some other microorganism (Stewart et al. 2012; Davis et al. 2011). Temperature is also the other important factor which influences the

growth and their survivability. Microorganisms can endure an extensive variety of temperatures in their natural environment though the most common microbial isolations are incubated between the temperature range 27 °C–37 °C.

3.6.2 rRNA and Other Genes as Phylogenetic Markers

In mobile organisms rDNA and rRNA are ubiquitous in nature, including bacteria. They may encode the RNA structural elements in the ribosome and translation tools of the cell. These are accordingly crucial. Within prokaryotes, three genes which encode the 5S, 16S, and 23S subunits of rRNA described by way of the sedimentation and in eukaryotes genes of rRNA genes are structured by an unusual approach with 5S, 5.8S, 18S, and 28S yield. In prokaryotes, the modern molecular taxonomy is dependent on the relatedness of these sequences found in microorganisms (Woese 1987). Variations in the sequences of 16S RNA had been initially used to suggest whatever we understand now as a different vicinity of life to be Archaes, magnificent from Eukaryota and Eubacteria (Woese et al. 1990). The genes of rRNA have finish the benchmark in the analysis of culture independent communities of microorganisms, however, increment in the further diverse marker genes and even the whole genomes are being used.

3.6.3 Genetic Fingerprinting

Dissimilarity in the target sequence of DNA permits microbes recognition at one kind of taxonomic level depending on the rate of evolution of the target DNA sequence and sensitivity of the used technique. Prior to nucleic acids sequencing was once generally on hand and very little price on the levels desiring for the ecology of microorganisms, various strategies have been build up to appear at deviations in the sequences which are being analyzed. Generally, a marker gene from the DNA sample may be amplified by using the technique polymerase chain reaction (PCR) (Mullis et al. 1986). Some scientists uncovered the amplified product by denaturing or enzyme restriction resulting in the fragmentation pattern, during the separation with electrophoresis, which is insightful for the structure of microbial community. Such types of strategies are consisting of terminal limit fragment size polymorphism (TRFLP) (Liu et al. 1997) and Denaturing Gradient Gel Electrophoresis (DGGE) (Muyzer et al. 1993). An adaptation on it is to enlarge a size changeable region of DNA such as Internally Transcribed Spacer (ITS) in between 16S and 23S rRNA genes, as present in Automatic Ribosomal Intergenic Spacer Evaluation (ARISA) (Garcia et al. 1999). Automatic Ribosomal Intergenic Spacer Evaluation (ARISA) needs no additional treatment subsequent to preliminary PCR. This is feasible to determine the abundance and measurement of the residues and these reports can be utilize for graphs creation based on multidimensional scaling or leading object assessment, allowing the structure of microbial community or superior frequently deviations among relatively constructions of some of the microbial communities to

be visualized. Bands of fragments which are incomparable between the microbial communities can be extracted through gel and then sequenced to develop the perceptive of the microorganisms. These techniques of fingerprinting have been used significantly for cDNA and 16S rRNA gene resulting from reverse transcription of 16S rRNA for the learning of microbial communities of rhizosphere (Garbeva et al. 2008), even though additionally different purposeful and phylogenetic markers genes have been used (Haichar et al. 2012).

3.6.4 High Throughput Analysis of 16S rRNA Gene

The microbial ecology has reformed the most recent, affordable and accessible technique, High throughput sequencing such as Illumina's MiSeq and HiSeq structures (Bentley et al. 2008). This huge implementation of the technique by the community of scientists is because of their era of enormous quantities of sequence statistics with a decreased significant price per base pair (bp) in compare to universal sequencing method given by Sanger et al. (1977). Additionally they do not require any cloning process of PCR products prior to sequencing as it was used before. Totally based on a unique barcodevarious samples can be sequenced, pooled and then arranged downstream, an approach known as multiplexing. However, maximum research related with microbial ecology from long time has been carried out by using the pyrosequencing, Illumina's MiSeq and HiSeq arrangement and it may probably dominate in the future.

Sequencing and amplification of the variable region in the 16S rRNA gene isolated from soil samples is developed now. It has done the contribution to our admiration of variety of microorganisms found in innumerable rhizosphere. These are made up of rhizospheres of the model organisms, for example, potato crop plant (Inceoglu et al. 2011), maize (Peiffer et al. 2013), Arabidopsis thaliana (Lundberg et al. 2012; Tkacz et al. 2013b), and bushes like Oak (Uroz et al. 2010) and additionally applied sciences such as microarray and pyrosequencing have been used to search out concerning rhizosphere microbiomes of beta vulgaris (Mendes et al. 2011) and Zea mays (Bouffaud et al. 2012), even as clone libraries of 16S rRNA have been used in combination with shotgun metagenomics to search out regarding rice rhizosphere microbes (Knief et al. 2012).

The indispensable barrier for these techniques is that amplification of genomic DNA by PCR is essentially influenced via PCR primer designing. Normally these are exclusively successful in the detection of an accurate targeted microbial community and thus suggest an influenced prototype within the target group (Pinto and Raskin 2012). Even though, the complex atmosphere is engaged with the aid of microorganisms from all life domains. The eukaryotes which are consisting of nematodes, protozoa, oomycetes, and fungi are ubiquitous in nature available in the soil. They can be very important symbionts or plant pathogens; however, others may be bacterial grazers. Archaea performs so many essential biogeochemical reactions principally in agriculture land such as methanogenesis (Conrad et al.

2006) and oxidation of ammonia (Leininger et al. 2006). As viruses are mobile organisms so they are also determined at anywhere and any place and they can comprise an effect on the population dynamics of the host plant (Williams 2013). Participants of the microbial community present in the rhizosphere have communication with each other and as well as with host plant (Barea et al. 2005) so it is very important to grab and endeavor the complete range present in a microbiome. For this, requirement of sophisticated techniques analysis such as metagenomics, metatranscriptomics and metaproteomics is necessary, which simultaneously give permission for the assessment and evaluation of the communities of microorganisms throughout the life.

3.6.5 Metagenomics

In the precise way, a metagenome is the miscellaneous genomes of the all living organisms present in an exclusive surroundings. During implemention, exclusively genomes fractions are sampled from various microorganisms, though this technique is some space superior about that a targeted method by using the PCR. The genomic DNA of E. coli into a heterologous host from the soil and ocean has been cloned by using this unique metagenomic technique. The sequencing of the bacterial artificial chromosomes (BAC) to recognize the nature of the insert DNA and from which organism the inserted DNA isolated was analyzed. This type of research perceived a wide variety of microorganism community, the expression of a wide variety of determined genes and in addition the products of the genes such as proteins, enzymes, and antibiotics (Jiang et al. 2009). In addition, a heterologous host having bacterial artificial chromosome (BAC) or other unusual vector should be screened functionally for the specific result (Tett and Turner 2012). From the rhizosphere environment various examples are made up of antibiotics (Chung et al. 2008), resistance genes for nickel (Mirete et al. 2007), and new lipases enzymes (Lee et al. 2010). The process of cloning inside the heterologous host shows various barriers essentially (Temperton et al. 2009). Initially, the measurement of insertion sequence is controlled with the help of assembling then it is cloned inside the vector resulting in partiality for the large insertion sequences. Furthermore, introduction of the foreign genomic DNA may also consequence by producing the harmful product which is toxic to the cell. Then, such type of cells which contain these foreign DNA are not improved and as a result not represented in the succeeding investigation. Additionally there are various limitations to different colonies that may be selected and sequenced. The majority of the sequencing DNA is resultant from the insert. Additionally the quantitative data is omitted because of the discrete replication cost of the plasmids available in the host.

A prologue of high throughput sequencing has immensely developed the strength of accuracy and statistics of the authentic shot gun metagenomic techniques. These processes have been already to more accurately recognized and stimulated the communities of microorganisms in comparison to PCR (Shakya et al. 2013). Through this technique the whole genomes from the dominant microbes present in

extreme conditions such as acid mine drains have been sequenced (Tyson et al. 2004). Additionally, more complex samples yet no longer sensible, though it is now realistic to attain ample records on the abundance and presence of the genes which encode for precise metabolic cycles (Handelsman 2004) and however non coding RNA species (Weinberg et al. 2009).

Furnishing of taxonomic data can be done by using possibly all sequences, though many more times essential, ubiquitous in nature and gradually growing genes are markers presenting a customary impression of composition of taxonomy such as Mta Phyler (Liu et al. 2010). These include genes of rpoB, recA, dnaG, ribosomal RNA, HSP70, and EF-Tu (Wu and Eisen 2008). Otherwise, the essential genes would be thought as taxonomic markers, illuminating the organisms in the support of individual methods. Various genes which encode the enzymes essential for nutrient cycle are frequently used to fulfill this purpose such as catalytic subunit of nitrogenase enzyme is encoded by nifH gene (Ueda et al. 1995).

This technique of Metagenomics may be used in the detection of living organisms from every field of life existence such as Prokaryotes, Archaea, Eukaryotes, and viruses also by replacing the partiality of primer annealing and amplification by using PCR (Pinto and Raskin 2012). Though, it is controlled to identify the availability of an organism. Utilization of numerous microbes in the environment such as soil may be very low, thus these microbes build a very little contributions to the functioning of the ecosystem at the exact moment. The microbiome of rhizosphere is selected from the soil. The motivation of plant is to expand in the profusion of some taxa, even a reduction in the loads of other microorganisms. In addition, plants will impact the activity of microorganisms by passing on the sources of energy and carbon. Strategies of steady state isotope with 16S ribosomal RNA by using DGGE have analyzed that a subset rhizosphere microbe community is in specific by consuming the carbon derived by plant (Haichar et al. 2012).

3.6.6 Metatranscriptomics and the Challenges

The metatranscriptome or the pooling of complete RNA molecule from the community of microbes may affords a snap shot of broad gene expression of microbial community. In a metatranscriptome, the dominance of ribosomal RNA allows very robust profiling of the microbial community from all the province of life. This technique has been already implemented to analyze about crop plants rhizosphere (Turner et al. 2013), the soil (Urich et al. 2008). Additionally metatranscriptome contributes facts on the expression of small RNA and non-coding RNA, which perform essential regulatory functions in the microbial communities (Narberhaus and Vogel 2009). However in the metatranscriptome, the main core of attraction is to provide statistics on the dynamic metabolic pathways in the analyzed atmosphere (Fig. 3.1).

In the actively developing, complicated or in pure subculture microorganisms the transcriptomes are dominated through rRNA (Hewson et al. 2009). This can imply

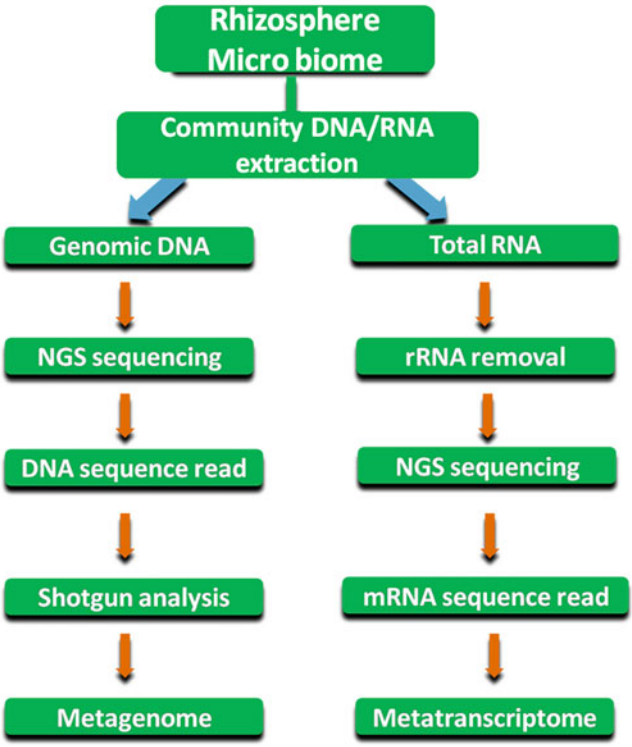

Fig. 3.1 Metatranscriptome analysis: A general overview to study the microbiome metatranscriptome

over 90% of RNA species. Though due to the power of sequencing now feasible with the development of high throughput sequencing (Bentley et al. 2008), the enrichment of the mRNA is very crucial during the transcriptome research. Ribosomal RNA dominance exclusively the 16S and 23S in prokaryotes can be very easily seen by using native agarose gel electrophoresis. However, elimination and purification of all these dominant 16S and 23S bands have been used for the enrichment of messenger RNA (McGrath et al. 2008). But during the enrichment this approach would have eliminated some amount of mRNA with equivalent molecular weight to the subunits of ribosomal RNA. Additionally it would not succeed to put off degraded ribosomal RNA or the 5S smaller subunit of rRNA. Moreover, enormous segments of RNA molecule are requisite and the extraction process can finish result in the degradation of RNA. Now this type of unbalanced system is not promoted for precious samples of atmosphere. This frequently gives low yield and requires high sampling efforts.

In most eukaryotic messenger RNA transcripts, the 3′ ends are poly adenylated (polyA), resulting with a polyA tail at the 3′ end (Zhao et al. 1999), and allow specific, eco-friendly, and easy reinstatement the utilization of complementary

columns of polyT or the magnetic beads. This process has been used to analyze the metatranscriptomes of a variety of soil atmospheres (Takasaki et al. 2013). Even though the mRNA of prokaryotes lacks the poly adenylated (polyA) tails so they cannot be recovered through this method. However, it is solely used to be in the fraction beneficial with the metatranscriptome of the soil (Botero et al. 2005). The transcripts of archaebacteria had no longer existed in the sample with enriched messenger RNA, but it has been confirmed by the use of quantitative reverse transcriptome polymerase chain reaction (Botero et al. 2005).

In the prokaryotic metatranscriptomes research the ribosomal RNA depletion is favored. Even though in this incomplete elimination of ribosomal RNA from the sample this continuously consequences, this is preferable to introducing partiality by taking solely a subset of messenger RNA. The ribosomal RNA (rRNA) in the pure cultures of single species is identical, allowing extremely eco-friendly exclusion. This has been done through a variety of available commercial kits, which is frequently used for E. coli. Incorporation of mixed population sufficient edition in the sequence of ribosomal RNA to come to be a task to most sequence structured depletion methods. Additionally the population is frequently not known that is why the lists of compatibility are not now particularly valuable. The sequence structured ribosomal RNA depletion methods are totally depend on subtractive hybridization. Whereas, longer probes or ribosomal RNA's complementary oligonucleotides connect to the rRNA in the sample. As a result both are removed by using the microspheres or magnetic beads. These techniques have been verified to be every superior fastidious and set up greatly less partiality than the enzymatic method as the terminator exonuclease enzyme (He et al. 2010). This subtractive hybridization is used with the help of a wide variety of kits which are commercially available. Several commercial kits are accessible as a solitary package that claims to dispose of 16S and 23S ribosomal RNA up to 95%. Some kits are very valuable for Gram positive and Gram negative bacteria and as well relatively a small number of eukaryotes such as plant, yeast, mouse, and human also. Bacterial kits state to get rid of 5S, 16S and 23S and ribosomal RNA upto 99% from B. subtilis and E. coli cultures. Availability of commercial kits are limited by means of the variety of sequence of their seize probes. The age of pattern seize probes has established high quality in the ribosomal RNA depletion in the ocean (Stewart et al. 2012). This concerned polymerase chain reaction amplification of the ribosomal DNA (rDNA) from the environment yet to be analyzed. Presence of T7 promoter at the 5′ end of the reverse primer permitted successive in vitro transcription, resulting in an extreme production of ribosomal RNA probes. Integration of biotinylated uracil and cytosine permitted probes retrieval by using the magnetic beads lined by streptavidin. Advantage of this process is the sample specificity of the probes. Even though, to seize all the ribosomal RNA in the sample, probes need to be generated for prokaryotes, eukaryotes and for archaea also and even then the primers useful for the amplification by PCR are not now universal and they will exclude some of the range. Additionally, the

probe technology is intensive mostly if a couple of units are required. Elimination of full 5S ribosomal RNA is regularly no longer profitable with such techniques both and if the generation of 5S ribosomal RNA probes takes place then the workload will increased similarly.

Substitutes of subtractive hybridization do not include so arbitrary priming reverse transcription to partiality for ribosomal RNA during the synthesis of cDNA and enzymatic degradation of ribosomal RNA. The only mRNA packages occupy the enzyme terminator exonuclease for the degradation of transcripts excluding a 5′ monophosphate, and leave intact messenger RNA. Though RNA extracted from environmental samples is in very specific conditions of degradation and, however, this package is being utilized to expend the ribosomal RNA in the metatranscriptomes of marine. Additionally, it has been verified to be much less kind at getting rid of rRNA. Even though there is an evidence of a synergistic extension in presumption.

On the other hand the enzyme successful for the depletion of rRNA is duplex unique nuclease, which is used during the normalization of eukaryotic cDNA libraries (Zhulidov et al. 2004) and genomic DNA libraries (Shagina et al. 2010) appreciably. It is very helpful in the degradation of any double standard nucleic acid such as RNA: RNA, DNA: DNA, and RNA: DNA. Its efficiency of rRNA removal has been tested recently (Yi et al. 2011). Ribosomal RNA depletion and the use of duplex unique nuclease (DSN) contains denaturing of cDNA pattern to recycled secondary organization, resulting in the single stranded molecules. Then the pattern of denaturation is maintained for an exclusive range of time at a lower temperature, after which the addition of duplex unique nuclease (DSN) takes place. Predominantly self-homologous and abundant ribosomal RNA derived cDNA molecules improve their duplexes and appear as a target for duplex unique nuclease (DSN), while the mRNA transcript with medium and low significance are not affected. Duplex unique nuclease (DSN) has been verified to be extra beneficial at doing away with ribosomal RNA and additionally it brings much less inequality (Yi et al. 2011). The disadvantage of many rRNA depletion techniques is the requirements of RNA quantity immensely, which is frequently very hard to achieve from the samples of environment. In addition, only single technique can be helpful to get rid of all the RNA present in the sample almost, which means there may be insufficient left to develop the sequencing library also. Duplex unique nuclease (DSN) protocol (Yi et al. 2011) conquers this with the help of producing cDNA from RNA molecule, by using the conserved tails which then can be used to create larger the cDNA after depletion resulting in enormous portions of messenger RNA enriched cDNA. This can directly be utilized to generate the sequencing libraries.

To overcome from the low yield of RNA from the environmental samples, it would allows more than one enrichment round and make sure that there is sufficient finishing after the treatment for justification, quantification and downstream processing. True amplification of RNA by using commercial kits has been utilized in the evaluation of microarray efficaciously. Additionally, the amplification may also be useful to generate the large RNA quantities in the metatranscriptomics from

the low amounts of valued opening material or after the enrichment of messenger RNA before sequencing process.

Before intending for sequencing process, it is quite vital to authenticate the success of mRNA enrichment. The most effective way to attain the mRNA share in a sample is to sequence it, though this is not repeatedly sagacious because of economic limitations and time. Utilization of capillary electrophoresis is utilized with the help of bioanalyzers, which are normally used to decide the depletion of ribosomal RNA mostly depends on the reduction or failure of the peaks showing 16S and 23S ribosomal RNA. Though, even various assays with high sensitivity performed with the help of such procedure do not accurately decide the levels of enrichment now. Additionally, qRT-PCR and quantitative PCR can be used to verify relative quantities of ribosomal RNA in an each sample prior and after the experiment. Template RNA quantity has to be the same for samples with or without treatment, so a unique RNA fluorescent dye is required for the correct quantification.

Till today the majority of research on metatranscriptomic has targeted on the marine environment (McCarren et al. 2010; Stewart et al. 2012), the place with the diversity of microbes, compactness and implement is little in compare to the soil, this generally results in less of ribosomal RNA in the metatranscriptomes of marine samples. Even though presently various environments have been studied by using this approach, which include lakes of freshwater (Vila-Costa et al. 2013), hydrothermal vents in deep ocean (Lesniewski et al. 2012) and guts of human (Ursell and Knight 2013), termites (Raychoudhury et al. 2011), nematodes (Bomar et al. 2011), and mice (Xiong et al. 2012). The study of metatranscriptomes for complex terrestrial environments such as plant and soil rhizospheres analyzed to date has been reserved to eukaryotes (Takasaki et al. 2013). Mostly, it is due to simple enrichment of mRNA taking achievement of polyA of mRNA transcripts in eukaryotes. An additional speculation introduced through the environment of soil is the availability of breakdown products of lignin, humic acids, which can be co-purify with nucleic acids and are inhibitory to many enzymes (Wang et al. 2012).

The main annoy in the research area of metatranscriptomic has been that some researchers have confined organic duplication or comparisons between incomparable atmosphere. Some studies have differences in day and night time metatranscriptomes in ocean and communities of lake (Vila-Costa et al. 2013). While, due to perturbations others have in distinguish adjustments in the transcriptomes (Ursell and Knight 2013). In marine metatranscriptomes the chronological dynamics have been evaluated by using maintenance tools and involuntary series. Including a wide range of sequencing reads that suit a very precise group of taxonomy or hit a gene in the metabolic pathway of two different environment can contribute solely comparisons. Additionally, an interior RNA admired allows the commitment of depth of sequencing and complete abundance of transcript (Moran et al. 2013). This protocol implementation, likewise recent updates in enrichment of messenger RNA and the huge quantity of given sequence makes it possible now for quantitatively and statistically metatranscriptomes observation isolated from more than one complex environment.

In case of non model microbes, assembly of de novo transcriptome can be cost effective approach, fragments of transcripts are formed and due to this subjected to gene annotation automatically by using the instruments such as KEGG datasets and gene ontology (Kanehisa and Gotz et al. 2008). Then mapping of short sequencing reads takes place to the assembled fragments to decide the quantities of transcript (Mortazavi et al. 2008). With the help of such information, it is feasible to conclude the relationship between phenotype and gene expression profiles (Ekblom and Galindo 2011). Though, such type of symbiotic connections can make it rigid to separate the RNA of plant from RNA of microorganisms. Specified complex nature of structure found during symbiosis and the errors in the database of sequencing, assemblies of transcripts may also include mistakes. In this case, the algorithms are not remarkable and enormous amount of mapping sequences can be vanished, these concerns can be partial the quantification and generate many false negative and false positives during the identification of gene (Xiao et al. 2014).

3.6.6.1 Characterization of the Metatranscriptome

Advancement in the sequencing techniques revolutionized metagenomic analysis and have also highly developed techniques targeting the study and understanding of expression of gene at universally. To understand the significant roles that expression of host gene performs at cellular and tissue level has become very important in the past time from the sophisticatedly explained differential display approach (Liang and Pardee 1992) to the universal transcriptome approach by using the microarrays (Schena et al. 1995). Recently, the initiation of extremely parallel sequencing and RNA sequencing has established exciting and novel prospects in the field of analysis of transcriptome by giving vibrant and imminent range formerly unbelievable (Wang et al. 2009). Additionally after gaining the insights from the explanation of expression profile of host gene, now we are also in the situation to analyze the gene expression of composite microbial communities at the specified environment that comprises the expression profile of gene of fungi and bacteria that can be cultured or not cultured. Datasets of metatranscriptome analysis thus accompaniment the datasets of metagenomics through explaining very precisely that which of the genes were annotated during the analysis of metagenome and are transcribed (Franzosa et al. 2014). Thus are facilitating the demonstration of the functions from a probable range of bacteria that are truly in use. With the help of such functional datasets, the active metabolic pathways can be recognized in the microbial communities and it can be linked with specific environmental situation (Shirley et al. 2015). Thus, metatranscriptomics provide the new perspective of information in comparison to metagenomics, because it can disclose the facts about the microbial communities that are transcriptionally active and just do not recognize the heritable content of bacterial communities as during the analysis of metagenome.

3.6.6.2 Metatranscriptomics Data Analysis

Database of a standard metatranscriptome incorporates millions of mRNA sequenced molecules. These sequenced molecules are known as RNA-Seq reads. However, as experiments on metatranscriptome are increasing in measurement and

number continuously so efficient and automated, high throughput sequencing analyses are essential to conclude the crude significance from the databases (Korf 2013). Over the previous years, various inclusive evaluation groups have been developed and are significantly followed and provide the uninterrupted results. These techniques are employed with the combination of specific bioinformatics tools to achieve the similar motive of concluding the expression stages of the gene and alteration in the different expression stages. Some steps of analysis are essential during the process and thus are equally exist during complete metatranscriptome analysis. These analytical steps are made up of the straining of non-mRNA sequences and same as the host reads, filtering and trimming of low quality nucleotides and reads, figure out of ORFs, mapping of reads with reference datasets, normalization and then calculation of the expression ranges of gene with various statistics (Wang et al. 2009).

In this process, a non-obligatory analytic step is the assembly of mRNA reads into contings. This can be performed after introductory filtering. After implementation, the assembly step is combined with the mapping of the contings to the genomes mentioned, when these are accessible. However, computationally an assembly step is very tricky and it entails superior experimental database for sequencing. It carries the attainable for the finding of facts related to expression of gene that is not conceivable to accepting this, for example, relationship between beginning and ending locations and neighboring genes. Deeper sequencing is required, experimentally to permit the assembly of contings and though, usually exclusively from a large sets of reads the considerable areas can be assembled (Morgan and Huttenhower 2014). The assembly step is exemplary in case in which successive gene annotations and a reference genome are not available extensively. The matches in which a reference genome is no longer available, the annotations of the sequenced transcripts are generally established with the help of similarity of the sequence to sequenced and annotated product. So the assembled transcripts are aligned by using the annotated datasets of protein with the help of computer software, for example, Blast2GO software (Conesa et al. 2005) and we get extremely comparable proteins then a comparable natural feature is inferred normally. A number of groups for the reconstruction of whole transcriptome have been developed. Primarily these are based on massive computational tools, usually depending on graph theoretic standards (Grabherr et al. 2011).

An additional vital problem during the evaluation and conclusion of data from metatranscriptomics is joining the complete DNA database and the evaluation of the RNA sequence statistics. During the analysis of these two different kinds of statistics alongside for a particular pattern allows us for the conclusion of the doubtlessly available genes vs really expressed genes (Shirley et al. 2015). In spite of the occurrence of the assembly step at the end of the RNA sequence evaluation and the process of postnormalization, an information outline is transformed into the values of relative gene expression and then it can be analyzed similarly in the comparison to the statistics analyzed in metagenomic and 16S RNA sequencing.

3.6.6.3 Future Scenario and Conclusions

To discover the metabolic interactions at community level, metatranscriptomics invention is a probably affluent technique. Although, the challenges to produce the metatranscriptomes database from the environmental mRNA is very simple in comparison to their assessment and their interpretation. Metatranscriptomics holds splendid plausible to find biological facts that may additionally be in any other case obscured by means of different genomic methodologies. This afford a correct snapshot under the specific conditions at a given time of profile of gene expression as an substitute than its capacity as indirect from DNA based metagenomic shotgun sequencing. Such as, metatranscriptomic interpretation of microbiome can also highly permit the clarification of beneficial changes that dictate the features of microbiome under given circumstance, its communications with the host plant and sensible modification that escort the beneficial microbiome conversion in the direction of a disease driving arrangement. In addition metatranscriptomics can also unlock an idea for the discoveries of regulatory mechanisms coordinate determined expression of gene, thus it uncovers that how microbe–microbe and microbe–host interactions regulate the activity of microbiome. Continue addition in the evaluation of a large number of microbiome techniques can make a crucial contribution in the direction of concluding a complicated and enormous mystery. Additionally, attracting the globe for integral techniques for 16S rRNA characterization such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics may benefit careful deliberation in illustration the cost choice limitations and availability of pattern are no longer excessive.

Potential to understand the facts of single transcriptome and metatranscriptomic is currently inhibited through the use of the accessibility of high quality, specifically annotated and phylogenetically diverse genomes with the inadequate information of cell metabolism of fungi, the place many mapped transcripts continue to be of imaginary characteristic and attributed features through the sequence homology might be doubtful also and by the way of lacking of databases of curated sequences for specific functions. Precisely annotated and high quality reference genomes are essential for all the metatrancriptome and transcriptome studies such as to identify transcripts in individual genomes, to endow statistics on order of the gene and community and to provide interpretation for the assessment of complex microbial community databases. Mission for sequencing the 1000 fungal genomes are trying to intensify the taxonomic size of sequence databases of fungal genome and are providing mixed genomes and transcriptomes to assist annotation. For being the most valuable, the database of genome sequence desires to be associated through expression of gene and various experimental databases. Such types of databases are under progress.

Constant emphasis needs to be located on the cell biology of a large taxonomic variety of fungi to assist definite responsibility of outline aspects of main metabolic pathways and gene features, mostly for the imaginary genes. The augmentation of research related with metatranscriptomics with improvement in metaproteome based on Mass Spectrometry analysis will supply accurate sensible assignments of transcribed genes (Mueller and Pan 2013) and limit our dependence on the techniques

based on sequence homology. Unremitting development of sequencing structures for guiding the longer sequence will improve the capacity additionally to record the transcriptional sequences to consider taxonomic communities and features.

Whereas the evaluation of microbiome metatranscriptomics holds assurance in the improvement of our grapple of the complex community behavior of the microbiome. Various challenges needs to be maintain to organize and adorn the general applicability and reproducibility during metatranscriptome study. Even with these tackles, the evaluation of metatranscriptomic of microbiome might be additionally of splendid cost during the transfer from an expressive microbiome characteristic to a reflective observation in the contribution of microorganism to disorder vulnerability and homeostasis. Such as, metatranscriptome assimilation into the research of microbiome can also permit to attain advanced grasp of its variety of functions in the physiology of rhizosphere.

As the databases based on sequence are continue becoming large and our research is becoming more complex, so enhancement in the computational speed and coping of databases are wished facilitating comparative studies. To overcome from the restrictions in the evaluation of transcriptome will need an intensive effort to improve our potential to specifically assign the functions of transcript, become attentive of various factors of main metabolic pathways and unscramble relationships amongst the microbes in the microbial communities. Doubtlessly this will contain the pioneering use of accessible computational resources as adequately as the diagram and advancement of novel techniques for assigning various functions. Continue sharing of this data through validated datasets publicly and various facts assets will encourage accurate explanation of databases of composite metatranscriptome. Metabolism of microbes at the cell and range of microbial community is controlled at different amazing factors such as material containing genome and its related closely regulatory features of transcription, post-transcriptional controls obligatory on the messenger RNA, post-translational modifications, and protein location in the cell and enzyme kinetics in the unusual atmosphere of biochemistry. Metatranscriptome based analysis provides a world view of metabolism of community the place considerable taxa and moderately the expresses genes are most likely to be represented. However, the area with small loads of microorganisms and genes with small stage of gene expression may be missed additionally. For being the most informative, analysis of metatranscriptomic ought to be assorted with the assessment of the detection and viability/growth of the members of microbial community, knowhow of the closely bio-geochemical environment and the things to do of expressed proteins and enzymes.

References

Adesemoye AO, Torbert HA, Kloepper KW (2009) Plant growth promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorhizal fungi. Nature 435(7043):824–827

Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic related compounds predominantly modulate the soil microbiome. J Biol Chem 288:4502–4512

Bais HP, Walker TS, Schweizer HP (2002) Root specific elicitation and antimicrobial activity of rosmerinic acid in hairy root cultures of sweet basil (Ocimum basilicum L.). Plant Physiol Biochem 40:983–995

Bais HP, Prithiviraj B, Jha AK, Ausubel FM, Vivanco JM (2005) Mediation of pathogen resistance by exudation of antimicrobials from roots. Nature 434:217–221

Barea JM, Pozo MJ, Azcon AC (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Bari R, Jones J (2009) Role of plant hormones in plant defense responses. Plant Mol Biol 69:473–488

Behm JE, Geurts R, Kiers ET (2014) Parasponia: a novel system for studying mutualism stability. Trends Plant Sci 19(12):757–762

Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG (2008) Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456:53–59

Berendsen RL, Pieterse CMJ, Bakker P (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Bertin C, Yang XH, Weston LA (2003) The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83

Besserer A, Puech PV, Kiefer P (2006) Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biol 4(7):1239–1247

Bittel P, Robatzek S (2007) Microbe associated molecular patterns (MAMPs) probe plant immunity. Curr Opin Plant Biol 10:335–341

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350

Bomar L, Maltz M, Colston S, Graf J (2011) Directed culturing of microorganisms using metatranscriptomics. MBio 2:e00012–e00011

Botero LM, D'Imperio S, Burr M, McDermott TR, Young M, Hassett DJ (2005) Poly (A) polymerase modification and reverse transcriptase PCR amplification of environmental RNA. Appl Environ Microbiol 71:1267–1275

Bouffaud ML, Kyselkova M, Gouesnard B, Grundmann G, Muller D, Moenne LY (2012) Is diversification history of maize influencing selection of soil bacteria by roots? Mol Ecol 21:195–206

Brandt LJ (2013) Intestinal microbiota and the role of fecal microbiota transplant in treatment of C. difficile infection. Am J Gastroenterol 108:177–185

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320(1–2):37–77

Bulgarelli D, Schlaeppi K, Spaepen S, ver van Loren TE, Schulze LP (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838

Cavaglieri L, Orlando J, Etcheverry M (2009) Rhizosphere microbial community structure at different maize plant growth stages and root locations. Microbiol Res 164:391–399

Chabaud M, Gherbi H, Pirolles E (2015) Chitinase resistant hydrophilic factors secreted by Frankia activate both Ca++ spiking and NIN gene expression in the actinorhizal plant Casuarina glauca. New Phytol 209(1):86–93

Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013) Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One 8:e55731

Chevrot R, Rosen R, Haudecoeur E (2006) GABA controls the level of quorum sensing signal in agrobacterium tumefaciens. Proc Natl Acad Sci U S A 103:7460–7464

Chung EJ, Lim HK, Kim JC, Choi GJ, Park EJ, Lee MH (2008) Forest soil metagenome gene cluster involved in antifungal activity expression in E. coli. Appl Environ Microbiol 74:723–730

Compant S, Clement C, Sessitsch A (2010) Plant growth promoting bacteria in the rhizosphere and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42(5):669–678

Conesa A, Gotz S, Garcia GJM, Terol J, Talon M, Robles M (2005) Blast 2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21 (18):3674–3676

Conrad R, Erkel C, Liesack W (2006) Rice cluster I methanogens, an important group of archaea producing greenhouse gas in soil. Curr Opin Biotechnol 17:262–267

Coronado C, Zuanazzi J, Sallaud C (1995) Alfalfa root flavonoid production in nitrogen regulation. Plant Physiol 108(2):533–542

da Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low nutrient environment. Plant Soil 245:35–47

Da Rocha UN, Andreote FD, De Azevedo JL, Van Elsas JD, Van Overbeek LS (2010) Cultivation of hitherto uncultured bacteria belonging to the Verrucomicriobio subdivision 1 from the potato (Solanum tuberosum L.) rhizosphere. J Soil Sediments 10:326–339

Davis KER, Sangwan P, Janssen PH (2011) Acidobacteria, Rubrobacteridae and Chloroflexi are abundant among very slow growing and mini colony forming soil bacteria. Environ Microbiol 13:798–805

Dawson JO (2007) Ecology of actinorhizal plants. In: Nitrogen fixing actinorhizal symbiosis. Nitrogen fixation: origin, application and research progress, vol 6, pp 199–234

De Angelis KM, Brodie EL, De Santis TZ, Andersen GL, Lindow SE, Firestone MK (2009) Selective progressive response of soil microbial community to wild oat roots. ISME J 3:168–178

De la Pena C, Badri DV, Lei Z (2010) Root secretion of defense related proteins is development dependent and correlated with flowering time. J Biol Chem 286(40):30654–30665

De Weert S, Vermeiren H, Mulders IHM, Kuiper I, Hendrickx N, Bloemberg GV (2002) Flagella driven chemotaxis towards exudates components is an important trait for tomato root colonization by Pseudomonas fluorescens. Mol Plant Microb Interact 15:1173–1180

Delaux PM, Sejalon DN, Becard G (2013) Evolution of the plant microbe symbiotic "toolkit". Trends Plant Sci 18(6):298–304

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72:313–327

Doornbos RF, Geraata BJP, Kuramae EE, Van Loon LC, Bakker P (2011) Effects of Jasmonic acid, ethylene and salicylic acid signaling on the rhizosphere bacterial community of Arabidopsis thaliana. Mol Plant Microb Interact 24:395–407

Dou DL, Zhou JM (2012) Phytopathogen effectors subverting host immunity: different foes, similar battleground. Cell Host Microb 12:484–495

Doyle JJ (1998) Phylogenetic perspectives on nodulation: evolving views of plant and symbiotic bacteria. Trends Plant Sci 3(12):473–478

Eilers KG, Lauber CL, Knight R, Fierer N (2010) Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. Soil Biol Biochem 42:896–903

Ekblom R, Galindo J (2011) Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity 107:1–15

Fasim F, Ahmed N, Parsons R, Gadd GM (2002) Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. FEMS Microbiol Lett 2013:1–6

Field KJ, Cameron DD, Leake JR (2012) Contrasting arbuscular mycorrhizal responses of vascular and non vascular plants to a simulated Palaeozoic CO2 decline. Nat Commun 3:835

Franzosa EA, Morgan XC, Segata N (2014) Relating the metatranscriptome and metagenome of the human gut. Proc Natl Acad Sci U S A 111(22):2329–2338

Galbally IE, Kirstine W (2002) The production of methanol by flowering plants and the global cycle of methanol. J Atmos Chem 43:195–229
Garbeva P, Van Elsas JD, Van Veen JA (2008) Rhizosphere microbial community and its response to plant species and soil history. Plant Soil 302:19–32
Garcia MJ, Acinas SG, Anton AI, Rodriguez VF (1999) Use of the 16S-23S ribosomal genes spacer region in studies of prokaryotic diversity. J Microbiol Methods 36:55–64
Gasper YM, Nam J, Schultz CJ, Lee LY, Gilson PR, Gelvin SB (2004) Characterization of the arabidopsis lysine rich arabinogalactan protein AtAGP17 mutant (rat1) that results in a decreased efficiency of agrobacterium transformation. Plant Physiol 135:2162–2171
Gotz S et al (2008) High throughput functional annotation and data mining with the Blast2GO suite. Nucl Acid Res 36:3420–3435
Grabherr MG, Haas BJ, Yassour M (2011) Full length transcriptome assembly from RNA seq data without a reference genome. Nat Biotechnol 29(7):644–652
Greenblum S, Turnbaugh PJ, Borenstein E (2012) Metagenomic system biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. Proc Natl Acad Sci U S A 109:594–599
Haichar FE, Roncato MA, Achouak W (2012) Stable isotope probing of bacterial community structure and gene expression in the rhizosphere of Arabidopsis thaliana. FEMS Microbiol Ecol 81:291–302
Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685
He SM, Wurtzel O, Singh K, Froula JL, Yilmaz S, Tringe SG (2010) Validation of two ribosomal RNA removal methods for microbial metatranscriptomics. Nat Methods 7:807–812
Hein JW, Wolfe GV, Blee KA (2008) Comparision of rhizosphere bacterial communities in Arabidopsis thaliana mutants for systemic acquired resistence. Microb Ecol 55:333–343
Hewson I, Poretsky RS, Dyhrman ST, Zielinski B, White AE, Tripp HJ (2009) Microbial community gene expression within colonies of the diazotroph, trichodesmium from the Southwest Pacific Ocean. ISME J 3:1286–1300
Holmer R, Rutten L, Kohlen W (2017) Commonalities in symbiotic plant microbe signaling. Adv Bot Res 82:187–221
Hortal S, Pera J, Parladé J (2008) Tracking mycorrhizas and extraradical mycelium of the edible fungus Lactarius deliciosus under field competition with Rhizopogon spp. Mycorrhiza 18:69–77
Hutchison CA, Venter JC (2006) Single cell genomics. Nat Biotechnol 24:657–658
Inceoglu O, Abu Al Sound W, Salles JF, Semenov AV, Van Elsas JD (2011) Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. PLoS One 6:23321
Jiang CJ, Ma GF, Li SX, Hu TT, Che ZQ, Shen PH (2009) Characterization of a novel beta glucosidase like activity from a soil metagenome. J Microbiol 47:542–548
Johnson NC, Jansa J (2017) Mycorrhizas: at the interface of biological, soil and earth science. In: Mycorrhizal mediation of soil. Elsevier, pp 1–6
Jones DJ, Dangl JL (2006) The plant immune system. Nature 444:323–329
Kennedy PG, Hortel S, Bergemann SE, Burns TD (2007) Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. J Ecol 95:1338–1345
Knief C, Delmotte N, Chaffron S, Stark M, Innerebner G, Wassmann R (2012) Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME J 6:1378–1390
Kohler A, Kuo A, Nagy LG (2015) Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutulists. Nat Genet 47(4):410–415
Korf I (2013) Genomics: the state of the art in RNA-seq analysis. Nat Methods 10(12):1165–1166
Lee MH, Hong KS, Malhotra S, Park JH, Hwang EC, Choi HK (2010) A new esterase EstD2 isolated from plant rhizosphere soil metagenome. Appl Microbiol Biotechnol 88:1125–1134
Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW (2006) Archaes predominate among ammonia oxidizing prokaryotes in soils. Nature 442:806–809

Lenton TM, Dahl TW, Daines SJ (2016) Earliest land plants created modern levels of atmospheric oxygen. Proc Natl Acad Sci U S A 113(35):9704–9709
Lesniewski RA, Jain S, Anantharaman K, Schloss PD, Dick GJ (2012) The metatranscriptome of a deep sea hydrothermal plume is dominated by water column methanotrophs and lithotrophs. ISME J 6:2257–2268
Liang P, Pardee AB (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257(5072):967–971
Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522
Liu B, Gibbons T, Ghodsi M, Treangen T, Pop M (2010) Accurate and fast estimation of taxonomic profiles from metagenomic shotgun sequences. BMC Genomics 12:S4
Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S (2012) Defining the core Aradidopsis thaliana root microbiome. Nature 488:86–90
Lutzoni F, Nowak MD, Alfaro ME (2018) Contemporaneous radiations of fungi and plants linked to symbiosis. Nat Commun 5:5451
Maillet F, Poinsot V, Andre O (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469(7328):58–63
Martin F, Kohler A, Murat C (2016) Unearthing the roots of ectomycorrhizal symbiosis. Nat Rev Microbiol 14(12):760–773
Martin F, Uroz S, Barker DG (2017) Ancestral alliances: plant mutualistic symbiosis with fungi and bacteria. Science 356(6340):4501
Martinez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG (2012) Gut microbiome composition is linked to whole grain induced immunological improvements. ISME J 7:269–280
Mathesius U, Watt M (2010) Rhizosphere signals for plant microbe interactions: implications for field grown plants. In: Luttge UE, Beyschlag W (eds) Progress in botany, vol 72, pp 125–161
McCarren J, Becker JW, Repeta DJ, Shi YM, Young CR, Malmstrom RR (2010) Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc Natl Acad Sci U S A 107:16420–16427
McGrath KC, Thomas HSR, Cheng CT, Leo L, Alexa A, Schmidt S (2008) Isolation and analysis of mRNA from environmental microbial communities. J Microbiol Methods 75:172–176
McLean JS, Lombardo MJ, Badger JH, Edlund A, Novotny M, Yee GJ (2013) Candidate phylum TM6 genome recovered from a hospital sink biofilm provides genomic insights into this uncultivated phylum. Proc Natl Acad Sci U S A 110:E2390–E2399
Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM (2011) Deciphering the rhizosphere microbiome for disease suppressive bacteria. Science 332:1097–1100
Micallef SA, Shiaris MP, Colon CA (2009) Influence of Arabidopsis thaliana accessions on rhizobacterial communities and natural variations in root exudates. J Exp Bot 60:1729–1742
Mirete S, De Figueras CG, Gonzalez Pastor JE (2007) Novel nickel resistance genes from the rhizosphere metagenome of plants adapted to acid mine drainage. Appl Environ Microbiol 73:6001–6011
Moran MA, Satinsky B, Gifford SM, Luo HW, Rivers A, Chan LK (2013) Sizing up metatranscriptomics. ISME J 7:237–243
Morandi D, Bailey J, Gianinazzi PV (1984) Isoflavonoid accumulation in soybean roots infected with vesicular arbuscular mycorrhizal fungi. Physiol Plant Pathol 24(3):357–364
Morgan XC, Huttenhower C (2014) Metagenomic analytic techniques for studying the intestinal microbiome. Gastroenterology 146(6):1437–1448
Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 5:621–628
Mueller RS, Pan C (2013) Sample handling and mass spectrometry for microbial metaproteomic analyses. Methods Enzymol 531:289e303
Mukerji KG, Manoharachary C, Singh J (2006) Microbial activity in the rhizosphere. Springer, New York, p 7

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H (1986) Specific enzymatic amplification of DNA in vitro – the polymerase chain reaction. Cold Spring Harbor Symp Quant Biol 51:263–273

Muyzer G, Dewaal EC, Uitterlinden AG (1993) Profiling of complex microbial-populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16S ribosomal RNA. Appl Environ Microbiol 59:695–700

Nadarajah K (2016) Induced systemic resistance in rice. In: Choudhary KD, Varma A (eds) Microbial-mediated induced systemic resistance in plants. Springer, Singapore, pp 103–124

Narberhaus F, Vogel J (2009) Regulatory RNAs in prokaryotes: Here, there and everywhere. Mol Microbiol 74:261–269

Navarro RA, Xu HAO, Kemppainen M (2015) Laccaria bicolor aquaporin LbAQP1 is required for Hartig net development in trembling aspen (Populus tremuloides). Plant Cell Environ 38 (11):2475–2486

Nguema OE, Vicre GM, Cannesan MA (2013) Arabinogalactan proteins in root – microbe interactions. Trends Plant Sci 18(8):440–449

Oldroyd GED, Murray JD, Poole PS, Downie JA (2011a) The rules of engagement in the legume-Rhizobial symbiosis. Ann Rev Genet 45:119–144

Oldroyd GED, Murray JD, Poole PS et al (2011b) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45(1):119–144

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL (2013) Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc Natl Acad Sci U S A 110:6548–6553

Pinto AJ, Raskin L (2012) PCR biases distort bacterial and archaeal community structure in pyrosequencing datasets. PLoS One 7:e43093

Pressel S, Bidartondo MI, Ligrone R et al (2014) Fungal symbioses in bryophytes: new insights in the twenty first century. Phytotaxa 9(1):238–253

Qiao Q, Wang F, Zhang J, Chen Y, Zhang C, Liu G (2017) The variation in the rhizosphere microbiome of cotton with soil type, genotype and developmental stage. Sci Rep 7:3940

Raaijmakers JM, de Bruijn I, Nybroe O (2010) Natural functions of lipopeptides from Bacillus and Pseudomonas: more than surfactants and antibiotics. FEMS Microbiol Rev 34(6):1037–1062

Ramachandran VK, East AK, Ramakrishnan K, Downie JA, Poole PS (2011) Adaptation of rhizobium leguminosarum to pea, alfalfa and sugar beet rhizospheres investigated by comparative transcriptomics. Genome Biol 12:R106

Raychoudhury R, Sen R, Cai Y, Sun Y, Lietze VU, Boucias DG et al (2011) Comparative metatranscriptomic signatures of wood and paper feeding in the gut of the termite Reticulitermes flavipes (Isoptera: Rhinotermitidae). Insect Mol Biol 22:155–171

Reinhold HB, Bunger W, Burbano CS, Sabale M, Hurek T (2015) Roots shaping their microbiome: global hotspots for microbial activity. Annu Rev Phytopathol 53:403–424

Remigi P, Zhu J, Young JPW (2016) Symbiosis within symbiosis: evolving nitrogen-fixing legume symbionts. Trends Microbiol 24(1):63–75

Rinku D, Suman C, Anju K, Rakesh K, Sneh G (2020) Symbiotic signaling: insights from arbuscular mycorrhizal Symbiosis. Plant Microbe Symb:75–103

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD (1996) Systemic acquired resistance. Plant Cell 8:1809–1819

Saharan B, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21:1–30

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74:5463–5467

Saravanan VS, Madhaiyan M, Thangaraju M (2007) Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium Gluconacetobacter diazotrophicus. Chemosphere 66:1794–1798

Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13:2844–2854

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270(5235):467–470
Shaffer JP, Uren JM, Gallery RE (2017) An endohyphal bacterium (Chitinophaga, Bacteroidetes) alters carbon source use by Fusarium keratoplasticum (F. solani species complex, Nectriaceae). Frontiers in. Microbiology 8:350
Shagina I, Bogdanova E, Mamedov IZ, Lebedev Y, Lukyanov S, Shagin D (2010) Normalization of genomic DNA using duplex-specific nuclease. BioTechniques 48:455–459
Shakya M, Quince C, Campbell JH, Yang ZMK, Schadt CW, Podar M (2013) Comparative metagenomic and rRNA microbial diversity characterization using archaeal and bacterial synthetic communities. Environ Microbiol 15:1882–1899
Shi SJ, Richardson AE, O'Callaghan M, DeAngelis KM, Jones EE, Stewart A (2011) Effects of selected root exudate components on soil bacterial communities. FEMS Microbiol Ecol 77:600–610
Shirley B, Alejandra V-L, Fernanda CG (2015) Combining metagenomics, metatranscriptomics and viromics to explore novel microbial interactions: towards a systems-level understanding of human microbiomes. Comput Struct Biotechnol J 13:390–401
Singh BK, Millard P, Whiteley AS, Murrell JC (2004) Unravelling rhizosphere-microbial interactions: opportunities and limitations. Trends Microbiol 12:386–393
Singh BK, Bardgett RD, Smith P, Reay DS (2010) Microorganisms and climate change: terrestrial feedbacks and mitigation options. Nat Rev Microbiol 8:779–790
Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier, Academic Press, Amsterdam
Spatafora JW, Chang Y, Benny GL (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108(5):1028–1046
Spoel SH, Dong XN (2012) How do plants achieve immunity? Defence without specialized immune cells. Nat Rev Immunol 12:89–100
Spor A, Koren O, Ley R (2011) Unravelling the effects of the environment and host genotype on the gut microbiome. Nat Rev Microbiol 9:279–290
Stewart FJ, Ulloa O, DeLong EF (2012) Microbial metatranscriptomics in a permanent marine oxygen minimum zone. Environ Microbiol 14:23–40
Strullu DC, Kenrick P, Pressel S (2014) Fungal associations in Horneophyton ligneri from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: novel insights into ancestral plant-fungus symbioses. New Phytol 203(3):964–979
Strullu DC, Selosse MA, Kenrick P (2018) The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. New Phytol 220:1012–1030
Stursova M, Zifcakova L, Leigh MB, Burgess R, Baldrian P (2012) Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiol Ecol 80:735–746
Takasaki K, Miura T, Kanno M, Tamaki H, Hanada S, Kamagata Y (2013) Discovery of glycoside hydrolase enzymes in an avicel adapted forest soil fungal community by a metatranscriptomic approach. PLoS One 8:e55485
Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263
Temperton B, Field D, Oliver A, Tiwari B, Muhling M, Joint I (2009) Bias in assessments of marine microbial biodiversity in fosmid libraries as evaluated by pyrosequencing. ISME J 3:792–796
Tett AJ, Turner TR (2012) Poole PS, Genomics and the rhizosphere. eLS. Wiley
Torsvik V, Ovreas L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5:240–245
Turner T, Ramakrishnan K, Walshaw J, Heavens D, Alston M, Swarbreck D (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME J 7:2248–2258
Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428:37–43

Ueda T, Suga Y, Yahiro N, Matsuguchi T (1995) Remarkable N2-fixing bacterial diversity detected in rice roots by molecular evolutionary analysis of nifH gene-sequences. J Bacteriol 177:1414–1417

Urich T, Lanzen A, Qi J, Huson DH, Schleper C, Schuster SC (2008) Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 3:e2527

Uroz S, Buee M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2:281–288

Ursell LK, Knight R (2013) Xenobiotics and the human gut microbiome: metatranscriptomics reveal the active players. Cell Metab 17:317–318

Van der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

Van Elsas JD, Chiurazzi M, Mallon CA, Elhottova D, Kristufek V, Salles JF (2012) Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc Natl Acad Sci 109:1159–1164

Van Nguyen T, Pawlowski K (2017) Frankia and actinorhizal plants: symbiotic nitrogen fixation. In: Rhizotrophs: plant growth promotion to bioremediation microorganisms for sustainability, vol 2. Springer, Singapore, pp 237–261

Vandenkoornhuyse P, Quaiser A, Duhamel M (2015) The importance of the microbiome of the plant holobiont. New Phytol 206(4):1196–1206

Venturi V, Keel C (2016) Signaling in the rhizosphere. Trends Plant Sci 21(3):187–198

Vila-Costa M, Sharma S, Moran MA, Casamayor EO (2013) Diel gene expression profiles of a phosphorus limited mountain lake using metatranscriptomics. Environ Microbiol 15:1190–1203

Wagg C, Bender SF, Widmer F, Van der Heijden MGA (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc Natl Acad Sci U S A 111:5266–5270

Wang H, Moore MJ, Soltis PS, Bell CD, Brockington SF, Alexandre R, Davis CC, Latvis M, Manchester SR, Soltis DE (2009) Rosid radiation and the rapid rise of angiosperm-dominated forests. Proc Natl Acad Sci U S A 106:3853–3858

Wang Y, Hayatsu M, Fujii T (2012) Extraction of bacterial RNA from soil: challenges and solutions. Microbes Environ 27:111–121

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629–1633

Weinberg Z, Perreault J, Meyer MM, Breaker RR (2009) Exceptional structured noncoding RNAs revealed by bacterial metagenome analysis. Nature 462:656–659

Werner GDA, Cornwell WK, Sprent JI et al (2014) A single evolutionary innovation drives the deep evolution of symbiotic N2-fixation in angiosperms. Nat Commun 5:1–9

Williams SCP (2013) The other microbiome. Proc Natl Acad Sci U S A 110:2682–2684

Willmann R, Lajunen HM, Erbs G (2011) Mediate bacterial peptidoglycan sensing and immunity to bacterial infection. Proc Natl Acad Sci U S A 108(49):19824–19829

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271

Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms - proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A 87:4576–4579

Wu M, Eisen JA (2008) A simple, fast and accurate method of phylogenomic inference. Genome Biol 9:151

Xiao CL et al (2014) FANSe2: a robust and cost-efficient alignment tool for quantitative next-generation sequencing applications. PLoS One 9:e94250

Xie F, Williams A, Edwards A, Downie JA (2012) A plant arabinogalactan like glycoprotein promotes a novel type of polar surface attachment by rhizobium leguminosarum. Mol Plant-Microbe Interact 25:250–258

Xiong X, Frank DN, Robertson CE, Hung SS, Markle J, Canty AJ (2012) Generation and analysis of a mouse intestinal metatranscriptome through Illumina based RNA-sequencing. PLoS One 7: e36009

Yao J, Allen C (2006) Chemotaxis is required for virulence and competitive fitness of the bacterial wilt pathogen Ralstonia solanacearum. J Bacteriol 188:3697–3708

Yi H, Cho YJ, Won S, Lee JE, Yu HJ, Kim S et al (2011) Duplex-specific nuclease efficiently removes rRNA for prokaryotic RNA-seq. Nucleic Acids Res 39:e40

Zhao J, Hyman L, Moore C (1999) Formation of mRNA 3'ends in eukaryotes: mechanism, regulation, and interrelationships with other steps in mRNA synthesis. Microbiol Mol Biol Rev 63:405–445

Zhuang X, Gao J, Ma A (2013) Bioactive molecules in soil ecosystems: masters of the underground. Int J Mol Sci 14(5):8841–8868

Zhulidov PA, Bogdanova EA, Shcheglov AS, Vagner LL, Khaspekov GL, Kozhemyako VB (2004) Simple cDNA normalization using Kamchatka crab duplex-specific nuclease. Nucleic Acids Res 32:15

Chemical Signal Dissemination Through Infochemicals

4

Randeep Kumar, Chandini, Ravendra Kumar, Om Prakash, Rakesh Kumar, and A. K. Pant

Abstract

Every species has its own way of communication with diverse range of complexity that is relatively simplified in case of higher organisms while relatively complex in lower organisms like insects. Infochemical mediated chemical signal dissemination is very unanimous mode of communication in insects. Inability of lower organisms to emit acoustic signals enables them to adopt the infochemical mediated communication. Infochemicals comprise all forms of chemicals involved in communication process whether it is intraspecific or interspecific. Chemicals responsible for intraspecific communication are termed as pheromones while it is allelochemicals in case of interspecific communication. Infochemicals based communication has procured its efficiency in various environmental conditions such as rocks, aquatic, soil, and air, etc. Chemically these infochemicals represent diverse range of functional groups such as hydrocarbons, proteins, peptides, lactones, terpenes, amino acids, carbohydrates, lipids, phenolics, etc. A number of signal emitters and receptors are present in insects regulated by a collection of genes for its better effectiveness. Chemical diversity and the gene regulated signal dissemination are the two factors responsible for specificity of the infochemical communication. These infochemicals regulate a

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range of social functions in insects such as mating, aggregation, trailing, alarming, protection from enemies, aphrodisiac, etc. Also, utilization of these infochemicals is an alternate way of providing the pathways for insect management by mating disruption, mass trapping, monitoring of pest infestation, mass annihilation, etc. Thereby, these infochemicals can be an important component of sustainable management of insect pests and also Integrated Pest Management (IPM).

Keywords

Infochemicals · Pheromones · Allelochemicals · Infochemical effect · Pest management

4.1 Introduction

Infochemicals stands for the communication or sharing of information through the use of some chemical signals or cues in some small organisms or insects. Every organism has its own way of communication by the use of acoustic signals of some optimal frequency but this is not so in the case of minor organisms so they intended the use of some chemical compounds to communicate and to perform their activities. Often the term semiochemicals is confused with the infochemicals. Communication mediated through chemicals signals dissemination most often collectively termed as semiochemicals but nowadays it is advised to use the term infochemicals instead of semiochemicals to be more appropriate. Infochemicals represent the sharing of information among the individuals of same or different species resulting in the physiological or behavioral changes in either one or both the sender and receiver species. The behavioral changes represent the stimulation or inhibition of a behavior pattern on the receipt of any stimuli and hence regulation of the expressions (Foster and Harris 1997).

4.1.1 Infochemical Effect

Sometimes infochemical transmission of signals gets interrupted with the anthropogenic substances and also may interfere with the communication process. This is because of the fact that the chemical cues responsible for possible communication in insects may be identical as the chemical released from humans. So the interruption of chemical cues of some minor organism by anthropogenic sources is called as infochemical effect (Klaschka and Kolossa-Gehring 2007; Klaschka 2008). The infochemical effect can have various effects on an individual that may be lethal or sublethal irrespective of toxicity of the infochemicals. When the infochemical effect leads to mating disruption of a species it is sublethal, while when the effect leads to non-recognition of the predator it can have lethal effect.

4.1.2 Adaptability of Infochemicals

There are several stimuli that persist in the environment that evokes various chemical signal transmission and also responsible for biological activities of any population. Even a single organism receives overplus of stimuli every day. These stimuli are responsible for the survival and existence of organisms. These chemical stimuli or cue has adapted to be superior over other sources of stimuli such as acoustic signals.

This infochemical signaling persists in a wide range of environmental conditions such as in aqueous environment, in the air, in the rocks, etc. Also, these signals transverse in the wave manner and are independent of the presence of the light. These signals are not so effective in case of higher organisms like humans while very much effective in case of lower organisms such as insects. The effectiveness of these chemical signals may be attributed to the highly developed olfactory or chemo-receptors in the insects which is not so adapted in case of higher organisms. These stimuli after emission from its source get significantly reduced after traveling towards long distance receiver organisms. In other words, it can be stated that the concentration of the chemical stimuli gets reduced over distance. These signals lasts more than the sound time range but not more than those of the morphological changes and possess substantial time limit. Also, it possesses the intermediate spatial limit that is lower than the penetrating range of the sound waves but more than the range of touch and taste. These effects are due to the degradation of compound responsible for chemical signaling. In fact, infochemical communication is not the one to one communication as it is more effective when there are a large numbers of olfactory receptors, more numbers of chemical compounds present in the signal, and the odor qualities too. The universality of the infochemical communication being that it does not require any physical signal to be transformed into biochemical signal.

4.2 Classification of Infochemicals

Primarily the broad category infochemicals are classified as per its function which distinguishes the infochemical into two groups that are pheromones and allelochemicals. Again, the pheromones are classified based on its intended function that is the chemical employed in various uses such as mating attraction, danger alarming, social aggregation, marking territory, etc. Also, a category of infochemicals that is allelochemicals are distinguished into three sections that are kairomones, allomones, and synomones. Based on the chemical compounds falling under both the category, allelochemicals comprise the much broader group than that of the pheromones (Wyatt 2010; Law and Regnier 1971; Regnier and Law 1968; Dicke and Sabelis 1988; Nordlund and Lewis 1976).

4.2.1 Pheromones

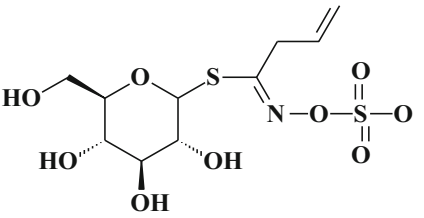
Pheromone word has been derived from two Greek words "*pherein*" and "*horman*" which mean to carry and to excite, respectively. Pheromones are defined as those substances which are released outside the body unlike the hormones which are released inside the body and also these pheromones are meant to be utilized by the individuals of the same species (Karlson and Lüscher 1959). Basically pheromones fall under the class of intraspecific communication. Several classes of compounds secreted by various insects fall under this category such as bombykol (*Bombyx mori*), gyptol (*Porthetria dispar*), gyplure (*Porthetria dispar*), 9-oxodecenoic acid (*Apis mellifera*), 9-hydroxydec-trans-2-enoic acid, isopentyl acetate (honey bee), methyl-n-pentyl ketone (*Iridomyrmex pruinosus*), propyl isobutyl ketone (*Tapinoma* sp.), citral (*Atta sexdens rubropilosa*), citronellal (*Acanthomyops claviger*), a furan, dendrolasin (*Lasius fuliginosus*), 2,α-dimethyl-3-isopropylidene-cyclopropyl propionate (*Periplaneta americana*) (Jacobson et al. 1960; Gary 1962, 1963; Callow and Johnstonn 1960; Butler et al. 1964; Chadha et al. 1962; Blum et al. 1963; Wilson and Pavan 1959; Boch et al. 1962; Wharton et al. 1963). Chemical structures of some of the important allelochemicals along with their producing species have been illustrated in Fig. 4.1.

4.2.1.1 Trailing Pheromones

Most of the chemical substances used for marking are meant for terrestrial or trail marking and these substances are used by the individuals of same species having well-developed olfactory or gustatory organs. These substances are generally secreted on the ground or over other substrate towards which the insects are directed to crawl. These substances being identified intraspecifically based on the polarity or the chemical constituents of the pheromones released (MacGregor and Thorpe 1948; Carthy 1951). These types of pheromones are observed in several insects such as *Eciton hamatum* (F.), *Myrmelachista ramulorum* (Wheeler) and *Paratrechina longicornis* (Latreille), *Lasius fuliginosus*, *Atta texana* (Buckley) and *Tetramorium guineense* (F.); *Solenopsis saecissima* (Blum and Portocarrero 1964; Blum and Wilson 1964; Carthy 1951; Moser and Blum 1963; Blum and Ross 1965; Wilson 1959).

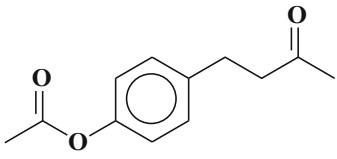
4.2.1.2 Aggregation Pheromones

Many of the insect species need to form aggregation for many objectives such as hibernation, estivation, mating, protection from predators, etc. These insects may belong to several orders such as hemiptera, ephemeroptera, isoptera, hymenoptera, etc. These social gathering may occur temporarily or permanently as per the need of the insect species. In general social insects like ants, honey bee require permanent aggregation. Colonies of honey bees are the part of permanent or persistent aggregation while aggregation of mating purposes is a temporary type. Pheromones released for such aggregations may be secreted by any sex of that species or even may be by both the sexes depending on the species to species and also may be occasional or several times as per requirements of a particular species (Caspary and



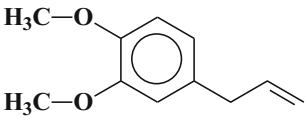
Sinigrin

(***Pieris brassicae***)



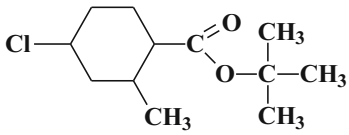
Cue lure

(***Bacterocera cucurbitae***)



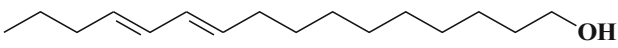
Methyl eugenol

(***Bactrocera dorsalis***)



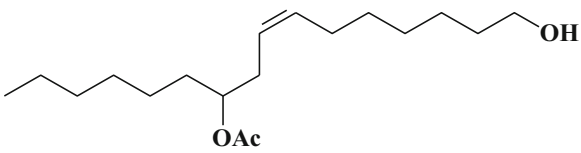
Trimedlure

(***Ceratis capitata***)



Bombykol

(***Bombyx mori***)



Gyptol

(***Portheria dispar***)

Fig. 4.1 Chemical structures of some of the important pheromones along with their producing species

Downe 1971; Spieth 1940; Nixon and Ribbands 1952). These types of pheromones are observed in several insects such as *Vespula* spp., *Trigona iridipennis*, *Polistes* sp., *Andrena flavipes*, *Schistocerca gregaria*, *Semiadalia undecimnotata*,

Dendroctonus frontalis (Morimoto 1960; Butler 1965; Norris and Richards 1963; Hodek 1960).

4.2.1.3 Alarm Pheromones

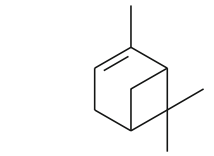
Many insect species utilize various means to ensure its security from its enemies or predators. Some of the species exudates some species-specific secretions which act as an alarm to the whole species population. Many a times such secreted substances were not referred to as the pheromones as they do not induce any behavioral changes in the alarmed members of that species. But also in some cases like ant species *Formica rufa* secreting formic acid in order to save the other members from predators were called as pheromones. Since, these secretions are mainly utilized in protection purposes therefore the alerting substances would be more appropriate than that of the alarming substances (Maschwitz 1964a; Wilson 1963, 1965). These types of pheromones are observed in several insects such as *Vespula germanica* (F.), *V. vulgaris* (L.), *Ponera coarctata*, *Myrmecina graminicola*, *Bombus lucorum, B. hortorum* (L.), *B. hypnorum* (Maschwitz 1964a, b; Moore 1964; Stuart 1963).

4.2.1.4 Sex Attractant Pheromones

Sex attractant scents or lures are usually produced by females and attract males exclusively. However, there are a few species in which the roles of the sexes are reversed in this respect and the males produce scents that attract the females. Aggregations of insects for sexual reproduction sometimes result from one or other of the sexes emitting an olfactory pheromone that attracts both sexes. Olfactory sex attractants serve to bring male and female insects that are out of sight of one another close enough for visual and tactile attractants to come into play. Although many, probably most, olfactory sex attractants are species-specific, there are enough exceptions known to show that such specificity is not essential to prevent indiscriminate cross-mating. Even with closely related species occupying the same general habitat, interspecific mating is unusual in nature (although less so in the artificial conditions of the laboratory), probably because of slight but important differences in courtship behavior. These types of pheromones are observed in several insects such as *Anthonomus grandis*, Belostoma indica, *Harpobittacus australis*, *H. nigriceps*, *Hepialus hectus* (L.), *Leucophaea maderae*, *Lasius neoniger, L. alienus*, and *Acanthomyops claviger* (Law et al. 1965; Richards 1927; Engelmann 1965; Bornemissza 1964; Keller et al. 1964; Butenandt and Tam 1957).

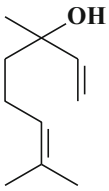
4.2.2 Allelochemicals

Unlike pheromones, allelochemicals comprise the infochemical communication in between the individuals of different species. Comparatively, allelochemicals comprise more number of compounds than that of the pheromones. Allelochemicals are classified on the basis of species that benefitted of the communication like kairomones in which recipient species is benefitted, allomones in which the emitting species is benefitted, and synomones in which both the emitter and recipient species



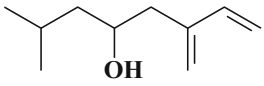
α-Pinene

(*Dendroctonus brevicomis*)



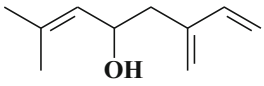
Linalool

(*Ips pini*)



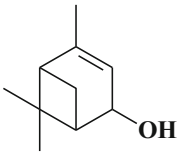
Ipsenol

(*Ips paraconfusus*)



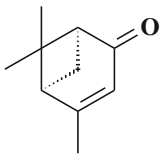
Ipsdienol

(*Ips paraconfusus, Ips pini*)



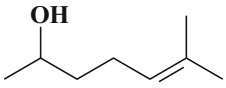
Cis-verbenol

(*Ips paraconfusus, Ips pini*)



Verbenone

(*Dendroctonus brevicomis*)



Sulcatol

(*Gnathotrichus sulcatus*)

Fig. 4.2 Chemical structures of some of the important allelochemicals along with their producing species

are benefitted (Karlson and Lüscher 1959; Wyatt 2010; Nordlund and Lewis 1976). Chemical structures of some of the important allelochemicals along with their producing species have been illustrated in Fig. 4.2.

4.2.2.1 Kairomones

The term kairomones have been derived from a Greek word *Kairos* which means opportunistic. The kairomones stands for the type of chemical cues responsible for

the chemical communication in between two different species where the responding species is benefitted. These type of secretions are released by the plants, insects, and even by the higher animals like humans also. As lactic acid constituents in the human sweat acts as an attractant to *Aedes aegypti*, helpful for their feeding. Even the secondary metabolites produced by the plant can act as chemical cues to the herbivore which stimulates them for aggregation and also indicates a site of food source. An insect that is American bolas spiders release chemicals to attract male moths prey which indirectly acts as a sex pheromones to the female moths (Raji et al. 2019; Torto 2009).

4.2.2.2 Allomones

The term allomones have been derived from two Greek words *allos* + *hormon* which mean to excite others. In this type of chemical cues, chemical released by one organism induces a specific response by the other organism of some different species than the sender species while the communication attained being favorable to the sender species. Most of the time these allomones act as defensive secretions as it is poisonous, noxious, or deterrent to the predators. Many of the plant constituents have been identified to be antifeeding, insecticidal, deterrent to the pest species. Sometimes the plants release its secondary metabolites such as alkaloids, cardiac glycosides, cyanogenic glycosides, etc. which acts defensive to the phytophagous insects (Torto 2009; Kumar et al. 2019a, b; Wink 2018).

4.2.2.3 Synomones

In this type of chemical communication chemical released by emitter species is recognized and responded by the recipient individual of some different species while the communication being favorable to both the emitter and recipient species. The chemical communication regulates the specific reaction to the specific chemical release. The infochemical release by the *Ips paraconfusus* constitutes cis-verbenol, ipsenol, and ipsdienol, while in case of *Ips pini* it is linalool, cis-verbenol, and ipsdienol. While sharing the common habitat, ipsenol acts as repellent to the *Ips pini*, while linalool acts as repellent to the *Ips paraconfusus* so as to avoid competition for habitat or food (Andersson 2012; Torto 2009).

4.3 Chemical Nature of Infochemicals

It is the chemical specificity of the infochemical that enables its effectivity at a very low concentration. It requires a specific concentration of the chemical cues that is in between the high tissue concentration and low background concentration. A very minimal amount of around nanomolar concentration is required for its effective communication. Sometimes, an effective biological activity is achieved even when there is no peak observed in Gas Chromatography (GC). A vast diverse range of chemical group constituents present in chemical cues responsible for infochemical communication involves carbohydrates, proteins, lipids, hydrocarbons, aldehydes, alcohols, glycopeptides, oligosaccharides, carbonic acids, lactones, phenolics,

flavonoids, steroids, terpenoids, etc. Apart from the diverse functional groups, stereochemistry and the chirality of the chemical cues are also responsible for its specificity. In general the infochemical cues are a complex mixture of various chemicals. A sender releases a specific complex mixture of infochemicals at a very specific low concentration which is very essential to stimulate recipient receptor and hence the effective communication is achieved. Both the qualitative and quantitative compositions are critical for the infochemical communication to be specific and effective (Klaschka 2008; Rasmussen et al. 2003; Larcher 2003; Boller 1995; Zou and Buck 2006).

4.4 Function of Infochemicals

There are broad ranges of functions that prevail influencing insect's behavior depending on the species to species. Usually multitude of chemical cues exists that is the one chemical stands for more than one messages. Following are the most common functions identified resulting of the chemical release and detection such as:

• Aphrodisiac	• Social hierarchy
• Attractants	• Search for food
• Anti-aphrodisiacs	• Search for ovipositional sites
• Aggregation	
• Alarm	• Protection from predators and parasitoids
• Defense	
• Mating	

Some of these functions associated with the specific chemicals released by the insect species have been listed in Table 4.1.

4.5 Infochemicals Communication

As stated earlier the infochemical communication is very effective in lower organisms like insects due to highly active olfactory organs preset in them much more than that of the higher animals. The infochemical communication specificity is based on the release of structural specific chemicals by the sender and thereby the capability of the receiver to detect the chemical released. These release and detection based communication may be either intraspecific just like as pheromones or inter-specific like as allelochemicals and also may be medium to long time or spatial ranges.

Just like other ways of communication, infochemical communication too requires three components: one is the sender responsible for release or encoding the signal, second one is the receptor where the signal is detected based on the specificity of the receptors, and the third one is the response through decoding of the signal and thereby appropriate action. Sender releases a specific type of chemicals in specific

Table 4.1 Some important infochemicals responsible for crucial function in the insects

S. No.	Function	Insects	Infochemicals	References
1.	Aphrodisiac	*Lygus hesperus*	Myristyl acetate	Brent et al. (2017)
		Drosophila melanogaster	Cis-vaccenyl acetate, Heptacosadiene, Nonacosadiene	Laturney and Billeter (2016), Yew et al. (2009)
2.	Anti-aphrodisiac	*Heliconius melpomene*	(E)-β-Ocimene, Hexyloctadecenoate, Heneicosane	Schulz et al. (2008), Darragh et al. (2019)
3.	Defense	Amazon Azteca ants	6-Methyl-5-hepten-2-one-2,3-butadione, Acetoin	Jardine et al. (2020)
		Dolichovespula maculata	Dimethylaminoethanol, 2,5-dimethylpyrazine, 2-heptadecanone	Jimenez et al. (2016)
		Leptopilina ryukyuensis	(-)-Iridomyrmecin	Böttinger et al. (2019)
4.	Aggregation	*Cimex lectularius*	(2E, 4E)-Octadienal, (E)-2-Hexenal, Nonanal, Decanal	Liu et al. (2017)
		Tribolium confusum	1-Tetradecene	Kheloul et al. (2019)
		Murgantia histrionica	Murgantiol	Lancaster et al. (2018)
5.	Alarm	*Solenopsis invicta Apis mellifera Rhopalosiphum padi*	2-Ethyl-3,6-dimethylpyrazine, Isopentyl acetate, E-β-Farnesene	Du et al. (2019)
		Vespa velutina	Undecen-2-one, Undecene-2,10-dione	Cheng et al. (2017)
		Halyomorpha halys	(E)-2-Decenal	Zhong et al. (2018)
6.	Mating	*Anoplophora glabripennis*	(3R,5S)-3,5-Dimethyldodecanoic acid	Hanks and Millar (2016)
		Sitotroga cerealella	(7Z,11E)-Hexadecadien-1-ol acetate	Ma et al. (2016)
		Monochamus saltuarius	2-Undecyloxy-1-ethanol, Ipsenol, Ipsdienol, Limonene	Lee et al. (2017)
7.	Search for oviposition site	*Lobesia botrana, Eupoecilia ambiguella*	Linalool oxide, Cumene, (-)-Perillaldehyde, (R/S)-Limonene	Markheiser et al. (2020)
		Rhynchophorus ferrugineus	Oleic acid	Mazza et al. (2016)
8.	Search for food	*Trogoderma variabile, Trogoderma granarium*	Myristic acid, Palmitic acid, Stearic acid	Morrison et al. (2020)

situations in order to produce a particular effect. These signals are very specific and vary as per the response required and depend on the type of receiver organisms (interspecific/intraspecific communication). The specificity and effectiveness of the infochemical communication are based on the structural specificity or the chemical group of the infochemicals released by the sender and also based on the genes involved in the functioning of the receptor present in receiver organisms. These signals after release get diffused in the environment and should contact the receptors present in the receiver organisms for the appropriate action. These receptors are also very specific in nature as it only detects the specific signals only. And the specificity of the receptors is achieved by the presence of the odorant binding proteins in the olfactory receptors. These proteins on reception of the signal undergo configurational changes in order to produce a particular electrophysiological response. These electrophysiological changes get transmitted to the brain for the perception of the odor and thereby the receiver reaction occurs. After perception of the signal these signals needed to be discontinued so these stimuli get disintegrated by the degrading enzymes present in the receiver organisms (Freitag et al. 1998; Young and Trask 2002; Zhao and Firestein 1999; Ferrari et al. 2007).

In general three different proteins are involved in the perception of any signal such as odor binding proteins, odor receptor proteins, and the odor degrading enzyme proteins. In a study it was revealed that there are a total of 57 genes responsible for encoding the receptor proteins in *Drosophila melanogaster*. For an effective communication to be achieved, complex formation between the odorant and the odorant binding proteins is mandatory. Infochemical communication comprises the formation of a dynamic communication web consisting of a varied number of signals, released multiple times having broad ranges of action and also released at various concentrations to produce multiple signals for the receivers. Even after the complexity of the communication web the effective communication is attributed to the presence of large number of genes involved in the specific recognition of the signal with proper recognition and appropriate response. Just like the diversity in the signals released by the sender, the responses to an infochemical signal are also diversified. In fact, the interspecific and intraspecific communication can be achieved simultaneously. Just like an appropriate signal consists of several stimuli, an appropriate response also composed of multiple reactions as per the requirement (Field et al. 2007; Polya 2003; Klaschka 2008). Schematic diagram representing infochemical communication in insects has been illustrated in Fig. 4.3.

4.6 Uses of Infochemicals in Insect Pest Management

Infochemicals are basically used for the communication among the lower organisms mainly for their own purposes like defense mechanism, aggregation, trail marking but still can be basis for pest management practices when utilized in a proper way such as monitoring and detection, mass trapping and annihilation and mating disruption as well (Suckling 2000; Byers 2014; El-Sayed et al. 2006).

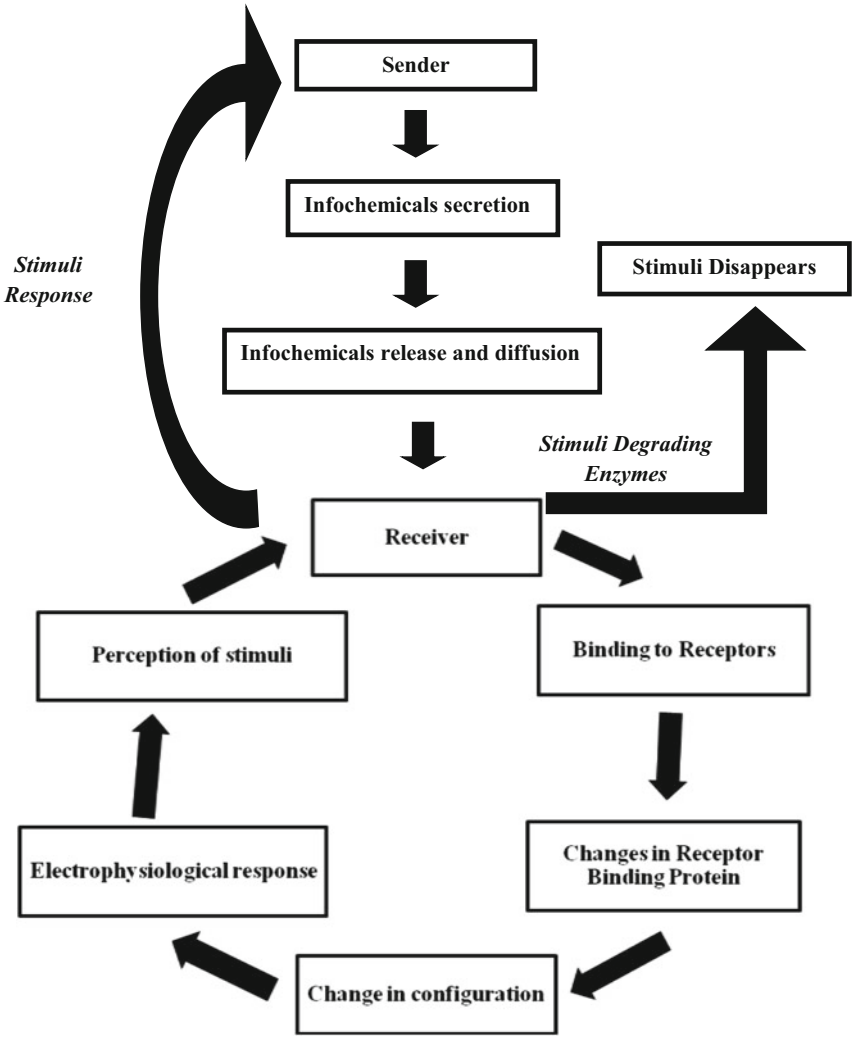


Fig. 4.3 Schematic diagram representing the infochemical communication pathway

4.6.1 Monitoring and Detection

Pheromones are the chemical substances that are secreted and detected by the organisms of same species for their intraspecific communication. Purpose of insect pest monitoring at an early stage requires the installment of pheromones incorporated traps at various places of the fields. In fact, the newly infested fields should be monitored and detected prior to the deployment of management practices. Prior forecasting of the pests is very much required for identification of the pest species before their establishment in the field and also for the preparation of specific

pheromones to be used. Pest detection using pheromones is an eco-friendly approach of the pest management practices. Pheromones approach is a highly sensitive method as it can be used to be effective at a very low concentration and is able to monitor a wide range of insects from large to small insects. Also, this approach is found to be highly effective against the insects which have attained resistance against other management practices. Mostly effective pheromones have been discovered from coleopteran pests (Wall et al. 1987; El-Sayed et al. 2006; Kumar 2016; Liebhold and Tobin 2008; Pereyra and Sánchez 2006).

4.6.2 Mass Trapping

Mass trapping is a unique strategy involving lure, trapping, and killing a large mass of insects at a particular place in the field. Usually a device incorporated with pheromones in it is installed to lure and to concentrate most of the pest species at a particular place in or outside the field and thereby the application of the insecticidal efficient components to kill the pest masses collectively. In general, sex pheromones are usually used for this purpose due to its higher efficiency and also to control all the male individuals of that particular insect species. Doing this, it enables the control of one mating individuals for further inhibition of egg production and also for the decrement of pest population in the field. Quantity of the traps to be installed depends on the level of infestation. Generally, `20–25 traps per ha are used in the green house condition and 40–50 traps per ha are used in the field conditions. This method of pest management approach is also the efficient, eco-friendly and the cost-effective approach of pest management. Only the cost involved is the use of insecticides which can be remediated by the use of approach alternative to synthetic chemicals use such as use of plant extracts, essential oils, biological control agents, and other cultural practices having insecticidal, antifeeding efficacy (Bolckmans 2009; Giblin-Davis et al. 1996; Kumar 2016; Oehlschlager et al. 2002).

4.6.3 Mating Disruption

Mating disruption is a method involving the false alarm to the mating population and also to deviate them to mate other than the infestation to the crops. In this method usually the female sex pheromones are used and are placed outside the place to attract all the male species away from the field attracted towards the false sex alarm and gets devoid of mating. Furthermore, there is no egg laying and the pest population gets suppressed minimizing the level of infestation in the field crops. Usually this method involves the use of high doses of pheromones than that of other two approaches at an elevated concentration of 80–100 traps per ha. Also this method involves the installment of the infochemicals at several discrete locations. Again, mating disruption techniques are also an eco-friendly, cost-effective, and efficient approach of pest management practices (Witzgall et al. 1997; Kumar 2016; Cork et al. 2001).

4.7 Summary and Conclusions

Infochemicals represent chemical cues released by the insects responsible for their basic necessities such as reproduction, defense, orientation, and social behavior. Apart from that they have serious impact on ecology and ecotoxicology. Several sources influence the infochemical mediated communication including anthropogenic sources called infochemical effect. Infochemical compounds being non-pollutant, specific, cost-effective, and eco-friendly in nature are utilized in pest management practices. But the ongoing researches are slow and steady. Utilization of such components can lead to development of green strategies in pest management for log duration of time. There are several research gaps or lacunae in knowledge to work upon. Genetic regulations and neural network influencing the infochemical communication are still needed to be elucidated. The adaptability of chemical cues and the range of infochemical use is still a researchable issue. Furthermore, the role of infochemical effect on the ecotoxicology has to be determined as its complexity is very well known but very little is understood so far till now.

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References

Andersson MN (2012) Mechanisms of odor coding in coniferous bark beetles: from neuron to behavior and application. Psyche

Blum MS, Portocarrero CA (1964) Chemical releasers of social behavior IV. The hindgut as the source of the odor trail pheromone in the Neotropical army ant genus Eciton. Ann Entomol Soc Am 57(6):793–794

Blum MS, Ross GN (1965) Chemical releasers of social behavior V. source, specificity, and properties of the odour trail pheromone of *Tetramorium guineense* (F.)(Formicidae: Myrmicinae). J Insect Physiol 11(7):857–868

Blum MS, Wilson EO (1964) The anatomical source of trail substances in formicine ants. Psyche 71 (1):28–31

Blum MS, Warter SL, Monroe RS, Chidester JC (1963) Chemical releasers of social behavior I. Methyl-n-amyl ketone in *Iridomyrmex pruinosus* (Roger)(Formicidae: Dolichoderinae). J Insect Physiol 9(6):881–885

Boch R, Shearer DA, Stone BC (1962) Identification of iso-amyl acetate as an active component in the sting pheromone of the honey bee. Nature 195(4845):1018–1020

Bolckmans K (2009) Integrated pest management of the exotic invasive pest Tuta absoluta. In: International Biocontrol Manufacturers Association and Research Institute of Organic Agriculture (eds.) Proceedings of the 4th annual biocontrol industry meeting internationals, Lucerne, Switzerland

Boller T (1995) Chemoperception of microbial signals in plant cells. Annu Rev Plant Biol 46 (1):189–214

Bornemissza GF (1964) Sex attractant of male scorpion flies. Nature 203(4946):786–787

Böttinger LC, Hofferberth J, Ruther J, Stökl J (2019) Semiochemicals mediating defense, intraspecific competition, and mate finding in *Leptopilina ryukyuensis* and *L. japonica* (Hymenoptera: Figitidae), parasitoids of Drosophila. J Chem Ecol 45(3):241–252

Brent CS, Byers JA, Levi-Zada A (2017) An insect anti-antiaphrodisiac. Elife 6:e24063

Butenandt A, Tam N (1957) Über einen geschlechtsspezifischen duftstoff der wasserwanze *Belostoma indica* Vitalis (*Lethocerus indicus* Lep.). Hoppe-Seyler's Zeitschrift für Physiol Chem 308(1):277–283

Butler CG (1965) Sex attraction in *Andrena flavipes* panzer (Hymenoptera: Apidae), with some observations on nest-site restriction. Proc R Entomol Soc Lond 40:77

Butler CG, Callow RK, Chapman JR (1964) 9-Hydroxydec-trans-2-enoic acid, a pheromone stabilizing honeybee swarms. Nature 201(4920):733–733

Byers JA (2014) Simulation of mating disruption and mass trapping with competitive attraction and camouflage. Environ Entomol 36(6):1328–1338

Callow K, Johnstonn C (1960) The chemical constitution and synthesis of queen substance of honeybees *(Apis mellifera)*. Bee World 41:152–153

Carthy JD (1951) The orientation of two allied species of British ant, II. Odour trail laying and following in *Acanthomyops fuliginosus* (Lasius). Behaviour:304–318

Caspary VG, Downe AER (1971) Swarming and mating of *Chironomus riparius* (Diptera: Chironomidae). Canadian Entomol 103(3):444–448

Chadha MS, Eisner T, Monro A, Meinwald J (1962) Defence mechanisms of arthropods VII: citronellal and citral in the mandibular gland secretion of the ant *Acanthomyops claviger* (Roger). J Insect Physiol 8(2):175–179

Cheng YN, Wen P, Dong SH, Tan K, Nieh JC (2017) Poison and alarm: the Asian hornet *Vespa velutina* uses sting venom volatiles as an alarm pheromone. J Exp Biol 220(4):645–651

Cork A, Alam SN, Das A, Das CS, Ghosh GC, Farman DI, Hall DR, Maslen NR, Vedham K, Phythian SJ, Rouf FMA (2001) Female sex pheromone of brinjal fruit and shoot borer, *Leucinodes orbonalis* blend optimization. J Chem Ecol 27(9):1867–1877

Darragh K, Orteu A, Byers KJ, Szczerbowski D, Warren IA, Rastas P, Pinharanda AL, Davey JW, Garza SF, Almeida DA, Merrill RM (2019) A novel terpene synthase produces an anti-aphrodisiac pheromone in the butterfly Heliconius melpomene. bioRxiv:779678

Dicke M, Sabelis MW (1988) Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? Funct Ecol:131–139

Du Y, Grodowitz MJ, Chen J (2019) Electrophysiological responses of eighteen species of insects to fire ant alarm pheromone. Insects 10(11):403

El-Sayed AM, Suckling DM, Wearing CH, Byers JA (2006) Potential of mass trapping for long-term pest management and eradication of invasive species. J Econ Entomol 99(5):1550–1564

Engelmann (1965) Pheromones and mating behaviour. In: Proc. XII. Int. Congr. Ent., London, pp 289

Ferrari MC, Messier F, Chivers DP (2007) Degradation of chemical alarm cues under natural conditions: risk assessment by larval woodfrogs. Chemoecology 17(4):263–266

Field LM, Birkett M, Dufour S, He X, Hooper A, Pickett J, Zhou JJ (2007) Prospects for coupling new molecular approaches to chemical analysis. In: Contribution at the 23rd ISCE annual meeting in Jena, Germany

Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest-management. Annu Rev Entomol 42(1):123–146

Freitag J, Ludwig G, Andreini I, Rössler P, Breer H (1998) Olfactory receptors in aquatic and terrestrial vertebrates. J Comp Physiol A 183(5):635–650

Gary NE (1962) Chemical mating attractants in the queen honey bee. Science 136(3518):773–774

Gary NE (1963) Observations of mating behaviour in the honeybee. J Apic Res 2(1):3–13

Giblin-Davis RM, Oehlschlager AC, Perez A, Gries G, Gries R, Weissling TJ, Chinchilla CM, Peña JE, Hallett RH, Pierce HD Jr, Gonzalez LM (1996) Chemical and behavioral ecology of palm weevils (Curculionidae: Rhynchophorinae). Florida Entomol 79:153–167

Hanks LM, Millar JG (2016) Sex and aggregation-sex pheromones of cerambycid beetles: basic science and practical applications. J Chem Ecol 42(7):631–654

Hodek J (1960) Hibernation-bionomics in Coccinellidae. Gas Esl Spol Ent 57:1–20

Jacobson M, Beroza M, Jones WA (1960) Isolation, identification, and synthesis of the sex attractant of gypsy moth. Science 132(3433):1011–1012

Jardine KJ, Luani RDO, Rodrigues TB, Spanner GC, Rodrigues JR, Menezes VS, Sampaio I, Oliveira DC, Gimenez BO, Higuchi N, Chambers JQ (2020) Volatiles defenses of Amazon Azteca ants (repellent ants). bioRxiv

Jimenez SI, Gries R, Zhai H, Derstine N, McCann S, Gries G (2016) Evidence for a nest defense pheromone in bald-faced hornets, *Dolichovespula maculata*, and identification of components. J Chem Ecol 42(5):414–424

Karlson P, Lüscher M (1959) 'Pheromones': a new term for a class of biologically active substances. Nature 183(4653):55–56

Keller JC, Mitchell EB, Mckibbenn G, Davich TB (1964) A sex attractant for female boll weevils from males. J Econ Entomol 57:609–610

Kheloul L, Kellouche A, Bréard D, Gay M, Gadenne C, Anton S (2019) Trade-off between attraction to aggregation pheromones and repellent effects of spike lavender essential oil and its main constituent linalool in the flour beetle *Tribolium confusum*. Entomol Exp Appl 167 (9):826–834

Klaschka U (2008) Odorants–potent substances at minor concentrations: the ecological role of infochemicals. In: Pharmaceuticals in the environment. Springer, Berlin, pp 305–320

Klaschka U, Kolossa-Gehring M (2007) Fragrances in the environment: pleasant odours for nature. Environ Sci Pollut Res 14(1):44–52

Kumar J (2016) Infochemicals: an effective and environment friendly management of insect pests for sustainable agriculture. Int J Agric Invent 1:218–224

Kumar R, Kumar R, Prakash O, Srivastava RM, Pant AK (2019a) Chemical composition, in vitro antioxidant, anti-inflammatory and antifeedant properties in the essential oil of Asian marsh weed *Limnophila indica* L.(Druce). J Pharm Phytochem 8(1):1689–1694

Kumar R, Kumar R, Prakash O, Srivastava RM, Pant AK (2019b) GC MS analysis of the hexane extract of *Limnophila indica* (L.) Druce, its total phenolics, in-vitro antioxidant, anti-inflammatory and antifeeding activity against Spilosoma obliqua. J Entomol Zool Stud 7 (1):970–975

Lancaster J, Khrimian A, Young S, Lehner B, Luck K, Wallingford A, Ghosh SKB, Zerbe P, Muchlinski A, Marek PE, Sparks ME (2018) De novo formation of an aggregation pheromone precursor by an isoprenyl diphosphate synthase-related terpene synthase in the harlequin bug. Proc Natl Acad Sci 115(37):E8634–E8641

Larcher W (2003) Physiological plant ecology: ecophysiology and stress physiology of functional groups. Springer, Berlin

Laturney M, Billeter JC (2016) *Drosophila melanogaster* females restore their attractiveness after mating by removing male anti-aphrodisiac pheromones. Nat Commun 7(1):1–11

Law JH, Regnier FE (1971) Pheromones. Annu Rev Biochem 40(1):533–548

Law JH, Wilson EO, McCloskey JA (1965) Biochemical polymorphism in ants. Science 149 (3683):544–545

Lee HR, Lee SC, Lee DH, Choi WS, Jung CS, Jeon JH, Kim JE, Park IK (2017) Identification of the aggregation-sex pheromone produced by male *Monochamus saltuarius*, a major insect vector of the pine wood nematode. J Chem Ecol 43(7):670–678

Liebhold AM, Tobin PC (2008) Population ecology of insect invasions and their management. Annu Rev Entomol 53:387–408

Liu F, Xiong C, Liu N (2017) Chemoreception to aggregation pheromones in the common bed bug, *Cimex lectularius*. Insect Biochem Mol Biol 82:62–73

Ma M, Chang MM, Lei CL, Yang FL (2016) A garlic substance disrupts odorant-binding protein recognition of insect pheromones released from adults of the angoumois grain moth, *Sitotroga cerealella* (Lepidoptera: Gelechiidae). Insect Mol Biol 25(5):530–540

MacGregor EG, Thorpe WH (1948) Odour as a basis for orientated movement in ants. Behaviour:267–296
Markheiser A, Rid M, Biancu S, Gross J, Hoffmann C (2020) Tracking short-range attraction and oviposition of European grapevine moths affected by volatile organic compounds in a four-chamber Olfactometer. Insects 11(1):45
Maschwitz UW (1964a) Alarm substances and alarm behaviour in social Hymenoptera. Nature 204 (4956):324–327
Maschwitz U (1964b) Gefahrenalarmstoffe und Gefahrenalarmierung bei sozialen Hymenopteren. Z Vgl Physiol 47(6):596–655
Mazza G, Inghilesi AF, Longo S, Cervo R (2016) Male chemical cues mediate the female preference for egg deposition site in *Rhynchophorus ferrugineus*. Bull Insectol 69(1):41–48
Moore BP (1964) Volatile terpenes from *Nasutitermes soldiers* (Isoptera, Termitidae). J Insect Physiol 10(2):371–375
Morimoto R (1960) On the social co-operation in *Polistes chinensis antennalis* Perez. IX studies on the social hymenoptera of Japan. Kontyd 28:198–208
Morrison WR, Grosdidier RF, Arthur FH, Myers SW, Domingue MJ (2020) Attraction, arrestment, and preference by immature *Trogoderma variabile* and *Trogoderma granarium* to food and pheromonal stimuli. J Pest Sci 93(1):135–147
Moser JC, Blum MS (1963) Trail marking substance of the Texas leaf-cutting ant: source and potency. Science 140(3572):1228–1228
Nixon HL, Ribbands CR (1952) Food transmission within the honeybee community. Proc R Soc Lond Ser B Biol Sci 140(898):43–50
Nordlund DA, Lewis WJ (1976) Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. J Chem Ecol 2:211–220
Norris MJ, Richards MO (1963) Laboratory experiments on gregarious behaviour in ovipositing females of the desert locust, (*Schistocerca gregaria* (forsk.)). Entomol Exp Appl 6(4):279–303
Oehlschlager AC, Chinchilla C, Castillo G, Gonzalez L (2002) Control of red ring disease by mass trapping of *Rhynchophorus palmarum* (Coleoptera: Curculionidae). Fla Entomol 85 (3):507–513
Pereyra PC, Sánchez NE (2006) Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). Neotrop Entomol 35(5):671–676
Polya G (2003) Biochemical targets of plant bioactive compounds: a pharmacological reference guide to sites of action and biological effects. CRC press
Raji JI, Melo N, Castillo JS, Gonzalez S, Saldana V, Stensmyr MC, DeGennaro M (2019) *Aedes aegypti* mosquitoes detect acidic volatiles found in human odor using the IR8a pathway. Curr Biol 29(8):1253–1262
Rasmussen LEL, Lazar J, Greenwood DR (2003) Olfactory adventures of elephantine pheromones, vol 31, p 137
Regnier FE, Law JH (1968) Insect pheromones. J Lipid Res 9(5):541–551
Richards OW (1927) Sexual selection and allied problems in the insects. Biol Rev 2(4):298–364
Schulz S, Estrada C, Yildizhan S, Boppré M, Gilbert LE (2008) An antiaphrodisiac in *Heliconius melpomene* butterflies. J Chem Ecol 34(1):82–93
Spieth HT (1940) Studies on the biology of the Ephemer-Optera. II. The nuptial flight. J NY Entomol Soc 48(4):379–390
Stuart AM (1963) Studies on the communication of alarm in the termite *Zootermopsis nevadensis* (Hagen), Isoptera. Physiol Zool 36(1):85–96
Suckling DM (2000) Issues affecting the use of pheromones and other semiochemicals in orchards. Crop Prot 19(8–10):677–683
Torto B (2009) Chemical signals as attractants, repellents and aggregation stimulants. Chem Ecol 1:186
Wall C, Garthwaite DG, Smyth JB, Sherwood A (1987) The efficacy of sex-attractant monitoring for the pea moth, *Cydia nigricana*, in England, 1980–1985. Ann Appl Biol 110(2):223–229

Wharton DRA, Black ED, Merritt C Jr, Jacobson M, Beroza M (1963) Sex attractant of the American cockroach. Science:1257–1261

Wilson EO (1959) Source and possible nature of the odor trail of fire ants. Science 129 (3349):643–644

Wilson EO (1963) Pheromones. Sci Am 208(5):100–115

Wilson EO (1965) Chemical communication in the social insects. Science 149(3688):1064–1071

Wilson EO, Pavan M (1959) Glandular sources and specificity of some chemical releasers of social behavior in dolichoderine ants. Psyche 66(4):70–76

Wink M (2018) Plant secondary metabolites modulate insect behavior-steps toward addiction? Front Physiol 9:364

Witzgall P, Unelius RC, Rama F, Chambon JP, Bengtsson M (1997) Mating disruption of pea moth, *Cydia nigricana*, and codling moth, *Pomonella*, using blends of sex pheromone and attraction antagonists. IOBC WPRS Bull 20:207–216

Wyatt TD (2010) Pheromones and signature mixtures: defining species-wide signals and variable cues for identity in both invertebrates and vertebrates. J Comp Physiol A 196(10):685–700

Yew JY, Dreisewerd K, Luftmann H, Müthing J, Pohlentz G, Kravitz EA (2009) A new male sex pheromone and novel cuticular cues for chemical communication in Drosophila. Curr Biol 19 (15):1245–1254

Young JM, Trask BJ (2002) The sense of smell: genomics of vertebrate odorant receptors. Hum Mol Genet 11(10):1153–1160

Zhao H, Firestein S (1999) Vertebrate odorant receptors. Cell Mol Life Sci CMLS 56(7–8):647–659

Zhong YZ, Tang R, Zhang JP, Yang SY, Chen GH, He KL, Wang ZY, Zhang F (2018) Behavioral evidence and olfactory reception of a single alarm pheromone component in *Halyomorpha halys*. Front Physiol 9:1610

Zou Z, Buck LB (2006) Combinatorial effects of odorant mixes in olfactory cortex. Science 311 (5766):1477–1481

Nitrogen Fixation Through Genetic Engineering: A Future Systemic Approach of Nitrogen Fixation

5

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Abstract

One of the major limiting factors of growing plants is nitrogen. Nitrogen is a very important nutrient because it is a constituent of major genomic or structural portion of the organism due to the presence of nucleic acid (DNA and RNA), ATP, NAD and amino acid (Protein). The abundant amount is present in the atmosphere but not be utilized by plants and animals directly. From several decades, plant biotechnologists inspired about improvement of genetically modified N_2 fixation. In this chapter, we discuss biological nitrogen fixation and how much recent progress occurred in genetic engineering of nitrogenase into bacteria and plants to perform their own biological nitrogen fixation. Researchers are inspired to develop transgenic bacteria capable of producing novel symbiotic relationships with non-legumes, as well as engineering transgenic plants to express its own nitrogenase. Several advances have been made in synthetic biology, as well as our understanding of biochemistry and genetics of the nitrogenase enzyme in last four decades, which has made the apparently distant and for long unapproachable dream more possible.

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Diazotroph · Biological N_2 fixation (BNF) · nif gene · Nitrogenase · Promoter

5.1 Introduction

In every organism, nitrogen is a primary ingredient of biomolecules such as amino acids (proteins) and nucleic acids (DNA and RNA). The natural source of nitrogen uptake includes biological nitrogen fixation, decomposing organic matters and the small amounts from due to the physical process of nitrogen fixation, e.g. thunderstorm or rainfall, and automobile exhausts. The common limitation in modern agriculture for crop productivity is bioavailable nitrogen even though N_2 constitutes around 78% of the atmosphere. The high yielding varieties of cereal crops, rice, wheat, etc. need huge quantity of industrially produced fertilizer to overcome the limitation of nitrogen. The Haber-Bosch process has been providing needed nitrogen fertilizers from atmospheric nitrogen since the beginning of twentieth century. It was a major step forward because of its low cost and full substitution from previously used mineral fertilizers which were non-renewable (Poliakoff et al. 2002; Cherkasov et al. 2015).

In contrast to cereal crops, a small group of prokaryotes (bacteria and archaea), some of which are free-living and some in symbiotic associations with leguminous plants, are not only capable of reducing atmospheric N_2 but also enriched the soil. The process of fixing nitrogen involves enzyme nitrogenase, which is a multiple subunit protein encoded by the nif genes by the process well-known as biological nitrogen fixation (BNF) (Burns and Hardy 1975; Andersen et al. 1980; Singh et al. 2016). The median global value of BNF has been estimated around 88 Tg N $year^{-1}$ (52–130 Tg N $year^{-1}$) in natural terrestrial ecosystems. At least third part of it comes from free-living sources (Davies-Barnard and Friedlingstein 2020). From the ecological point of view, biological NF has also several advantages over industrial fertilizer production, that is, self-regulation, utilization of renewable and environmentally abundant substance such as carbohydrates and agriculture or forestry wastes treatment (Nuntagij et al. 1989; Balis et al. 1996; Cherkasov et al. 2015; Singh et al. 2019).

Several bacteria live in close proximity to the plant root forming the loose association, and some even invade or spread within plant tissue. One of the approaches could be to enhance the N_2-fixing efficiency, colonization ability, density, and NH_3 release of these bacteria. This approach can be considered most suitable until the utilization of transgenic plants face technical hurdles in several countries as it does not involve genetic modification. Improvement of biomass accretion, nitrogen content, and seed yield by inoculation of N_2-fixing strain of *P. protegens* Pf-5 X940 into maize and wheat rhizoplane is one such example (Burén and Rubio 2018).

Biological fixation of nitrogen can also be improved by the development of a novel symbiotic relationship in non-legume cereals, such as rice, wheat, and corn, to form nodules or nodule-like structure by associating with N_2-fixing bacteria (Santi

et al. 2013; Zhang et al. 2017). These structures provide a low-O_2 environment, as well as rich supply of carbon sources. For this purpose, it is necessary to modify plants capable of interacting with bacteria and to get recognized as a suitable host. Unay and Perret (2019) demonstrated the nodules development on roots of cowpea by synthetic plasmid having a small set of selected nodulation genes assembly. Their results confirm that by understanding the molecular bases for symbiotic NF would better the possibility of transforming non-symbiotic bacteria into proficient rhizobia.

The most challenging strategy proposes to transfer nif gene into the genome of plants (Curatti and Rubio 2014). These transgenic plants would then synthesize N_2 fixing system and also regulate some amount of fixed nitrogen without requiring the bacterial interactions. There are several obstacles such as the genetic differences and frangibility of the nif regulon, requirement of specific biochemical conditions for nitrogenase assembly and function, for example, necessity for protection from oxygen, due to its extreme oxygen sensitivity (Robson and Postgate 1980; Dixon and Kahn 2004; Temme et al. 2012; Poza-Carrión et al. 2014; Yang et al. 2020). Allen et al. (2017) demonstrated the expression of 16 nif proteins within plant mitochondrial matrix, providing experimental evidence to the strategy that transgenic plants in the future can be self-sufficient in utilizing atmospheric nitrogen.

5.2 Nitrogen Fixation

The atmosphere consists of large quantities of molecular nitrogen (N_2), where it undergoes complex biogeochemical cycle. Although the free N_2 does not react with other elements easily because of its stable triple bond, it is subjected to several processes such as fixation, nitrification, denitrification, and nitrate leaching. Nitrogen fixation is a process of conversion of "dihydrogen," or nitrogen gas into bioavailable ammonia. This reaction is necessary for agriculture and various other natural processes that hold and support the life on the planet and can be carried out by both industrial and natural processes and requires high energy input and a series of reduction steps due to high activation energy of the reactions (Saha et al. 2017; Cherkasov et al. 2015).

Natural processes to fix nitrogen involve lightning (physical), photochemical reactions and biological nitrogen fixation (biological). Lightning involves the formation of highly reactive hydroxyl free radicals, free oxygen atoms, and free hydrogen atoms by the conversion of water vapor and oxygen that form nitric acid (HNO_3) by attacking molecular nitrogen (N_2). Nitrogen photochemical reactions can also fix nitrogen by the reaction of gaseous nitric oxide (NO) and ozone (O_3) that produce nitric acid. The nitric acid afterward falls on to Earth with rain. The remaining fixation results from the cardinal reaction by nitrogenase complex in blue-green algae (cyanobacteria) or bacteria through biological nitrogen fixation (Schlesinger 1997; Taiz and Zeiger 2002).

Due to the high demand of nitrogen fertilizer, several methods have been used to fix nitrogen industrially. Among them, Haber-Bosch process is the significant way of nitrogen fixation since its establishment in 1913, in which N_2 reacts with hydrogen

under extreme conditions. The high pressure (about 200 atmospheres) and elevated temperature (about 200 °C) are necessary factors due to the high activation energy of the reaction. There exist some environmental concerns associated with the process because of its very intensive energy consumption and requires non-renewable raw material to generate hydrogen. Besides intensive inputs to agricultural production worldwide, one-third amount of fertilizer is subjected to leaching and emission as greenhouse gas. Therefore, researchers are looking for substitute methods to fix atmospheric nitrogen.

An alternative way of the industrial nitrogen fixation is the utilization of plasma in nitrogen fixation. NF with plasma involves conversion of air component into valuable products using only electricity. This method uses renewable energy sources and produce nitrogen fertilizers without greenhouse gas emissions, essentially with no waste and uses no solvents. One of the cons of the thermal plasma method is the requirement of high temperatures and low energy efficiency. Another alternative method involves the use of metallorganic complexes to fix nitrogen. It is a low yield process with fast catalyst decomposition and extremely expensive compounds. Its advantage lies in the possibility to investigate reaction mechanisms (Dance 2010; Cherkasov et al. 2015).

From an agricultural point of view, biological nitrogen fixation is most vital, as the output of nitrogen fertilizers through industrial process not often meets demands. Due to in situ utilization, nitrogen fixed by the biological process has fewer tendencies of leaching and volatilization. It is the need of the hour to reduce the dependency on chemical fertilizers due to their interference in the nitrogen cycle, nitrogen oxide emissions, acidification of soil and water eutrophication. Therefore, enhancing the biological nitrogen fixation is the way forward for sustainable agriculture (Capone 2001; Dixon and Kahn 2004).

5.3 Structure of Nitrogenase Complex

These nitrogen-fixing bacteria or diazotrophic bacteria execute one of the most intriguing chemical reactions in nature. Nitrogenase enzyme catalyzes the ATP-dependent reduction of approximately one dinitrogen per second (optimum turnover frequency) into ammonia. At least, each nitrogenase contains two metalloprotein component: dinitrogenase reductase (Fe protein) and dinitrogenase (MoFe protein), both homologous to each other. Dinitrogenase reductase functions in MgATP hydrolysis and reduces dinitrogenase protein and transfer electrons, while dinitrogenase binds and reduces dinitrogen molecules (Burris and Roberts 1993; Einsle and Rees 2020).

On the basis of the metal cluster at the active site on dinitrogenase protein, three classes of molybdenum, vanadium, and iron-based nitrogenase complex are recognized. Mo-nitrogenase abundant one, which in Mo-limiting condition can utilize V and Fe. *Streptomyces thermoautotrophicus* has a fourth class of a superoxide-dependent nitrogenase (Eady et al. 1978; Hoffmann-Findeklee et al. 2007; Saha et al. 2017). The most investigated nitrogenase enzyme is the

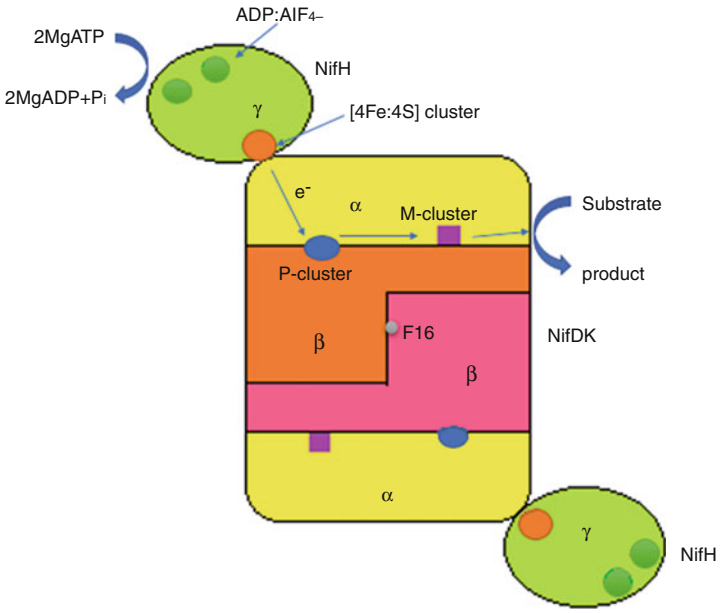

Fig. 5.1 Diagrammatic representation of Mo-nitrogenase complex structure: MoFe protein and the Fe protein (nifDK-MoFe protein, nifH-Fe protein) (**a**) α, β, and γ subunit (**b**) location and function of 4Fe:4S, ADP:AlF_{4-}, P-cluster, M-cluster (FeMoco), and F16 in nitrogenase complex

Mo-dependent nitrogenase. The structure of the nitrogenase, especially the structure of FeMo-protein's central unit, has been extensively reviewed and extensively researched (Sickerman et al. 2019; Burén and Rubio 2018; Einsle and Rees 2020).

The dinitrogenase reductase is a dimer of ~60 kD (γ2) having one [4Fe–4S] cubane between the two γ monomers (nifH) (Peters and Szilagyi 2006; Zhang et al. 2015). The dinitrogenase (MoFe protein), a ~ 230 kD tetramer, consists of two α and β subunit each. α and β-subunits forms a αβ dimer (nifDK). MoFe protein comprises metal centers of three types, that is, P-clusters, M cluster, and Fe16 (Fig. 5.1). There are two "P-clusters" [8Fe:7S] serving as the initial acceptor of electrons from the Fe protein at the α- and β-subunit interfaces. Two active sites buried in each α-subunits, a [7Fe:9S:Mo:C:R-homocitrate] cluster called the FeMo-cofactor or M cluster represents the site of substrate reduction. Two F16, a mononuclear iron site between the two β-subunits is the third cluster. Electrons flow from the [4Fe:4S] cluster (in Fe protein) to the P-cluster to the M cluster during substrate turnover. Sufficient buildup of protons and electrons is required for substrate reduction. (Morrison et al. 2015; Georgiadis et al. 1992; Peters et al. 1997; Lee et al. 2018; Einsle and Rees 2020).

During the reaction, Fe protein transfers electrons to MoFe protein forming a transient complex with ensuant ATP hydrolysis. ATP binding lowers the reduction potential of dinitrogenase reductase (Fe protein) by conformation change permitting it to interact with dinitrogenase (Brill 1980). The cluster of Fe protein is proposed to cycle between the $[Fe4S4]^{2+}$ and $[Fe4S4]^{+}$ states during normal catalytic reaction

(Burgess and Lowe 1996; Sickerman et al. 2019). In the assembly of FeMoco, different metal clusters containing nitrogenase-related proteins nifB and NifEN are also involved (Lee et al. 2018). Examination among the nitrogen-fixing species revealed the highly conserved basic framework of nitrogenase MoFe proteins with some differences. There is a greater variation around the P cluster and at the docking surface of Fe protein. It may suggest that active site, where the intermolecular and intramolecular electron and proton transfer occurs, could be less sensitive to the sequence and structure variations (Zhang et al. 2015).

Sixteen molecules of ATP are utilized to reduce a single N_2 molecule, two ATP molecules for every electron transfer in optimum conditions (Burgess and Lowe 1996, Sickerman et al. 2019).

$$N_2 + 8H^+ + 8e^- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i.$$

Two electrons of eight produce an ineludible by-product hydrogen and show the "electron efficiency" of the process around 75%.

Generally, requirements of energy source range between 20 and 30 molecules of MgATP in de novo nitrogen fixation due to less efficiency of process under natural conditions in contrast to optimum laboratory conditions. The ferredoxin or flavodoxin supply the requisite electrons for the reaction while photosynthetic processes and decomposed organic compound provide ATP in N_2-fixing photoautotrophs and nitrogen-fixing heterotrophs, respectively (Saha et al. 2017).

In order to introduce nitrogenase complex in eukaryotic system, the most important challenge is its oxygen sensitivity. Both enzymes have half-life of 0.5–0.75 s for Fe protein and 10 min for MoFe protein in air. In natural conditions, diazotrophs have developed various approaches to overcome this situation. Some of the examples are formation of thick-walled heterocysts in cyanobacterium *Anabaena*, regulation of gas permeability in actinorhizal plants, and the presence of oxygen-binding heme protein leghemoglobin (Taiz and Zeiger 2002).

5.4 Nitrogen-Fixing Groups Diversity and Associations

Nitrogen fixation occurs only in prokaryotes, and the capability to fix N_2 is extensively dispersed around both the archaeal and bacterial domains paraphyletically. These diazotrophic organisms show considerable biodiversity, as well as a wide range of physiology. Phylogenetic groups of bacteria such as actinomycetes, cyanobacteria, green sulfur bacteria, firmibacteria, proteobacteria, and methanogens in Archaea have the capability to fix the nitrogen (Raymond et al. 2004; Dixon and Kahn 2004).

Diazotrophs inhabited in a broad form of habitats including living free in soils and water, with grasses in associative symbioses, termite guts, actinorhizal associations with woody plants, and specialized nodules in legume roots. Major diazotrophs associations involve lichen, *Anabaena-Azolla*, *Frankia*-actinorhizal plants, and *Rhizobium*-leguminous plants. *Rhizobium*-legume symbiotic relationship was the first

established in 1888, but it has been utilized in agricultural since ancient times. *Rhizobia* have the capability to infect their host plant roots and form nodules in them specifically, that is, Genus *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium* nodulates peas, alfalfa (Lucerne), and soybean (Saha et al. 2017).

Among all the relationships, endophytic bacteria having ability to enter and colonize the roots interior can be promising for non-legume agriculture. Endophytes such as *Herbaspirillum seropedicae* have been reported from within the roots, stems, and leaves of crops such as maize, rice, and sugarcane. Other important diazotrophs involve saccharophilic bacterium *Acetobacter diazotrophicus* in association with sugarcane, sweet sorghum, and *Gluconacetobacter diazotrophicus* with *Coffea arabica* and *Ananas comosus* (Kirchhof et al. 1997; Jimenez-Salgado et al. 1997; Tapia-Hernández et al. 2000). Diazotrophs have an advantage of lesser competition for nutritional sources with other microorganisms, as well as low oxygen environment required for the expression and activity of nitrogenase (James et al. 1994).

5.5 Genetics of Nitrogen Fixation

For improvement of biological nitrogen fixation, considerable efforts have been made by workers, reviewed extensively previously (Dixon and Postgate 1972; Masepohl et al. 2002; Wang et al. 2013; Mus et al. 2016). Nitrogenase metalloenzymes complex involves multigene assembly regulation pathway system for its synthesis and functional activity (Rubio and Ludden 2008; Hu and Ribbe 2013; Allen et al. 2017; Singh et al. 2020). Earliest well-studied BNF work had been carried out on *Klebsiella pneumonia*. The nif genes encode complex metalloenzymes nitrogenase (enzyme with metal cofactor) responsible for nitrogen fixation. It is an ATP-dependent process of formation of ammonia catalyzed by reduction of dinitrogen. The nif gene of *K. pneumoniae* is composed of 25 kb of DNA and contains about 21 contiguous genes arranged and transcribed in eight operons (Dean and Jacobson 1992; Swain and Abhijita 2013). Study suggested that many of structural and regulatory genes are involved in regulation of nif gene functions (Table 5.1).

Most progress have been made on agronomically important legume crops, genera Rhizobium and Bradyrhizobium (Shantharam and Mattoo 1997). In *Rhizobium meliloti*, the fixABCX gene is first recognized earlier (Kallas et al. 1985; Earl et al. 1987; Mahmud et al. 2020). Gram-negative bacterium *Sinorhizobium meliloti* having genetically modified commercial strain (RMBPC-2) contain genes that regulate nitrogen fixation from plant to bacteria by nitrogenase enzymes (Chowdhury et al. 2008). Many rhizobium species such as *Bradyrhizobium japonicum*, *Sinorhizobium meliloti* and *Mesorhizobium loti* genomes have been sequenced. The rhizobial specificity against legumes has wide range. Rhizobial species such as *Rhizobium loti* and *Rhizobium etli* have different host choices (Phaseolus spp. for *Rhizobium etli* and Lotus spp. for *Rhizobium loti*), but produce identical Nod factors. However, many species such as *Bradyrhizobium japonicum* and *B. elkanii* have mutual hosts (Phaseoleae (P), Glycine spp. typically) but produce different Nod factors.

Table 5.1 The nif gene/product and their functions

Sr. No.	nif gene/product	Function/activity	References
1.	nifD and nifK	Encodes for the α subunit and β subunit of dinitrogenase protein, respectively	Beringer and Hirsch (1984)
2.	nifH	Dimeric protein dinitrogenase reductase	Roberts et al. (1978)
3.	nifS and nifU gene	Assembly of Fe-S clusters	Hu and Fay (2007)
4.	nifA, nifB, nifE, nifN, nifQ, nifS, nifV, nifW, nifX, and nifZ	Functionality of nif genes, FeMoco biosynthesis, assembly, and maturation of electron transport	Masepohl et al. (2002)
5.	nifA	Essential for constitute expression of nif gene transcription	Dixon (1998)
6.	nifH, nifM, nifS, and nifU	Maturation of Fe protein	Saha et al. (2017)
7.	nifE and nifN	Scaffold for FeMo-co biosynthesis	Allen et al. (1995), Saha et al. (2017)
8.	nifB gene product	Sulfur and iron containing precursor of FeMo-co	Allen et al. (1995)
9.	nifV	Encodes the homocitrate synthase and required for the FeMoCo synthesis	Saha et al. (2017)
10.	nifW	Protects the dinitrogenase from oxygen inactivation and give stability to protein complex	Cheng (2008)
11.	nifF	Encodes flavodoxin protein that shifts electrons to nitrogenase	Thorneley et al. (1992)
12.	nifJ	Regulates the synthesis of nifF protein, encodes the pyruvate oxidoreductase protein that shifts electrons to flavodoxin protein from the pyruvate	Shah et al. (1983)
13.	nifB, nifE, and nifN products	Essentials for normal functionality of the FeMo-cofactor center in component I	Saha et al. (2017)
14.	nifL	Repressor of nitrogenase	Dixon (1998), Beringer and Hirsch (1984)

Moreover, *R. etli* and *R. tropici* nodulate *Proteus vulgaris* but have different Nod factors (acetyl-fucosylated and sulfated, respectively).

5.6 Approach of Nitrogen Fixation

Diazotrophic bacteria are well-suitable organism for approaching nitrogen fixation due to the capability to fix the nitrogen by both types symbiotically and free-living mechanism. For genetic manipulation, nitrogen fixation genes (nif, fix) and

nodulation (nod, nol, and noe) genes are targeted to improve N_2 fixation. When *Ensifer adhaerens*, Gram-negative soil bacterium, get *Rhizobium tropici* CFN299 symbiotic plasmid, it forms and regulates the functional nitrogen-fixing nodules on both hosts.

5.7 Symbiotic Plasmid

Plasmids of rhizobium contains nitrogen fixation gene (nif and fix genes), nodulation affecting genes (nod, nol, and noe), some polysaccharide production genes (*lps* and *exo* genes) and other cellular functions (Denarie et al. 1992). The well-studied N_2 fixation bacterial genome of *K. pneumoniae* structural and functional similarity to non-nitrogen-fixing bacterium *E. coli*. Both species could have genes transferable and expressed in one another. Under the suitable circumstance parental strain of *K. pneumoniae* and *E. coli* carrying the nif plasmid pRDI was alike (Beringer and Hirsch 1984). Factors such as fixed N2, oxygen and temperature suppress the nif gene expression which gets over by the nifL gene deletion from gene constituent. It permitted the constant level of expression of nif gene.

5.8 Genetic Manipulation of N_2 Fixation in Prokaryotes

The hope of developing new forms of transgenic bacteria has encouraged since Dixon and Postgate (1972) had effectively transferred the nif gene from *K. pneumoniae,* a diazotroph to a non-diazotroph *Escherichia coli. Escherichia coli* is used as a model organism after the evolution of genetic engineering technology from past several decades. The most prominent objective of choosing *E. coli* is that it gives an idea about how many genes are necessary for the genetic manipulation experiments. *E. coli* is the first successfully genetic manipulated diazotroph, carrying an entire set of 20 nif genes of *K. oxytoca* by applying the N15 isotope system (Dixon and Postgate 1972). Recently, recombinants *E. coli* have been successfully expressed the nif genes system from diverse diazotrophs. Study suggested that nifFJ genes and nifSU of K. *oxytoca* enhanced the nitrogenase action of the recombinant *E. coli* while nifQ or nifM have no role in increasing the nitrogenase activity of nitrogen fixation (Li et al. 2016). Co-expression of *K. oxytoca* nifSU genes and *Paenibacillus pfoABfldA* genes (encodes electron transporter activity of complex nitrogenase metalloenzymes) gives 50.1% increased activity of wildlife strain of *Paenibacillus* (Li and Chen 2020). Under the regulation of the T7 promoter and native nifB promoter, recombinant *E. coli* having nine nif genes cluster from nitrogen fixer Paenibacillus polymyxa WLY78 (D, K, H, B, E, N, X, A and V) successfully produces the active nitrogenase (Wang et al. 2013). Several bioinformatics analysis such as phylogenetic investigation of nif genes revealed the horizontal nitrogenase gene transfer among different microorganisms (Raymond et al. 2004; Kechris et al. 2006; Latysheva et al. 2012).

5.9 Genetic Manipulation of N_2 Fixation in Eukaryotes

Genetic manipulation involves the manipulation of nitrogen-fixation genes for improvement of fixation strategies of nitrogen. However, structural and regulatory genes occur in nitrogen-fixing species are conserving in the environment. As nitrogen-fixation-mediated microorganisms are adapted in host, they are not capable to assimilate the fixed nitrogen (Peters et al. 1982). Scientists have made many attempts of nif gene incorporation in eukaryotic cells. Prokaryotic cells have polycistronic mRNA differentiating from monocistronic mRNA with binding site at 5′ end. For incorporation of prokaryotic nif gene in the eukaryotic genome, it will be essential to identification of nif promoter, ability of host to recognize the nif codon by DNA-dependent RNA polymerase and mRNA translation process. Introduction of nif gene into non-leguminous plant's protoplast have been carried out by many workers (Shanmugam and Valentine 1975; Charpentier and Oldroyd 2010; Geurts et al. 2012). *P. radiate*-associated modified fungus mycelia can fix the nitrogen (Pandey 1978). Strain of *Burkholderia* species grow with rice seedling can fix the good amount of nitrogen for rice (Baldani et al. 2000; Swain and Abhijita 2013).

Plant organelles such as mitochondria and chlorophyll are suitable locations on plant for nif genes expression for nitrogenase activity (Good and Beatty 2011; Stokstad 2016). Mitochondria suitability can be justified by the following conditions. (1) The complex nitrogenase is a highly oxygen-sensitive enzyme, required reluctant environment, high concentration of ATP, and accessible S-adenosylmethionine, Fe, Mo and homocitrate for metalloenzymes biosynthesis and activity (Hu and Ribbe 2013; Allen et al. 2017). (2) Mitochondrial matrix having enzymes for oxygen consumption could provide normal functioning conditions of oxygen-sensitive enzymes such as nitrogenase. (3) Furthermore, mitochondria is a major site of synthesis of plant biosynthetic proteins and metalloenzymes, therefore this give suitable functional conditions equal to nif protein (Balk and Pilon 2011). Sixteen nif proteins (B, D, E, F, H, J, K, M, N, Q, S, U, V, X, Y, and Z) expression from the diazotroph *Klebsiella pneumoniae* were successfully targeted within plant mitochondrial matrix (Allen et al. 2017).

The nitrogenase metalloenzymes have structural as well as function similarity with chlorophyll biosynthesis enzyme of *Chlamydomonas reinhardtii*. They have close genome similarity between nifH and chlL. Therefore, transcriptional factors activate the chlL gene and can also trigger ability of the nifH gene. The gene sequence of chlL gene can be substituted by the nifH gene. Dinitrogenase reductase, a dimeric protein, consists of two identical subunits and are product of nifH gene (Roberts et al. 1978). Another strategy also includes the transformation process that includes the introduction of nitrogenase enzyme into a chloroplast.

The promise of plant biotechnology and diversity among groups fixing and assimilating atmospheric nitrogen has inspired researchers to device various strategies to extend BNF benefit to the non-legumes. The main objective has been to reduce the dependency of energy intensive industrial process to produce nitrogen fertilizer, as well as to increase crop productivity to meet the demands of increasing global population. Besides alternative solutions are actively sought for all the

processes that have a considerable ecological impact. Genetically modified nitrogen fixation process is an alternate source of nitrogen fixation which reduces the dependency on harmful synthetic chemical nitrogen fertilizers for sustainable agriculture and great concern about soil and air environmental health.

References

Allen RM, Chatterjee R, Ludden PW, Shah VK (1995) Incorporation of iron and sulfur from NifB cofactor into the iron-molybdenum cofactor of dinitrogenase. J Biol Chem 270:26890–26896

Allen RS, Tilbrook K, Warden AC, Campbell PC, Rolland V, Singh SP, Wood CC (2017) Expression of 16 nitrogenase proteins within the plant mitochondrial matrix. Front Plant Sci 8:287

Andersen K, Shanmugam KT, Lim ST, Csonka LN, Tait R, Hennecke H, Scott DB, Hom SS, Haury JF, Valentine A, Valentine RC (1980) Genetic engineering in agriculture with emphasis on nitrogen fixation. Trends Biochem Sci 5(2):35–39

Baldani VLD, Baldani JI, Dobereiner J (2000) Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol Fert Soils 30:485–491

Balis C, Chatzipavlidis J, Flouri F (1996) Olive mill waste as a substrate for nitrogen fixation. Int Biodeterior Biodegradation 38(3–4):169–178

Balk J, Pilon M (2011 Apr) Ancient and essential: the assembly of iron-sulfur clusters in plants. Trends Plant Sci 16:218–226. https://doi.org/10.1016/j.tplants.2010.12.006

Beringer JE, Hirsch PR (1984) Genetic engineering and nitrogen fixation. Biotechnol Genet Eng Rev 1:65–88

Brill WJ (1980) Biochemical genetics of nitrogen fixation. Microbiol Rev 44(3):449–467

Burén S, Rubio LM (2018) State of the art in eukaryotic nitrogenase engineering. FEMS Microbiol Lett 365(2):fnx274

Burgess BK, Lowe DJ (1996) Mechanism of molybdenum nitrogenase. Chem Rev 96 (7):2983–3012

Burns RC, Hardy RW (1975) Nitrogen fixation in bacteria and higher plants. Springer, New York

Burris RH, Roberts GP (1993) Biological nitrogen fixation. Annu Rev Nutr 13(1):317–335

Capone DG (2001) Marine nitrogen fixation: what's the fuss? Curr Opin Microbiol 4(3):341–348

Charpentier M, Oldroyd G (2010) How close are we to nitrogen-fixing cereals? Curr Opin Plant Biol 13:556–564

Cheng Q (2008) Perspectives in biological nitrogen fixation research. J Integr Plant Biol 50 (7):786–798

Cherkasov N, Ibhadon AO, Fitzpatrick P (2015) A review of the existing and alternative methods for greener nitrogen fixation. Chem Eng Process 90:24–33

Chowdhury MKA, Hoque MI, Sonnino A (2008) Biosafety of genetically modified organisms: basic concepts, methods and issues. Proceedings of biotechnology and biosafety workshop. Dent Gaz 2008:21–30

Curatti L, Rubio LM (2014) Challenges to develop nitrogen-fixing cereals by direct nif-gene transfer. Plant Sci 225:130–137

Dance I (2010) Mimicking nitrogenase. Dalton Trans 39(12):2972–2983

Davies-Barnard T, Friedlingstein P (2020) The global distribution of biological nitrogen fixation in terrestrial natural ecosystems. Global Biogeochem Cy 34(3):e2019GB006387

Dean DR, Jacobson MR (1992) Biochemical genetics of nitrogenase. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation. Chapman and Hall, New York, pp 763–834

Denarie J, Debelle F, Rosenberg C (1992) Signaling and host range variation in nodulation. Annu Rev Microbiol 46(1):497–531

Dixon R (1998) The oxygen-responsive NIFL-NIFA complex: a novel two-component regulatory system controlling nitrogenase synthesis in γ-Proteobacteria. Arch Microbiol 169:371–380

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2 (8):621–631

Dixon RA, Postgate JR (1972) Genetic transfer of nitrogen fixation from *Klebsiella pneumoniae* to *Escherichia coli*. Nature 237(5350):102–103

Eady RR, Issack R, Kennedy C, Postgate JR, Ratcliffe HD (1978) Nitrogenase synthesis in *Klebsiella pneumoniae*: comparison of ammonium and oxygen regulation. Microbiology 104 (2):277–285

Earl C, Ronson C, Ausubel FJ (1987) Genetic and structural analysis of the *Rhizobium meliloti fixA*, *fixB*, *fixC*, and *fixX* genes. J Bacteriol 169:1127–1136

Einsle O, Rees DC (2020) structural enzymology of nitrogenase enzymes. Chem Rev 120 (12):4969–5004

Georgiadis MM, Komiya H, Chakrabarti P, Woo D, Kornuc JJ, Rees DC (1992) Crystallographic structure of the nitrogenase iron protein from Azotobacter vinelandii. Science 257 (5077):1653–1659

Geurts R, Lillo A, Bisseling T (2012) Exploiting an ancient signalling machinery to enjoy a nitrogen fixing symbiosis. Curr Opin Plant Biol 15:1–6

Good AG, Beatty PH (2011) Fertilizing Nature: A Tragedy of Excess in the Commons. PLoS Biol 9 (8):e1001124. https://doi.org/10.1371/journal.pbio.1001124

Hoffmann-Findeklee C, Gadkari D, Meyer O (2007) Superoxide dependent nitrogen fixation. In: Hu Y, Fay AW. P-cluster maturation on nitrogenase MoFe protein. Proc Natl Acad Sci U S A 104(25):10424–10429

Hu Y, Fay AW (2007) P-cluster maturation on nitrogenase MoFe protein. Proc Natl Acad Sci U S A 104(25):10424–10429

Hu Y, Ribbe MW (2013) Biosynthesis of the iron-molybdenum cofactor of nitrogenase. J Biol Chem 288(19):13173–13177. https://doi.org/10.1074/jbc.R113.454041

James EK, Reis VM, Olivares FL, Baldani JI, Döbereiner J (1994) Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. J Exp Bot 45(6):757–766

Jimenez-Salgado T, Fuentes-Ramirez LE, Tapia-Hernandez A, Mascarua-Esparza MA, Martinez-Romero E, Caballero-Mellado J (1997) Coffea arabica L., a new host plant for *Acetobacter diazotrophicus*, and isolation of other nitrogen-fixing acetobacteria. Appl Environ Microbiol 63 (9):3676–3683

Kallas T, Coursin T, Rippka RJ (1985) Different organization of *nif* genes in nonheterocystous and heterocystous cyanobacteria. Plant Mol Biol 5:321–329

Kechris K, Lin JC, Bickel PJ, Glazer AN (2006) Quantitative exploration of the occurrence of lateral gene transfer by using nitrogen fixation genes as a case study. PNAS 103:9584–9589

Kirchhof G, Reis VM, Baldani JI, Eckert B, Döbereiner J, Hartmann A (1997) Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. In: Opportunities for biological nitrogen fixation in rice and other non-legumes. Springer, Dordrecht, pp 45–55

Latysheva N, Junker VL, Palmer WJ, Codd GA, Barker D (2012) The evolution of nitrogen fixation in cyanobacteria. Bioinformatics 28:603–606

Lee CC, Hu Y, Ribbe MW (2018) Reduction and condensation of aldehydes by the isolated cofactor of nitrogenase. ACS Cent Sci 4(10):1430–1435

Li Q, Chen S (2020) Transfer of nitrogen fixation (*nif*) genes to non-diazotrophic hosts. Chem Bio Chem 21(12):1717–1722. https://doi.org/10.1002/cbic.201900784

Li XX, Liu Q, Liu XM, Shi HW, Chen SF (2016) Using synthetic biology to increase nitrogenase activity. Microb Cell Factories 15:43. https://doi.org/10.1186/s12934-016-0442-6

Mahmud K, Makaju S, Ibrahim R, Missaoui A (2020) Current progress in nitrogen fixing plants and microbiome research. Plants (Basel) 9(1):97. https://doi.org/10.3390/plants9010097

Masepohl B, Drepper T, Paschen A, Gross S, Pawlowski A, Raabe K, Riedel KU, Klipp W (2002) Regulation of nitrogen fixation in the phototrophic purple bacterium *Rhodobacter capsulatus*. J Mol Microbiol Biotechnol 4:243–248

Morrison CN, Hoy JA, Zhang L, Einsle O, Rees DC (2015) Substrate pathways in the nitrogenase MoFe protein by experimental identification of small molecule binding sites. Biochemistry 54 (11):2052–2060

Mus F, Crook MB, Garcia K, Costas AG, Geddes BA, Kouri ED, Paramasivan P, Ryu MH, Oldroyd GE, Poole PS, Udvardi MK (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Appl Environ Microbiol 82(13):3698–3710

Nuntagij A, de Lassus C, Sayag D, André L (1989) Aerobic nitrogen fixation during the biodegradation of lignocellulosic wastes. Biol Wastes 29(1):43–61

Pandey KK (1978) Novel techniques of gene transfer and plant improvement: an appraisal of transformation in eukaryotes. New Phytologist 81:685–704

Peters JW, Szilagyi RK (2006) Exploring new frontiers of nitrogenase structure and mechanism. Curr Opin Chem Biol 10(2):101–108

Peters GA, Calvert HE, Kaplaw D, Ito O, Toia RE (1982) The Azolla-Anabaena symbiosis: morphology, physiology and use. Israel J Bot 31:305–323

Peters JW, Stowell MH, Soltis SM, Finnegan MG, Johnson MK, Rees DC (1997) Redox-dependent structural changes in the nitrogenase P-cluster. Biochemistry 36(6):1181–1187

Poliakoff M, Fitzpatrick JM, Farren TR, Anastas PT (2002) Green chemistry: science and politics of change. Science 297(5582):807–810

Poza-Carrión C, Jiménez-Vicente E, Navarro-Rodríguez M, Echavarri-Erasun C, Rubio LM (2014) Kinetics of *nif* gene expression in a nitrogen-fixing bacterium. J Bacteriol 196(3):595–603

Raymond J, Siefert JL, Staples CR, Blankenship RE (2004) The natural history of nitrogen fixation. Mol Biol Evol 21(3):541–554

Roberts GP, Macneil T, Macneil D, Brill WJ (1978) Regulation and characterization of protein products coded by the nif genes of Klebsiella pneumoniae. J Bacteriol 136:267–279

Robson RL, Postgate JR (1980) Oxygen and hydrogen in biological nitrogen fixation. Annu Rev Microbiol 34(1):183–207

Rubio LM, Ludden PW (2008) Biosynthesis of the iron molybdenum cofactor of nitrogenase. Annu Rev Microbiol 62:93–111. https://doi.org/10.1146/annurev.micro.62.081307.162737

Saha B, Saha S, Das A, Bhattacharyya PK, Basak N, Sinha AK, Poddar P (2017) Biological nitrogen fixation for sustainable agriculture. In: Agriculturally important microbes for sustainable agriculture. Springer, Singapore, pp 81–128

Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. Ann Bot 111(5):743–767

Schlesinger WH (1997) Biogeochemistry. An analysis of global change, 2nd edn. Academic, San Diego, 588 p.

Shah VK, Stacey G, Brill WJ (1983) Electron transport to nitrogenase. Purification and characterization of Pyruvate: flavodoxin oxidoreductase the nifJ Gene product. J Biol Chem. 258:12064–12,068

Shanmugam KT, Valentine RC (1975) Molecular biology of nitrogen fixation. Science 187:919

Shantharam S, Mattoo AK (1997) Enhancing biological nitrogen fixation: an appraisal of current and alternative technologies for N input into plants. In: Ladha JK, de Bruijn FJ, Malik KA (eds) Opportunities for biological nitrogen fixation in rice and other non-legumes, Springer, Dordrecht, pp 205–216

Sickerman NS, Hu Y, Ribbe MW (2019) Nitrogenases. In: Metalloproteins. Humana Press, New York, NY, pp 3–24

Singh RP, Manchanda G, Singh RN, Srivastava AK, Dubey RC (2016) Selection of alkalotolerant and symbiotically efficient chickpea nodulating rhizobia from north-west Indo gangetic plains. J Basic Microbiol 56:14–25

Singh RP, Manchanda G, Yang Y, Singh D, Srivastava AK, Dubey RC, Zhang C (2019) Deciphering the factors for nodulation and symbiosis of Mesorhizobium associated with Cicer arietinum in northwest India. Sustainability 11:7216

Singh RP, Anwar MN, Singh D, Bahuguna V, Manchanda G, Yang Y (2020) Deciphering the key factors for heavy metal resistance in gram-negative bacteria. In: Singh R, Manchanda G, Maurya I, Wei Y (eds) Microbial versatility in varied environments. Springer, Singapore

Stokstad E (2016) The nitrogen fix. Science 353:1225–1227. https://doi.org/10.1126/science.353.6305.1225

Swain H, Abhijita S (2013) Nitrogen fixation and its improvement through Genetic engineering. J Global Biosci 2(5):98–112

Taiz L, Zeiger E (2002) Plant physiology, 3rd edn. Sinauer Associates, Sunderland, pp 67–86

Tapia-Hernández A, Bustillos-Cristales MR, Jiménez-Salgado T, Caballero-Mellado J, Fuentes-Ramirez LE (2000) Natural endophytic occurrence of Acetobacter diazotrophicus in pineapple plants. Microb Ecol 39(1):49–55

Temme K, Zhao D, Voigt CA (2012) Refactoring the nitrogen fixation gene cluster from *Klebsiella oxytoca*. Proc Natl Acad Sci U S A 109(18):7085–7090

Thorneley RN, Abell C, Ashby GA, Drummond MH, Eady RR, Huff S (1992) Posttranslational modification of Klebsiella pneumoniae flavodoxin by covalent attachment of coenzyme a, shown by 31P NMR and electrospray mass spectrometry, prevents electron transfer from the NifJ protein to nitrogenase. A possible new regulatory mechanism for biological nitrogen fixation. Biochemistry 31:1216–1224

Unay J, Perret X (2019) Synthetic plasmids to challenge symbiotic nitrogen fixation between rhizobia and legumes. In: Methods in rhizosphere biology research. Springer, Singapore, pp 3–18

Wang LY, Zhang LH, Liu ZZ, Zhao DH, Liu XM, Zhang B, Xie JB, Hong YY, Li PF, Chen SF, Dixon R, Li JL (2013) A minimal nitrogen fixation gene cluster from Paenibacillus sp. WLY78 enables expression of active nitrogenase in *Escherichia coli*. PLoS Genet 9:e1003865

Yang YJ, Liu L, Singh RP, Meng C, Ma S, Jing C, Li Y, Zhang C (2020) Nodule and root zone microbiota of salt-tolerant wild soybean in coastal sand and saline-alkali soil. Front Microbiol 11:2178

Zhang LM, Morrison CN, Kaiser JT, Rees DC (2015) Nitrogenase MoFe protein from Clostridium pasteurianum at 1.08 Å resolution: comparison with the Azotobacter vinelandii MoFe protein. Acta Crystallogr Sect D Biol Crystallogr 71(2):274–282

Zhang JJ, Yang X, Chen G, de Philippe L, Singh RP, Wang ET, Chen WF (2017) Mesorhizobium muleiense and Mesorhizobium gsp. Nov. are symbionts of Cicer arietinum L. in alkaline soils of Gansu, northwest China. Plant Soil 410:103–112

Functional AM Fungi in the Rhizosphere of Fruit Crops

6

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Abstract

Mycorrhizal fungi can affect the nutrient acquisition, biochemical characters and water transportation for stressed plants. This chapter summarizes these effects on various fruit crops, which may be very useful for organic cultivation of fruit crops and also for expanding the fruit cultivation in low fertility degraded soil with less expenditure and minimum reduction to yield.

Keywords

Mycorrhizae · Colonization · Glomus · Plant nutrition · Subtropical climate

6.1 Overview

Most of the increase in population (approximate 9 billion by the year 2030) will be in developing countries, where malnutrition, food availability and its shortages persist. Hunger and malnutrition are the two major global challenges in developing counties. In this situation, horticultural crops, especially fruit crops can ensure nutritional security. Thus, increasing demand of fruit produce can only be met by exploiting the marginal, saline and drought-prone areas for cultivation, where main constraints are poor soil structure, high salt content and minimal access to irrigation. Several studies have shown that some beneficial fungi belonging to AMF (Arbuscular mycorrhizae

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fungi) are identified to form rhizospheric symbiotic association with plants belonging to angiosperms. Mycorrhizal associated intracellularly or extracellularly with plants and may colonize the roots and showed mutualistic relationship. In this association, fungus retrieves constant and direct access to mono- or dimeric carbohydrates, such as glucose and sucrose produced by the plant during photosynthesis. These carbohydrates are translocated from leaves to the root zone/tissues. Whereas the plant gains mineral nutrient and water access due to large surface area provided by fungus.

Mycorrhizal mycelia are much smaller in size than the smallest root and can explore a greater volume of soil, providing a suitable and effective condition for absorption of mineral nutrients such as phosphorus (Chandreshekara et al. 1995), nitrogen (Al-Karaki and Al-Raddad 1997), K, Mg and Ca (Liu et al. 2002), copper (Li et al. 1991), Zn and Ni (Jamal et al. 2002) and iron (Caris et al. 1998). Mycorrhizal provides better drought tolerance, increased photosynthetic rate, direct hyphal water uptake from the soil, enzymatic activity to enhance defence and osmotic stress (Auge 2001) in the fruit crops. Mycorrhizal fungi are also potential bio-control agents against plant pathogens such as fungi (*Phytophthora*, *Gaeumannomyces*, *Fusarium*, *Thielaviopsis*, *Pythium*, *Rhizoctonia*, *Sclerotium*, *Verticillium* and *Aphanomyces*) and nematodes (*Rotylenchus*, *Pratylenchus* and *Meloidogyne*) by giving competition to pathogens for colonization sites and enhance the expression of the genes for plant defence. In addition, mycorrhizae showed increased lignifications of root endodermal cells, enhanced *peroxidase* activity associated with epidermal and hypodermal cells and high accumulation of mRNAs encoding *chitinases* and a β-1, 3-endoglucanase in and around cells (Azcon and Barea 1996). Moreover, mycorrhizal fungi are also capable of heavy metals (such as cadmium, nickel, zinc, copper and lead.) bioremediation due to high heavy-metal binding capacity into the roots that restricts their movements into shoot tissues (Gaur and Adholeya 2004).

Thus, AM fungi can be an efficient option for fruit plant propagation for the healthy and vigorous plants so as to expand the area of fruit cultivation in drought, saline or heavy-metal-contaminated lands.

6.2 Effect of Mycorrhizal Fungi on Tropical and Subtropical Fruit Plants

6.2.1 Mango

The AM fungi are naturally harbouring in the rhizosphere Mango crop. AM fungi enhance the nutrient content and plant growth over long time period of time. Mycorrhizal colonization and sporulation showed significant effect of annual seasons on native mycorrhizal fungi in mango crop (Harinikumar and Bagyaraj 1988). In the tropic and subtropical environment, maximum colonization and sporulation occurred during the winter (November to January) season and in summer season (April to June) were unfavourable for the growth of AM/VAM fungi. There

was a negative correlation between temperature (ambient and soil) and mycorrhizal proliferation, while a positive correlation between relative humidity and mycorrhizal activities.

In this connection, the rootstocks of mango (*Mangifera indica* L.), 3-year-old varieties including Chandrakaran, Bappakai, Totapuri, Olour, Necker, Vellakulamban, Peach and Vellakulamban rhizospheres, were analysed for the AM fungi at 15–30 cm depths for spore load and root colonization (Sukhada 2012). In Totapuri, mycorrhizal spores were highest as compared to the Vellakulamban, Peach, Olour and Bappakai at 15 cm depth. Observed spores belong to the *Acaulospora*, *Glomus* and other genera. By morphology characterization, *Glomus fasciculatum* and *Glomus mosseae* were identified. In Vellakullamban and Totapuri rootstocks, the root colonization was observed greater.

Under field conditions, compared to uninoculated rootstocks, hybrids of mango including Aruna, Arka and Puneeth grafted on Totapuri rootstocks (AM inoculated) and in early 10–12 days produced shoots (Sukhada 2012).

The plant health parameters studied by observation of number of branches, soil P (available), leaf Cu, Zn and P enhanced greatly in plants colonized with AM compared to uninoculated plants under 2 years of AM fungi application.

Krishna et al. (2008) studied in vitro the effect of mycorrhization on biochemical status of mango cv. Amrapali shoot tip culture. This study was conducted to see contribution of phenols and oxidative enzymes in the mango explants browning at pre- and post-culture stages during mycorrhizal treatment for achieving lowest browning rate.

Plant treated with mycorrhizae had higher phenol and enzyme activities (in vivo). Due to mycorrhization, it could mitigate the oxidative stress and heals faster the cut portion of a wound and also phenolics leaching reduction and their oxidation in the medium. Plant with mycorrhizal treatment had better level of peroxidises, PAL and PPO, and these enzymes play a key role to reduce plant pathogen population and their pathogenicity or strengthening the lignification process of newly formed cell walls.

The better growth and survival of mycorrhizal explants was recorded because of greater antioxidant levels in mycorrhizal plant system despite the higher production of oxidative enzymes. Non-mycorrhizal plant secrete phenolic compounds responsible for browning of explant while very less amount of such chemical secrete when plant in relation with mycorrhizal association.

6.2.2 Banana

The world's major fruit crops is banana, but its yield losses (approximately 50%) are due to infection by *R. similis* (Speijer et al. 2000; Sarah et al. 1996). After roots penetration or infection of any other part, this nematode invaded entirely in the root length (i.e. the pre-infectional phase).

In banana, nematodes infect through root penetration and intercellularly travel to the cortical parenchyma and feeding on the surrounding cells of the cytoplasm. This

leads to creating cavities and dead cells, observed as necrosis. After infectional phase, the nematode reproduction and development occurred.

Elsen et al. (2008) studied the AMF bio-control agent against such kind of nematodes in banana cultivar Grand Naine. The mycorrhizal treatment with *Glomus intraradices* inoculum (300 g) of rhizosphere was inoculated as a layer. After 6 weeks, mycorrhizal colonization allows good root development in split-root set-up in the both sides. After that the right side of the split-root set-up was inoculated with 1000 nematodes. The presence of *G. intraradices* showed a shield effect against nematodes *R. similis* and *P. coffeae*. In the experiment, 72% of nematode (*R. similis*) population got reduced significantly under the co-inoculation + AMF treatment (AMF on both sides) compared with the control treatment.

Vos et al. (2012) at the pre-infectional mycorrhiza-induced resistance showed in banana the level of *R. similis* infection, and mycorrhizal root exudates observed negative impacts on the nematode infecting, host behaviour and this leads to the subsequent reduction of nematode penetration.

Mycorrhizal colonization can lead to overall plant vigour that include increased root branching, which helps to elicit the negative impact nematode infection (Stoffelen et al. 2000).

The AM fungi symbiosis influences the exudation from root that influences the host metabolism (Jones et al. 2004; Wuyts et al. 2006).

Marschner and Baumann (2003) observed that in the mycorrhizal plants, root exudate composition changes the rhizosphere microbial population, which leads to an effect on nematode population in the soil.

Therefore, for parasitic nematodes, AM fungi inoculation induces the systemic resistance in a root system of banana.

6.2.3 Papaya

Papaya (*Carica papaya* L.) is widely cultivated around the world and a highly appreciated fruit crop. Under field conditions, papaya showed a high diversity of AM fungi in its rhizosphere.

Available phosphorus in the soil governs the species richness. The AM fungi are very important for sustainable cropping systems and the soil biota (Bethlenfalvay and Barea 1994).

In this regard, AM colonization is adversely affected by maintaining high P levels and low soil pH in papaya crop.

According to Weber and Amorim (1994), Trindade et al. (2000) under fumigated and unfumigated soils (controlled conditions), the papaya plants were observed with high capabilities to form AM and observed to be benefited by AM.

Mamatha et al. (2002) studied that in the field conditions, the papaya trees respond to inoculation of *G. mosseae* and *G. caledonium* (with mixed culture/efficient AM fungi), reducing the need for P fertilization by 50%.

According to Khade et al. (2010), AM fungi aided in plant for efficient mineral uptake specially phosphorus by influencing activity of root phosphatase. It showed

the positive relationship between root colonization of AM fungi and root phosphatase activities of papaya plant under different field conditions. Meloidogyne species of nematode causes damage to papaya plant as papaya is susceptible to this nematode, and it is a major limitation for production of papaya in dry environment (Singh and Nath 1996).

According to Ramakrishnan and Rajendran (1998) plant developments and its functions were hampers by the root tissues gall formation, nematode infection and overall vigour of the plant system.

Jaizme-Vega et al. (2006) stated that the nematode reduction (only 8 nematodes per g^{-1} of root and a reproduction rate of nearly 0.12) was showed more impact by the inoculation of *G. manihotis* alone or combined with PGPR.

6.2.4 Grapes

AM fungal colonization changes the N fertilization in grapevine and is affected by berry composition. Karagiannidis et al. (2007) studied that the effect on grapevine root colonization and sporulation by AM fungal is highly influenced by different nitrogenous fertilizers, different nutrition, and composition of berry. In this study, the pot trail (for 3.5-year) was performed by supplying grapevine plants with different N forms such as calcium nitrate, urea, ammonium nitrate, or ammonium sulphate.

In one year of study, three plants per pot of old grapevine were transplanted. Each pot treated with solution of nutrients and rates in mg kg^{-1} soil such as 25.4 KCl, 29.7 K_2SO_4, 162 $Ca_5(PO_4)_3OH$, 27.6 $MgCl_2.6H_2O$, that give 90, 80, 10 kg ha^{-1}, P, K, and Mg, respectively. Applied nitrogen either as ammonium (NH_4^+), amidic (N in urea), nitric (NO_3^-) or the combination of the N forms ($NH_4^+ + NO_3^-$) in quantities in mg kg^{-1} soil as 147.63 $CO(NH_2)_2$, 314.31 $(NH_4)_2SO_4$, 390.5 $Ca(NO_3)_2$ or 190 NH_4NO_3 gives 200 kg N ha^{-1}.

In the transplant hole, 30 g of inoculum was applied. The *Glomus mosseae* spores, hyphae and maize roots (colonized) were applied in the inoculum. The plants were maintained by pruning every year during mid-winter. Root colonization (avg. 57–77%) was observed by AM fungi in the grapevine plants.

In the treatment, NO_3–N favoured the development of spores while urea treated showed colonization of root (AM) reduction in comparison to the other treatments of nitrogenous form and the limited sporulation.

As compared to the non-mycorrhizal plants, the mycorrhizal plants showed almost two fold shoot dry weight and leaves about 50% more.

The different nitrogenous forms showed no impact on uninoculated plants and also showed no difference in dry weight or number of leaves regardless of the N source. Comparatively mycorrhizal plants showed greater dry weight with NO_3–N, lower with NH_4–N and urea, and moderate with NH_4^+ with NO_3^- (combined); mycorrhizal plants treated with $(NH_4)_2SO_4$ had a lower number of leaves compared to other N treatments.

There were no significant effects of the AM fungal colonization in the total soluble solids. Whereas berries (non-mycorrhizal) have more acids as compared to the berries treated with mycorrhiza, but for NH_4NO_3 was statistically true only and observed opposite effect of urea.

In the presence of NO_3^- with mycorrhizal grapevine showed better growth as it happened due to in the xylem this form is readily mobile and may be stored root vacuoles, or shoot, or other storage organs. Mycorrhizal grapevine in the presence of NO_3^- showed better growth due nitrogen in this form is readily mobile or may be stored in root vacuoles or shoot, or other storage organs.

6.3 Fruit Plants Suitable for Sub-Tropical Climate

6.3.1 Citrus

Among the fruit tree crops, citrus is one of the leading fruit crops. Citrus plants normally grown between latitudes 40°N and 40°S. In the field conditions, most citrus plants strongly depend on mycorrhizal association due to lack root hairs, because it facilitates the root hairs for minerals and uptake of water (Wu and Zou 2009). Citrus with AM fungi showed better citrus seed germination by mutualistic symbiosis with seed and competing with pathogens for space and nutrients.

Barman et al. (2007a, b) studied that as compared to the uninoculated plant, AM fungi-treated plant showed higher vigour than control, and softwood grafting done on inoculated stocks recorded significantly better graft success and graft survival percentage.

Studies on AM fungi effects on citrus has established that AM fungi can enhance citrus plant vigour by enhancing uptake of phosphorus and microelements like Zn, Cu and so on in the sterilized soil and non-fertile desert soils (Daft and Nicolson 1966; Hattingh and Gerdemann 1975; Timmer and Layden 1978; Menge et al. 1977).

Due to extramatricular hyphae of the fungus, nutrient absorption got enhanced and proliferating beyond the root nutrient depletion zone. Wang et al. (2008) studied the iron absorption by red tangerine (*Citrus reticulata*) and trifoliate orange (*Poncirus trifoliata*) under the influence of *Glomus versiforme* in sand culture under different pH values, that is pH of 5.0, 6.0, 7.0 or 8.0 for trifoliate orange and pH of 5.2, 6.2, 7.2 or 8.2 for red tangerine.

With the increasing pH value, the colonized root length in percentage reduced from 51.3 to 28.3% in *P. trifoliata* and reduced significantly in *C. reticulata* from 55.2 to 27.2%. As compared to the uninoculated one, (control) the AM seedlings observed greater dry shoot weights.

For *C. reticulata*, pH level (pH 7.2 and 8.2) showed significant differences in shoot dry weights. The contents of chlorophyll were decreased by 30.7% in AM seedlings and inoculated controls by 26.5%, suggesting that in the rhizosphere the iron absorption and its translocation to the shoots significantly enhanced *G. versiforme.*

Seedlings of *P. trifoliata* inoculated with AM fungi observed higher chlorophyll contents than uninoculated controls significantly, but there were no effects found when inoculated with *C. reticulata.*

With AM fungi, seedlings observed higher contents of iron compared to non-mycorrhizal seedlings. However, in *C. reticulata* at high pH, no significant differences were observed between AM and uninoculated control.

The plants with AM also observed higher ferric chelate reductase activity in the root compared to non-mycorrhizal controls, indicating that the reduction of Fe^{3+} to Fe^{2+} was enhanced by *G. versiforme*. At high pH level (pH 6.0–8.0), the *P. trifoliata* showed significant differences between AM and non-mycorrhizal seedlings but no such effects were observed in *C. reticulate*. The ratios of P/Fe and 50 (10P + K)/Fe were used to observe iron deficiency (chlorosis) status because with severity in chlorosis, the increase in P and decrease in iron occurred because roots of plant in Fe deficiency were used to excrete H^+ into the soil at higher rates which contributed to the conversion of HPO_4^{2-} to $H_2PO_4^-$, which was more easily absorbed by plants, resulting in higher P concentrations in plants. For iron absorption, the pH levels of 6.0 and 6.2 observed optimum values by the rootstocks of two citrus.

6.3.2 Guava

The effect of mycorrhizal fungi on the plant vigour, uptake of nutrients and exchange of gas in micro-propagated guava (*Psidium guajava* L.) plantlets was investigated for adaptation and plant establishment (Estrada-Luna et al. 2000).

Asexually propagated guava plantlets by tissue culture were grown for 18 weeks in a glasshouse. With a mixed endomycorrhiza, isolated plantlets (half in number) were inoculated from Mexico, ZAC-19 including *G. albidu*, *G. claroides* and *Glomus diaphanum.*

Ashton-modified nutrient solution was used for plantlets fertilized with long supplied 11 mg P ml $\pm$ 1. The measurements of gas exchange were taken at different time intervals, that is 2, 4, 8 and 18 weeks after treatment, using a portable photosynthesis system.

During transplant shock, all micropropagated guava plantlets were survived. After 6 weeks, greater shoot growth with mycorrhizal plantlets and production of leaf as compared to non-mycorrhizal plantlets were observed. The mycorrhizal plants with enhanced stomatal conductance and photosynthetic rates were also corresponded with this.

The plantlets with mycorrhizal observed enhanced leaf area, leaf, stem, shoot length and dry mass of root after 18 weeks. The higher photosynthetic rates observed were due to the higher P content in plantlets of mycorrhiza and enhanced content of Cu and Mg.

For phosphorylation processes, the Mg in chlorophyll is a major component and work as a cofactor to activate the most enzymes. For electron transport system, Cu is involved and is also a major component of the plastocyanin (chloroplast protein).

The container size was restricting the growth of the larger mycorrhizal plantlets and hence the exchange of gas was equivalent among treatments.

Non-mycorrhizal plantlet treatment observed increased leaf area ratios and specific leaf areas compared to plantlets with mycorrhiza treatment. In mycorrhizal plantlets, enhanced leaf tissue mineral levels of P, Mg, Cu and Mo were also observed. Root plantlets of guava (mycorrhizal) were massively found with arbuscules, vesicles and endospores. From that study, they concluded that plantlets of guava were greatly mycotrophic with 103% mycorrhizal dependency index.

6.3.3 Litchi

David et al. (2001) studied about the enhancement of growth of *Litchi chinensis* Sonn. trees arbuscular mycorrhizal fungi inoculation. Arbuscular mycorrhiza provide benefit to the litchi. In air-layers litchi were grown in ca. 95-l pots for 469 days in soil-free substrate spiked arbuscular mycorrhizal roots and observed that enhancement the vigour in all plant (litchi) health parameters.

Fertilization with high phosphorus (single-time application of ca. 1.32 g l^{-1} slow-release triple-superphosphate) observed no noticeable impact on mycorrhiza formation, litchi acclimatization, CO_2 assimilation (net) or vigour.

In South Florida, indigenous arbuscular mycorrhizal fungi inoculation observed enhanced the height and leaf production with great expansion of leaflet after 120 days of inoculation but did not affect CO_2 assimilation (net), stem diameter growth, or acclimatization and adaptation.

Inoculated plants leaflets recorded higher concentrations of Zn, K, Cu and P, lower concentrations of Mn, Ca and Mg as compared to the control plants, but Kjeldahl nitrogen in total and after 10 months inoculation, Fe concentrations did not differ significantly.

Mycorrhizae spiked litchi plants improves phosphorus bioavailability and become no longer limiting factor for overall developments. Regardless of the growth-limiting nutrient elements involved, they concluded that arbuscular mycorrhizal fungi indigenous to South Florida soils can substantially improve litchi air-layer growth in pots of soil-free medium.

6.3.4 Olive

Successful programmes of revegetation in soils where the water supply limits plant growth may require improvement of plant drought resistance through mycorrhizal inoculation (Requena et al. 2001).

In mycorrhizal fungus, the combined impact between (*Glomus intraradices*) and water stress on stomatal conductance, transpiration and photosynthetic rates, intrinsic water use efficiency and nutrient contents in leaves of *Olea europaea* L. subsp. *Sylvestris* and *Rhamnus lycioides* L. seedlings was studied by Caravaca et al. (2003). Soil water deficit was imposed for 6 weeks.

Plants with well-watered were maintained (each plant species) at water potential equivalent to capacity of field (−0.03 MPa) and plants in stress were maintained at water potential near to wilting point (average −0.06 MPa).

Water stress period, the inoculation of mycorrhizal and water regime had no significant effect on plant health parameters such as height, the basal diameter or shoot dry mass of the *O. europaea* and *R. lycioides* seedlings.

G. intraradices-colonized *O. europaea* seedlings under drought stress observed significant enhancement in photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_s) over their non-mycorrhizal counterparts (similar-sized).

However, there were no significant differences in P_N of inoculated and non-inoculated *R. lycioides* seedlings subjected to drought stress.

Wright et al. (1998) studied that the P_N may have been stimulated in inoculated *O. europaea* seedlings by the enhanced arising strength of sink from the extra requirements of carbon for the mycorrhizal fungus colonizing the roots. Water deficit in inoculated and non-inoculated *O. europaea* seedlings affected P_N and g_s in equal proportion, and thus the intrinsic water use efficiency (P_N/g_s) was not altered by AM colonization. However, the higher increases in g_s with respect to P_N in inoculated *R. lycioides* seedlings decreased P_N/g_s with respect to non-inoculated seedlings. The intrinsic water use efficiency is a physiological indicator of the drought tolerance of plants (Diaz and Roldan 2000). Under well-watered conditions, mycorrhizal *R. lycioides* seedlings showed higher instantaneous carbon gain at the expense of consuming available water. In contrast, the photosynthetic activity decreased under drought conditions.

It was stated that *O. europaea* seedlings inoculated with *G. intraradices* would be better suited to semi-arid environments than their non-mycorrhizal counterparts. Colonization by AM fungi diminished the drought tolerance of *R. lycioides*, resulting in lower intrinsic water use efficiency than for non-inoculated seedlings.

6.4 Fruit Plants Suitable for Arid and Semi-Arid Climate

6.4.1 Ber

To determine the influence of AM fungus, pot experiment was conducted in a nursery for plant vigour and mineral uptake in ber (*Ziziphus Mauritiana*) by Guissou (2009) in agroforestry systems (Sahelian). *Glomus aggregatum inoculated or untreated* plants were allowed to grow for 4 months in a sterilized phosphorus (P)-deficient sandy soil (2.18 ppm P). Treatment with *G. aggregatum* significantly increased plant health in terms of shoot height by 4.0 times as compared to the controls.

In the inoculated plants, the total dry weight produced was four times higher compared to the controls. It was observed that 91% of AM colonization of root and no mycorrhizal structures were found in the roots of uninoculated control plants.

Mycorrhizal dependency value was about 77% in jujube seedlings. In AM plants, N concentration in shoot was enhanced by 78.08%. *G. aggregatum* also significantly

increased P concentration by approximately 8.33-fold. The K concentration was significantly increased by 62.99% over the controls. *G. aggregatum* increased the Mg concentration by 2.67 fold.

The result showed clearly that AM inoculation with *Glomus aggregatum* is highly beneficial for jujube fruit trees. The absence of AM inoculation in jujube fruit trees could lead to a higher mortality of plants, which is highly dependent of AM fungi for its juvenile growth and development in P-deficient soils.

6.4.2 Avocado

The mycorrhizal fungi effect on the growth and nutrition of avocado (*Persea americana* mill.) seedlings was studied by Menge et al. (1980). The 'Topa Topa' seedlings of avocado were grown in loamy steamed sandy soil (pot trial) containing different fertilizers with either without fertilizer or absolute fertilizer (N, K, P, Ca, Mg, S, Zn, Mn, Cu, Fe, B, Mo), -Zn, -P, -P and -Zn, and -Zn + 10xP.

The *Glomus fasciculatum* (0-1 and 463) were used for seedlings treatment of *Citrus sp. and avocado tree* in pot culture inoculum and added 10 aliquots to each pot. Isolate 0–1 in *Citrus* sp. 463 in avocado were reisolated and proved for providing nutrient access.

In the Zn + 10xP fertilizer treatment, nonmycorrhizal plants (dry weight) were significantly influenced the growth that enhanced 142% and 133% over the no-fertilizer and complete fertilizer treatments, respectively.

It was observed that mycorrhizal plants were 98% larger on the average compared to the non-mycorrhizal plants with the same treatments of fertilizer, except those given -Zn + 10xP. The mycorrhizal isolates enhanced the uptake of P, N and Cu at all treatments of fertilizer, and Zn uptake was enhanced with all treatments of fertilizer.

Phosphorous fertilization enhanced the P concentrations in leaves of mycorrhizal seedlings but did not alter P concentrations in leaves of non-mycorrhizal plants. In mycorrhizal and non-mycorrhizal treated with 10xP fertilization enhanced the P concentrations in seedlings. Due to the destruction of mycorrhizal fungi, poor growth of avocado seedlings in steamed or fumigated soil was observed, and it can be related to the poor mineral nutrition.

One GF isolate (GF 0–1) proved to be superior to the other isolates depending on the host avocados mineral nutrition. Due to the rate of growth or ability to infect the differences between the isolates was found. The avocado seedlings growth could be improved due to mycorrhizal fungi added to fumigated or steamed soils in the nursery or greenhouse and it can also reduce the cost of fertilization.

6.4.3 Date Palm

Bouamri et al. (2006) studied in Morocco that the rhizosphere of date palm and the arbuscular mycorrhizal fungal species were associated. Based on one palm tree rhizosphere sample per site, ten soil sampling sites were tested (Fig. 6.1).

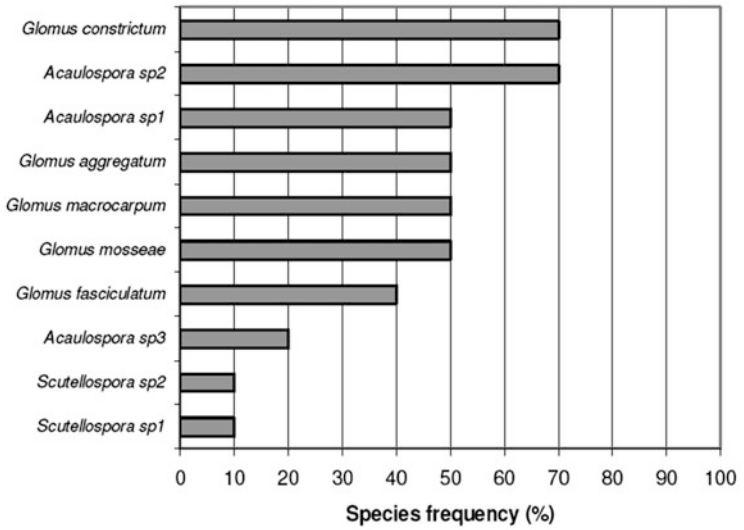

Fig. 6.1 *Phoenix dactylifera* L. rhizosphere survey for arbuscular mycorrhizal fungi diversity in an arid zone of South-West of Morocco

The soil and root samples (4 each) were harvested from each tree from 10 to 40 cm depth around the tree and mixed together for analysis.

Isolates including *G. macrocarpum*, *G. aggregatum*, *G. mosseae* and *Acaulospora* sp1 were found from 50% of the sites.

In semi-arid and arid habitats, *Glomus* species were found predominant in 40–70% range. Thus, in drought soil and salinity stresses, *Glomus* was considered the best genus for adaptation because of their dominance under those ecosystems.

6.4.4 Pomegranate

Aseri et al. (2008) studied the bio-fertilizers (*Azotobacter chroococcum*, *Azospirillum brasilense*, *Glomus mosseae* and *Glomus fasciculatum*) response in pomegranate cuttings followed by their acclimatization for transplantation in difficult field conditions such as Indian Desert (Thar), Rajasthan. Ten millilitres of cell suspension was used as inoculums for both bacterial types. The soil (10 g) with root bits (containing 8–10 propagules of AM fungal g^{-1} soil) were applied as inoculum.

The pomegranate of 6-month-old cuttings (semi-hard wood cuttings) were treated with 200 ppm solution of indole butyric acid for 12 h and then planted in polybags in the June month of the year 2000. In bio-fertilizers inoculated, cutting higher number of branches was observed as compared to uninoculated controls at 4 months after planting.

However, by using various bio-fertilizers, the noticeable differences were not found in terms of number of branches in the plants. While significant increase in leaf

area was recorded with the application of bio-fertilizers. With *A. brasilense,* the maximum increase was found followed by dual-inoculation treatment.

The shoot dry weight was enhanced by 16–36% using bio-fertilizer inoculation. In dual inoculation treatment the effect of variation was maximum, while *G. Fasciculatum* was observed minimum.

In *G. mosseae* and *A. brasilense* alone noticeable total chlorophyll were found but in dual-inoculated seedlings, the total chlorophyll was recorded highest. In the amino nitrogen and reducing sugar contents, the similar trend was observed, whereas with *A. brasilense* total phenols were recorded maximum followed by dual-inoculation treatment.

Bio-fertilizers with inoculation in soil showed significant enhancement in the soil enzyme activities such as *dehydrogenase, alkaline phosphatase, nitrogenase* and hydrolysis of fluorescein diacetate (FDA) in pomegranate rhizospheric soils than uninoculated control plants. The percent root colonization (after 4 months of inoculation) of AM fungi was enhanced by 15–38% compared to control. The dual-inoculation treatment had a maximum increase in the number of spores (203 spores/50 g soil) compared to control.

6.4.5 Mulberry

The soil inoculation effects with arbuscular mycorrhizal fungus *Glomus fasciculatum* (containing 2083 infective propagules/g) and a mycorrhizal helper bacterium *Bacillus coagulans* were investigated by Mamatha et al. (2002) for P fertilizer (at two levels) on mulberry plants (var. M5) already adapted in the field condition for one decade at Bangalore.

When plants were inoculated with AM fungi, the height of plant and number of leaves per plant in mulberry were maximal and given 50% recommended P. Mulberry plants are treated with AM fungus alone or AM fungus *plus B. coagulans.* This combination given is 50% P and majorly higher P concentration in leaf compared to the 50% or 100% P in treated or uninoculated plants.

6.5 Fruit Plants Suitable for Temperate Climate

6.5.1 Apple

Derkowska et al. (2008) studied the mycorrhization and organic mulches influence on mycorrhizal frequency and intensity in roots of one-year-old apple cv. ‘Gold Milenium’.

The above experiments were performed in different treatments including control, peat mulch, bark, sawdust, compost, straw and mycorrhizal substrate. When plants mulched with peat and bark observed the highest mycorrhizal frequency for the roots, while the lowest observed in mulched with sawdust in apple.

6.5.2 Plum

The mycorrhizal fungi effect on the growth and yield of 'Cacanska Lepotica' plum tree grafted on *Prunus tomentosa* rootstock was estimated by Slawomir and Aleksander (2010). In the first year (June month) of tree development, 1000 units per 1 plant mycorrhizal fungi dose was performed into the tree root systems. The growth of plum trees was significantly induced by mycorrhizal inoculum. The greater yield and yield efficiency per 1 cm^{-2} of trunk cross-sectional area was observed in mycorrhiza inoculation. However, there was no influence of mycorrhizal inoculum on the size of fruits.

6.5.3 Peach

According to Kipkoriony and Fusao (2006) in the mycorrhizal treatments, shoot length was significantly higher after 3 months and lowest were found in the non-mycorrhizal treatment without the charcoal amendment. In concomitant low shoot/root ratios, shoot growth was relatively lower in mycorrhizal seedlings treated with root-bark extracts and in treated non-mycorrhizal seedlings.

In mycorrhizal seedlings, shoot P was consistently higher irrespective of charcoal amendment or treatment with the extracts of root-bark. While in non-mycorrhizal seedlings, root P was better in the presence of activated charcoal, whereas the opposite was true for mycorrhizal seedlings.

Based on the above study, it is clear that due to release of phytotoxic substances upon decomposition (Allelopathic effect), peach root-bark extracts significantly inhibited growth of both mycorrhizal and non-mycorrhizal seedlings.

In the soil amendment, activated charcoal is very useful because of its ability to adsorb phytotoxic substances but the activated charcoal delayed the establishment of mycorrhizal symbiosis due to the adsorption of signal chemicals that exudates from respiring roots and play key role in the host and fungus signalling events that lead to the establishment of symbiosis. Therefore, after mycorrhizal symbiosis has been established, only then activated charcoal should be applied.

6.5.4 Kiwifruit

Calvet et al. (1989) studied that the inoculation of kiwifruit cv. Hayward seedlings and hardwood cuttings with *Glomus mosseae* showed that the roots of AM seedlings had more infection with hyphae and arbuscules than those of hardwood cuttings due to morphological differentiation at different levels.

There was a significant improvement in the percentage of vesicular infections between 1- and 2-month-old seedlings. The percentage of infected roots with active arbuscules did not significantly vary between 1- and 2-month-old seedlings, but there was a drastic change between 5- and 8-month-old cuttings.

6.5.5 Cherry

Yildiz et al. (2010) studied that the AMF effect on the growth and mineral absorption of rootstocks micro-propagated cherry was examined during adaptation, establishment and maintenance of the plant. The 'edabriz' and 'gisela 5′ (two cherry rootstocks) were propagated by using tissue culture and grown under greenhouse for 16 weeks.

Plantlets were treated using *Glomus clarum*, *G. caledonium*, *G. etunicatum*, *G. intraradices*, *G. mosseae*, cocktail (mixture of these species) and mycorrhiza (indigenous) into three different mixtures of substrates. All micropropagated cherry plantlets after transplanting were adopted successfully.

The greater nutrient uptake was observed in mycorrhizal plantlets compared to the non-mycorrhizal plantlets after 16 weeks of intervals.

Heavy colonization of AMF was observed in the inoculated cherry plantlets. During transplantation, the inoculation from in vitro to ex vitro culture indicated that the growth responses can be significantly induced by mycorrhizal association.

Healthier and higher Zn and P contents in the mycorrhizal cherry rootstocks were observed compared to controls for both rootstocks. *G. mosseae*. From Çukurova region, an indigenous AMF was isolated, and it also showed significantly enhanced plant vigour and nutrient absorption. Comparatively, 'Gisela 5' rootstocks had higher P and Zn contents compared to the Edabriz.

Based on the results, careful selection of compatible host/fungus/substrate combinations showed the maximum benefit. The performance of micropropagated plants may significantly enhanced by ensuring a stable mycorrhizal establishment during planting.

Horticultural woody plants when treated with efficient AMF (in vitro) exhibit significant survival and quality.

Moraes et al. (2004) observed that in vitro propagated plants were prone and lacking strength to adopt shock during transplant with great losses. There was a positive impact on plantlets due to mycorrhization (in vitro propagated) (Rai 2001; Kapoor et al. 2008).

From the results it is concluded that AMF inoculations improve plant vigour and development of micropropagated plants.

6.5.6 Blueberry

Gardes and Dahlberg (1996) studied that ericoid mycorrhizas are mostly found in the roots of dwarf shrubs throughout temperate and boreal ecosystems. Kasurinen and Holopainen (2001) studied mycorrhizal colonization of highblush blueberry (*Vaccinium corymbosum*) and its native relatives, wild bilberry (*V. myrtillus*) and bog whortleberry (*V. uliginosum*). Two highblush blueberry varieties investigated were 'North Country' and 'North Blue'. By using transmission electron microscopy (TEM) analysis, it is observed that in all species ericoid mycorrhizas formed hyphal coil inside the epidermal root cells. The highest mycorrhizal colonization was found

in the roots of wild bilberries (51%) by using stereomicroscopic view, whereas in ergosterol assay the highest total fungal biomass of roots was observed in bog whortleberry (209 μg g^{-1} of root dry weight). Thus, further step would be to test experimentally whether the mycorrhizas infecting the roots are truly beneficial to the highbush blueberries growing under agricultural field conditions.

6.6 Conclusion

The beneficial effects of AM mycorrhizal fungi were observed on plant health parameters and overall plants vigour. It is also observed as biocontrol agent with all other PGPR properties. This symbiotic interaction leads to the reduction of chemical fertilizers, pesticides and other agricultural inputs satisfactorily where they play the key role for sustainable horticultural/agricultural practices. By using biotechnological tools, AM fungi and rhizobacteria have the potential to be useful for benefiting plant health parameters.

According to Azcon and Barea (1997), the AM fungal inoculation process needs to be improved for the efficient application of AM fungal biotechnology in good horticultural crop plant production systems. In agro-ecosystems, mycorrhizal colonization is proved to be low input, as well as intensively managed practice. Undesirable or access use of phosphatic fertilizers and biocides leads to the disruptive to the association of mycorrhiza.

Presently, there are huge gaps in our experimentation and the application of the mycorrhizal association and the significance of AM fungal diversity in producing the several benefits including plant health and disease management in addition to the application effect on different agronomic practices, ecological balance.

For AM fungal physiology and function, our knowledge and understandings are limited, and their role in different environmental conditions and crops are needs to be updated with new tools and techniques so as to achieve effective AM fungi efficient use and manipulation for the long period agricultural sustainability. Nowadays, the cultivation of fruit plants and their application using micropropagation techniques and plant transformation under controlled condition makes it easy to inoculate plants with AM fungi. The AM fungi considered as plant strengtheners can become the basic tools for urban horticulture and natural farming systems that will promote future developments and will identify new demands and challenges for the mycorrhizal technology.

References

Al-Karaki GN, Al-Raddad A (1997) Effects of arbuscular fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in their drought resistance. Mycorrhiza 7:83–88

Aseri GK, Jain N, Panwar J, Rao AV, Meghwal PR (2008) Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of Pomegranate (*Punica granatum* L.) in Indian Thar Desert. Sci Hortic 117:130–135

Auge RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Azcon AC, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. Mycorrhiza 6:457–464

Azcon AC, Barea JM (1997) Applying mycorrhiza biotechnology to horticulture: significance and potentials. Sci Hortic 68:1–4

Barman P, Swamy GSK, Patil PB, Patil CP, Thammaiah N (2007a) Softwood grafting of seedless lime as influenced by Arbuscular Mycorrhizal fungi and different age of rootstocks. Karnataka J Agric Sci 20(3):691–693

Barman P, Swamy GSK, Patil PB, Patil CP, Thammaiah N (2007b) Effect of age of rootstocks on the success of softwood grafting and growth of lime grafts. Asian J Hortic 2(1):95–98

Bethlenfalvay GJ, Barea JM (1994) Mycorrhizae in sustainable agriculture. I. Effects on seed yield and soil aggregation. Am J Alter Agric 9:157–161

Bouamri R, Dalpé Y, Serrhini MN, Bennani A (2006) Arbuscular mycorrhizal fungi species associated with rhizosphere of *Phoenix dactylifera* L. in Morocco. Afr J Biotechnol 5(6):510–516

Calvet C, Pera J, Estaun V, Camprub A (1989) Vesicular-arbuscular mycorrhizae of kiwifruit in an agricultural soil: inoculation of seedlings and hardwood cuttings with *Glomus mosseae*. Agronomie 9:181–185

Caravaca F, Diaz E, Barea JM, Azcon-Aguilar C, Roldan A (2003) Photosynthetic and transpiration rates of *Olea europaea* subsp. *Sylvestris* and *Rhamnus lycioides* as affected by water deficit and mycorrhiza. Biol Plant 46(4):637–639

Caris C, Hoerdt W, Hwkins HJ, Roenheld V, George E (1998) Studies on the iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. Mycorrhiza 8:35–39

Chandreshekara CP, Patil VC, Sreenivasa MN (1995) VA-mycorrhiza mediated P effect on growth and yield of sunflower (*Helianthus annuus* L.) at different P levels. Plant Soil 176:325–328

Daft MJ, Nicolson TH (1966) Effect of *Endogene* mycorrhiza on plant growth. II. Influence of soluble phosphate on endophyte and host in maize. New Phytol 65(3):343–350

David PJ, Michelle SS, Bruce S, Jonathan HC (2001) Inoculation with arbuscular mycorrhizal fungi enhances growth of *Litchi chinensis* Sonn. trees after propagation by air-layering. Plant Soil 233:85–94

Derkowska E, Sas-Paszt L, Sumorok B, Szwonek E, Głuszek S (2008) The influence of mycorrhization and organic mulches on mycorrhizal frequency and intensity in apple and strawberry roots. J Fruit Ornam Plant Res 16:227–242

Diaz E, Roldan A (2000) Effect of reforestation techniques on the nutrient content, photosynthetic rate and stomatal conductance of *Pinus halepensis* seedlings under semi-arid conditions. Land Degrad Develop 11:475–486

Elsen A, Gervacio D, Swennen R, Waele DD (2008) AMF-induced bio-control against plant parasitic nematodes in *Musa* sp.: a systemic effect. Mycorrhiza 18:251–256

Estrada-Luna AA, Davies FT, Egilla JN (2000) Mycorrhizal fungi enhancement of growth and gas exchange of micro-propagated guava plantlets (*Psidium guajava* L.) during ex *vitro* acclimatization and plant establishment. Mycorrhiza 10:1–8

Gardes M, Dahlberg A (1996) Mycorrhizal diversity in artic and alpine tundra. New Phytol 133:147–157

Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86(4):528–534

Guissou T (2009) Contribution of arbuscular mycorrhizal fungi to growth and nutrient uptake by jujube and tamarind seedlings in a phosphate (P) - deficient soil. Afr J Microbiol Res 3(5):297–304

Harinikumar KM, Bagyaraj DJ (1988) The effect of season on VA mycorrhizae of leucaena and mango in a semi-arid tropic. Arid Soil Res Rehabil 2(2):139–143

Hattingh MJ, Gerdemann JW (1975) Inoculation of Brazilian sour orange seed with endomycorrhizal fungus. Phytopathology 65:10–13

Jaizme-Vega MC, Rodríguez-Romero AS, Barroso Núñez LA (2006) Effect of the combined inoculation of arbuscular mycorrhizal fungi and plant- growth promoting rhizobacteria on papaya (Carica papaya L.) infected with the root-knot nematode Meloidogyne incognita. Fruits 61:1–7

Jamal A, Ayub N, Usman M, Khan AG (2002) Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soybean and lentil. Int J Phytoremediation 4(3):203–221

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Kapoor R, Sharma D, Bhatnagar AK (2008) Arbuscular mycorrhizae in micro-propagation systems and their potential applications. Sci Hortic 116:227–239

Karagiannidis N, Nikolaou N, Ipsilantis I, Zioziou E (2007) Effects of different N fertilizers on the activity of *Glomus mosseae* and on grapevine nutrition and berry composition. Mycorrhiza 18:43–50

Kasurinen A, Holopainen T (2001) Mycorrhizal colonisation of highbush blueberry and its native relatives in central Finland. Agric Food Sci Finland 10:113–119

Khade SW, Rodrigues BF, Sharma PK (2010) Symbiotic interactions between arbuscular mycorrhizal (AM) fungi and male papaya plants: its status, role and implications. Plant Physiol Biochem 48(10–11):893–902

Kipkoriony LR, Fusao M (2006) Effect of mycorrhizal inoculation and activated charcoal on growth and nutrition in peach (*Prunus persica* Batsch) seedlings treated with peach root-bark extracts. J Jpn Soc Hortic Sci 75(6):463–468

Krishna H, Sairam RK, Singh SK, Patel VB, Sharma RR, Grover M, Nain L, Sachdev A (2008) Mango explants browning: effect of ontogenic age, mycorrhization and pre-treatments. Sci Hortic 118:132–138

Li XL, George E, Marschner H (1991) Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant Soil 136:41–48

Liu A, Hamel C, Elmi A, Costa C, Ma B, Smith DL (2002) Concentrations of K, Ca and Mg in maize colonised by arbuscular mycorrhizal fungi under field conditions. Can J Soil Sci 82 (3):271–278

Mamatha G, Bagyaraj DJ, Jaganath S (2002) Inoculation of field-established mulberry and papaya with arbuscular mycorrhizal fungi and a mycorrhiza helper bacterium. Mycorrhiza 12:313–316

Marschner P, Baumann K (2003) Changes in bacterial community structure induced by mycorrhizal colonization in split-root maize. Plant Soil 251:279–289

Menge JA, Lambright H, Johnson ELV (1977) Utilization of mycorrhizal fungi in citrus nurseries. Proc Int Soc Citricult 1:129–132

Menge JA, Larue J, Labanauskas CK, Jhonson ELV (1980) The effect of two mycorrhizal fungi upon growth and nutrition of avocado seedlings grown with six fertilizer treatments. J Am Soc Hortic Sci 105(3):400–404

Moraes RM, Andradez DE, Bedir E, Dayan FE, Lata H, Khan I, Pereira AMS (2004) Arbuscular mycorrhiza improves acclimatization and increases lignan content of micro-propagated mayapple (*Podophyllum peltatum* L.). Plant Sci 166:23–29

Rai MK (2001) Current advances in mycorrhization in micro-propagation. In Vitro Cell Dev Biol Plant 37:158–167

Ramakrishnan S, Rajendran G (1998) Influence of Meloidogyne incognita on yield components and physiological functions of papaya. Nematol Med 26:225–228

Requena N, Perez-Solis E, Azcon-Aguilar C, Jeffries P, Barea JM (2001) Management of indigenous plant-microbe symbiosis aids restoration of desertified ecosystems. Appl Environ Microbiol 67:495–498

Sarah JL, Pinochet J, Stanton J (1996) The burrowing nematode of bananas, Radopholus similis Cobb, 1913. Musa Pest Fact Sheet N° 1. The International Network for the Improvement of Banana and Plantain, Montpellier, France

Singh VS, Nath RP (1996) Pathogenicity of rootknot nematode Meloidogyne incognita on papaya. Indian J Nematol 26:115–116

Slawomir S, Aleksander S (2010) The influence of mycorrhizal fungi on the growth and yielding of plum and sour cherry trees. J Fruit Ornam Plant Res 18(2):71–77

Speijer PR, Gold CS, Karamura EB, Goossens B, Elsen A, De Waele D (2000) Rate of nematode infestation of clean banana planting material (Musa spp. AAA) in Uganda. Acta Hortic 540:461–470

Stoffelen R, Verlinden R, Xuyen NT, Swennen R, Wae DD (2000) Host plant response of *Eumusa* and *Australimusa* bananas (*Musa* spp.) to migratory endoparasitic and root-knot nematodes. Nematology 2:896–907

Sukhada M (2012) Arbuscular mycorrhizal fungi benefit mango (*Mangifera indica* L.) plant growth in the field. Sci Hortic 143(16):43–48

Timmer LW, Layden RF (1978) Stunting of citrus seedlings in fumigated soils in Texas and its correction by phosphorus fertilization and inoculation with mycorrhizal fungi. J Am Soc Hortic Sci 103:533–537

Trindade AV, Farias NG, Almeida FP (2000) Adequação do uso de esterco na obtenção de mudas de mamão (Carica papaya L.) inoculadas com fungos micorrízicos arbusculares. Brasília Pesq Agrop Bras 35:1389–1394

Vos C, Broucke DVD, Lombi FM, Waele DD, Elsen A (2012) Mycorrhiza-induced resistance in banana acts on nematode host location and penetration. Soil Biol Biochem 47:60–66

Wang M, Christie P, Xiao Z, Qin C, Wang P, Liu J, Xie Y, Xia R (2008) Arbuscular mycorrhizal enhancement of iron concentration by *Poncirus* trifol*i*ata L. Raf and *Citrus reticulata* Blanco grown on sand medium under different pH. Biol Fertil Soils 45:65–72

Weber O, Amorim SMC (1994) Adubação fosfática e inoculação de fungos micorrízicos vesículo-arbusculares em mamoeiro "solo". R Bras Ci Solo 18:187–191

Wright DP, Scholes JD, Read DJ (1998) Effects of VA mycorrhizal colonisation on photosynthesis and biomass production of *Trifolium repens* L. Plant Cell Environ 21:209–216

Wu QS, Zou YN (2009) Mycorrhiza has a direct effect on reactive oxygen metabolism of drought-stressed citrus. Plant Soil Environ 55(10):436–442

Wuyts N, Zar MZT, Swennen R, Waele DD (2006) Banana rhizodeposition: characterization of root border cell production and effects on chemotaxis and motility of the parasitic nematode *Radopholus similis*. Plant Soil 283:217–228

Yildiz A, Cagdas A, Aslihan A, Yesimyal C, Sedat S, Ibrahim O (2010) The effect of mycorrhiza in nutrient uptake and biomass of cherry rootstocks during acclimatization. Rom Biotechnol Lett 15(3):1–7

Importance of PGPRs in the Rhizosphere

7

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Abstract

Application of plant growth-promoting rhizobacteria (PGPR) for crop growth promotion and yield is an urgent need for sustainable agricultural production in view of increasing indiscriminate use of chemical fertilizers as well as plant nutrient deficiencies. Some PGPR strains have been identified and commercialized worldwide. The positive effect on crop growth and yields has been recorded. The application of PGPRs besides increasing crop growth and yield also reduces the cost of crop production and environmental risk. When PGPRs are applied in soil with different methods, they fix atmospheric nitrogen, solubilize plant nutrients, and also provide protection against soil-borne plant pathogens. Besides providing direct and indirect benefits to crop plants, the PGPRs also assist crop plants to tolerate drought and salt stress conditions. These PGPRs perform plausible mechanisms in the rhizosphere region, though abundantly documented but still remain greater scope for exploring various mechanisms. Generally, PGPRs are applied as biofertilizers and biocontrol agents, but their use as bioremediation, biodegredation, biostimulants, biopesticides, bio-osmoprotectants is very limited. Due to the nonjudicious application of chemical fertilizers, enormous opportunities are generated for the use and commercialization of PGPRs. So in this chapter, the role of PGPRs in the rhizosphere and activities performing in that zone are being described. The practical

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potential of PGPRs in crop production has also been discussed for commercial uses.

Keywords

Rhizosphere · PGPRs · Plant growth promotion · Induced systemic resistance · Tolerance to drought and salt stresses

7.1 Introduction

Soil adhered around root vicinity, called rhizosphere soil. This rhizosphere soil directly comes under influence of root exudates secreted from the plant root system (Badri and Vivanco 2009; Bais et al. 2006; Broeckling et al. 2008; Gransee and Wittenmayer 2000). In 1904, Hiltner first time described rhizosphere soil with the name of "rhizosphere effect." He observed that rhizosphere soil has an intense microbial population and activity compared with nonrhizosphere soil, bulk soil. Root exudate has recorded with triggering effect on microbial population located on rhizoplane (root surface), rhizosphere zone (buffer zone), and nonrhizosphere zone (bulk soil) (Hirsch and Mauchline 2012; Kristin and Miranda 2013; Toju et al. 2018; Turner et al. 2013; Zhang et al. 2017). Other than bulk soil, rhizoplane and rhizosphere zone are colonized more intensively by beneficial microbes, plant growth-promoting rhizobacteria (PGPRs), or harmful microbes [disease-causing microorganisms (DCMs) or plant pathogenic microbes (PPMs)] (Chaparro et al. 2012, 2014; Mitter et al. 2013; Pii et al. 2015). The beneficial microbes perform plant growth-promoting activities and provide additional nutrients availability (Adesemoye et al. 2008; Ahemad and Kibret 2014; Babalola 2010; Berendsen et al. 2012; Chauhan et al. 2015) while protecting from pathogenic microbes (Compant et al. 2005) and also tolerance to abiotic stresses (Berg et al. 2014; Fahad et al. 2015). The PGPRs can fix atmospheric nitrogen and mineralize and solubilize phosphorus, potash, silica, zinc, and oxidizing sulfur to the plants. The PGPR strain includes *Rhizobium, Azospirillum, Azotobacter, Gluconacetobacter, Bacillus, Pseudomonas, Burkholderia, Paenibacillus, Serratia*, etc. (Gupta et al. 2015; Jha and Saraf 2015; Lugtenberg and Kamilova 2009; Nehra and Choudhary 2015). *Rhizobium* is a well-known example of nodule-forming bacteria in legume crops where it fixes atmospheric nitrogen. Atmospheric nitrogen fixation by *Rhizobium* is a very sensitive process to oxygen. In the presence of oxygen, the biological nitrogen fixation pathway inhibited. *Azospirillum, Azotobacter, Gluconacetobacter*, etc. are atmospheric nitrogen-fixing bacteria of other nonlegume crops. *Bacillus* PGPR strain has better survival under adverse climatic conditions, while *Pseudomonas* is considered as better root-colonizing bacteria. These PGPR strains mineralize and solubilize fixed plant nutrients into unfix form, which is absorbed by the plant roots during the water translocation process (Bulgarelli et al. 2013, 2015; Itelima et al. 2018; Nelson 2004). Some PGPRs have been identified for restricting or limiting the population of pathogenic microbes. The population of soil-borne plant

pathogenic microbes is highly affected and reduced by the production of cell wall lytic enzymes, antimicrobial metabolites, and also competes for nutrients availability. Induced systemic resistance and phytohormone production have been reported as one of the mechanisms of PGPRs for managing plant diseases by manipulation in crop plants' physical and chemical properties. Some PGPR strains have been identified for providing tolerance to drought and salt stress through the production of osmoprotectants metabolites (Baez-Rogelio et al. 2017; Cardinale et al. 2015; Wani et al. 2016). The interaction between plant and PGPR strain is complex (Gray and Smith 2005; Jones et al. 2004; Leach et al. 2017), and PGPRs trigger cumulative effects on plant growth and yield (Bashan 1998; Bender et al. 2016; Vessey 2003), while root exudate provides a congenial atmosphere for the beneficial microbial population (Beattie 2015; Berg et al. 2016; Bossio et al. 1998; Evangelou and Deram 2014).

7.2 Rhizosphere

The soil region directly comes under the influence of root and/or root exudates, called the rhizosphere. The rhizosphere zone covers around 20–30 cm top of the undisturbed soil and consists of plant roots, soil matrix, and soil microflora and fauna. The soil microflora and fauna are bacteria, fungi, cyanobacteria, nematodes, protozoa, and even mites too. The soil provides physical supports as well as water and minerals to the plants. Plant roots secrete low-molecular-weight organic compounds such as sugars, amino acids, organic anions (OAs), and phenolics. They can easily be disintegrated and assimilated by soil microorganisms and serve as a substrate for microbes. The high-molecular-weight organic compounds (proteins, pigments, mucilage, and miscellaneous other substances) secreted by plant roots require additional extracellular enzymatic activity to break down before assimilation. Mucilage is a mixture of organic substances, released proton, oxygen, and water. In addition to organic substances, some inorganic substances like inorganic ions, H+, electrons, water, and siderophores are produced. These released substances make soil physical and chemical structural changes (Fig. 7.1) (Garcia-Pausas and Paterson 2011). Almost 20–50% of total photosynthates of the crop are secreted in the rhizosphere. These root exudates are preferably utilized by soil microbiota, and this effect is called the "rhizosphere effect," the first time described by Hiltner, 1904. The rhizosphere soil has 500 times more microbial load compared to the nonrhizospheric soil (bulk soil). The quality and quantity of secreted compounds depend on plant species cultivated and to a certain extent on soil physical and chemical properties (Andreote and Pereira 2017). Because of this reason, certain species of the bacteria and fungi can survive in this selective microenvironment, called phytomicrobiome (Badri et al. 2013). Surviving bacteria and fungi may have beneficial and harmful relationships to the crop plants. Some soil-borne plant pathogenic fungi are reported to cause economic damage to crops. The fungal genera are *Fusarium*, *Pythium*, *Phytophthora*, *Alternaria*, *Rhizoctonia*, etc. However, rhizosphere-residing bacterial population has a positive effect on

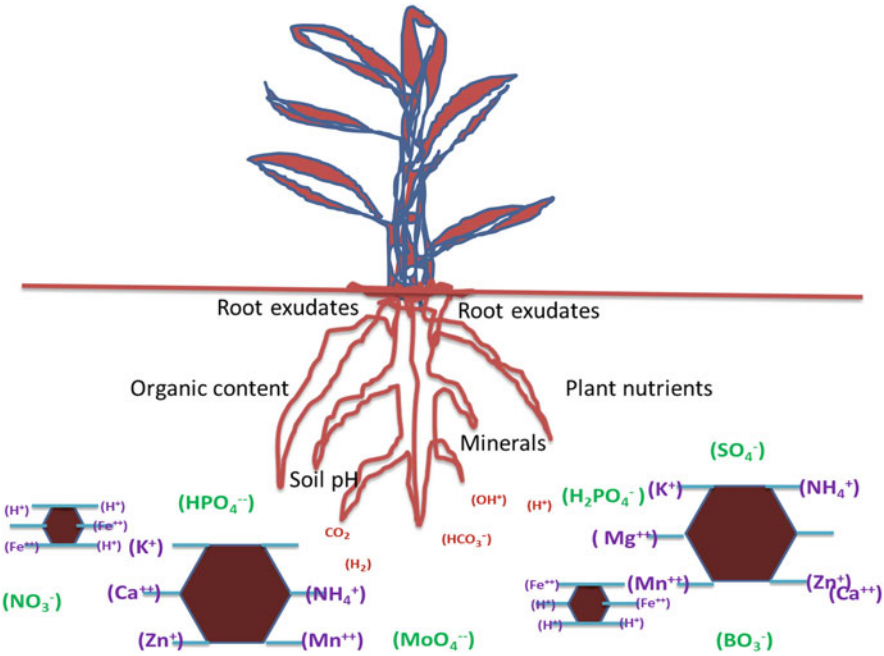

Fig. 7.1 Root exudates change soil physical and chemical properties

growth and crop yield called plant growth-promoting rhizobacteria (PGPRs). So far, well-known examples of PGPRs are *Rhizobium*, *Gluconacetobacter*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Azotobacter*, *Azospirillum*, *Burkholderia*, and Biocontrol agents (Babalola 2010). In the rhizosphere zone, a specific plant–soil–microbes interaction takes place. This interaction is mediated by chemical substances released by plants and microbes. In leguminous plants, some flavonoids are considered for playing a major role in nodule formation in plant roots (Dakora et al. 2015; Desbrosses and Stougaard 2011). Some researchers also emphasized that these flavonoids compounds may also be associated with vesicular-arbuscular mycorrhiza (VAM) colonization. Root colonization and the microbial population vary from crop growth stage. The grand growth and root elongation state of the crop are considered to have the greatest number of the rhizospheric microbial population. The ratio of the microbial population in the rhizosphere zone to bulk soil is always recorded 3–4 times more. Application of PGPR strain in the production of the agricultural crop through soil, seed, and root inoculation improves qualitative economic values and also avoids environmental risk efficiently (Fig. 7.2).

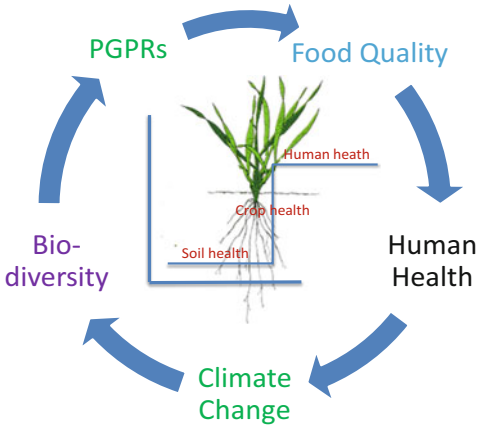

Fig. 7.2 PGPRs mediated ecological cycle while sustaining soil fertility and enhancing crop productivity

7.3 PGPRs in Rhizosphere: For Better Crop Growth and Yield

It has been estimated that almost more than a hundred million tonnes of nitrogen, phosphorus, and potassic fertilizers are applied in soil annually. Due to the nonjudicious application of these chemical fertilizers, tremendous detrimental effects on soil, water, and human beings besides the increased cost of crop production have been reported worldwide. Rhizosphere used to call root adhered soil region has intense microbial activity and also dynamic zone in the soil. In 1904, German agronomist Hiltner first time defined the term rhizosphere for the effect of legume roots on the surrounding soil. He recorded that more microbial activity at neighboring roots or root influenced soil. The reason for intense microbial activity is reported because 20% and 50% of their photosynthates released through the root (Bottner et al. 1988). The diverse group of low- and high-molecular-weight organic compounds are released in the rhizosphere. The soil microflora and fauna supported by these organic substances and microbial population in soil exert a beneficial effect on plant growth promotion and yield as well. Plant growth promotion itself describes the increased plant growth and crop yield occurred while treating seed or soil with certain plant growth-promoting bacteria. The plant growth-promoting rhizobacteria are free-living soil inhabitant bacteria. PGPRs improve seed germination, root formation, branching and tillering, fluorescence, fruit ripening in crop plants. Besides this, PGPRs also provide tolerance to biotic and abiotic stresses (Table 7.1). These plant growth-promoting attributes are finally visible in terms of increased seed germination, root formation, excessive branching and tillering, fluorescence, fruit ripening, and also tolerance to abiotic stresses. PGPRs are found in the rhizosphere of the crop plants (Kloepper et al. 1989). The effect of plant growth-promoting rhizobacteria on agricultural crops is reported by various researchers. Some PGPRs strain has been reported for plant growth-promoting attributes such as the cytokinin production by *Pseudomonas fluorescens* strain G20-18 (Bent 2006),

Table 7.1 Role of PGPRs in plant growth-promoting attributes of crop plants

S. no.	Plant growth-promoting attributes	Plant growth-promoting rhizobacteria	Agricultural crop	References
A. Biofertilizers: directly affecting plant growth promotion				
1.	Biological nitrogen fixation	*Rhizobium* spp., *Azotobacter chroococum*, *Azotobacter beijerinckii*, *Azotobacter vinelandii*, *Azospirillum brasilense*, *Azotobacter lipoferum*, *Gluconacetobacter diazotrophicus*, *Gluconacetobacter sacchari*	Legume crop, rice, sugarcane	Allen et al. (2017), Baldani et al. (2000), Bhattacharjee et al. (2008)
2.	Mineral solubilization (P, K, Zn, S, Silica, etc.)	*Pseudomonas fluorescens*, *Bacillus megaterium*, *Bacillus polymyxa*	Legume, cereals, vegetables, and cucumber crops	Oteino et al. (2015), Wyciszkiewicz et al. (2017), Altomar and Tringovska (2011)
3.	Plant growth regulating substance production	*Pseudomonas cepacia*, *P. fluorescens*, *Azotobacter chroococum*, *Azospirillum brasilense*	Wheat, rice, maize, barley	Bottini et al. (2004), Cohen et al. (2015), de Santi Ferrara et al. (2012), Etesami et al. (2015)
4.	Lowering of ethylene concentration	*Bacillus*, *Pseudomonas*	Rice	Etesami et al. (2014), Heydarian et al. (2016), Wang et al. (2016)
B. Biocontrol agents: indirectly affecting plant growth promotion				
1.	Antibiotics production	*Pseudomonas aureofaciens*, *P. fluorescens*	Wheat, tobacco, potato, groundnut, cotton	Akpa et al. (2001), Bender and Scholz-Schroeder (2004), Bender et al. (1999), Chang (1981), Fernando et al. (2005)
2.	Iron sequestration/ siderophores	Fluorescent pseudomonads	Tobacco	Keel et al. (1989), Pessi and Haas (2000), Rudrappa et al. (2008), Solomonson (1981), von Rohr et al. (2009)

(continued)

Table 7.1 (continued)

S. no.	Plant growth-promoting attributes	Plant growth-promoting rhizobacteria	Agricultural crop	References
3.	Synthesis of antifungal metabolites	*Pseudomonas fluorescens* strain CHAO	Tobacco, wheat	Budzikiewicz (1993), Delany et al. (2000), Dwivedi and Johri (2003)
4.	Production of fungal cell wall lysing enzymes	*Pseudomonas*, *Bacillus*	Wheat, rice, maize	Chernin and Chet (2002), Matthijs et al. (2007), Nagrajkumar et al. (2004), Pleban et al. (1997)
5.	HCN and ammonia production	Fluorescent *Pseudomonas, Enterobacter cloacae*	Tobacco	Askeland and Morrison (1983)
6.	Bacteriocins	*Rhizobium trifolii*, *R. leguminosarum*	Cowpea	Gray et al. (2006), Subramanian and Smith (2015)
7.	Plant defense activation	Fluorescent *Pseudomonas*	Cucumber, rice	Pieterse et al. (2014), Ortiz-Castro et al. (2009), Bent (2006)
8.	Efficient root colonization and competition for nutrients against soil-borne pathogens	*Bacillus*, *Pseudomonas fluorescens*, *Pseudomonas syringae*	Maize, tobacco, potato	Beneduzi et al. (2012), Sivasakthi et al. (2014), Benizri et al. (2001)
9.	Inducing plants for phytoalexins production	*Rhizobium leguminosarum*	Arabidopsis, bean, white bean	Wituszynska et al. (2013)

biological nitrogen fixation by *Rhizobium leguminosarum* strain MNF 710 and P solubilization by *Pseudomonas putida* strain GR12-2, etc. The PGPRs promote plant growth and crop yield by facilitation of the nutrients from the soil environment or by producing inhibitory substances to restrict the growth and minimize plant pathogenic load in soil.

7.3.1 Atmospheric Nitrogen Fixation

Nitrogen is one of the most important macronutrients for the plant. Conversion of gaseous atmospheric di-nitrogen into nongaseous ammonium nitrogen compound by microbial intervention is called biological nitrogen fixation. Further, gaseous ammonium nitrogen is oxidized in the form of nitrate. Both nitrogen forms are absorbed by

plants. Living entities depend on the availability of fixed nitrogen because nitrogen molecule is required for the biosynthesis of amino acids, proteins, nucleic acids, and other nitrogen-containing biomolecules. The plant makes different associations with beneficial microbes and symbiotic prokaryotic microorganisms like *Rhizobium meliloti, R. leguminosarum, Rhizobium phaseoli,* and *Rhizobium japonicum* in legume crops and asymbiotic microorganisms like *Azotobacter chroococum, Azotobacter beijerinckii, Azotobacter vinelandii, Azospirillum brasilense, Azotobacter lipoferum,* and *Gluconacetobacter diazotrophicus* in nonlegume crops. These rhizobacterial genera are identified as endophytic nitrogen fixers. The application of these endophytic nitrogen-fixing bacterial strains not only improves crop growth, yield, and crop productivity but also reduces chemical fertilizer's load. Biological nitrogen fixation is highly sensitive to the presence of oxygen, intensive energy input process, and involves functional and regulatory gene products. The nitrogenase protein complex consists of two metalloprotein subunits. The first one is composed of two different dimers (MoFe protein) which are encoded by nifD and nifK genes. This nitrogenase protein complex performs an actual reduction of atmospheric di-nitrogen. The second protein subunit is made of two similar dimers (Fe protein) which are encoded by the nifH gene. This site ensures ATP hydrolysis and electron transfer between subunits. Thus, acetylene reduction assay (ARA) is used as an indirect method to study the efficiency of the nitrogenase enzyme. Among the various biological nitrogen fixers, bacterial group belonging to rhizobia is well established and well-known example of this. The biological nitrogen fixers are generally called "diazotrophs." *Rhizobium* strain was used for the first time to develop the microbial product in the name of "Nitropin." Subsequently, a number of symbiotic and nonsymbiotic bacterial strains are isolated, screened, and identified. Worldwide several researchers used these nitrogen-fixing bacterial strain and recorded that the application of nitrogen fixers in the crops at the sowing, planting, and transplanting could reduce the fertilizer load up to 25–50% without compromising crop growth and yields. Bhattacharjee et al. (2008) reported that species of *Rhizobium* in legume crops like pea, gram, cowpea, pigeon pea, lentil, and bean can supply nitrogen sufficiently. *Gluconacetobacter, Azospirillum, Azotobacter, Bacillus*, and *Burkholderia* inoculation have been reported to enhance crop growth and yields in nonlegume crops (Allen et al. 2017; Baldani et al. 2000). The atmospheric fixed nitrogen is calculated in terms of the percentage of total plant nitrogen, protein, and yield increased. As per reports available, about 30–40 kg/ha/year nitrogen is fixed by seed/planting material bacterized with diazotrophs.

7.3.2 Phosphate Solubilization

Phosphorus is the second major macronutrients for better crop growth and yields. It is a constituent part of phosphorylated sugar, phospholipid, phytin, nucleotide, nucleic acid, and coenzymes. Soil pH plays a major role in its absorption by the plant roots (soil pH <4.0—H_3PO_4, between pH 4.0 and 7.0—H_2PO_4, between pH 7.0 and 10.0—HPO_4, and pH >10—PO_4). Phosphorus is available in the form

of orthophosphate, and plant takes phosphorus either in H_2PO_4 or in HPO_4. Maximum phosphorus is available in soil pH ranging from 6.0 to 7.0. The mobility of available phosphorus is very limited. In soil, phosphorus is available in the organic and inorganic state. The total phosphorus content in arid soil in India is reported around 700 kg/ha, but the only plant accessible phosphorus quantity is very low, 15–25 kg/ha. Phosphorus contributes to a total plant dry weight of around 0.2%. The early stage of crop growth requires a sufficient supply of phosphorus for primordial development, tillers formation, and photosynthetic processes. Deficiency of P nutrient resulted in stunted plant growth, twisted and tilted petioles, and leaflets. Soil is rich in phosphatic components but its availability to the crop plants is less. Besides this, almost 75–90% of applied phosphatic fertilizers easily chelate with calcium (Ca) and form calcium phosphates, with iron (Fe) and form iron phosphate, and with aluminum (Al) and form aluminum phosphate. This phosphorus precipitation occurs in acidic soil associated with Al and Fe compounds and mono-, di-, and tricalcium phosphate in calcareous soil (Stevenson 1986). Organic matter plays a major role in making organic phosphorus available. Organic matter contributes around 50–80% of the total organic phosphorus. Phytate, phospholipids and nucleic acid are the mother of soil organic phosphorus and utilized by the soil microbial population. Soil microbial populations especially PGPRs are capable to mineralize insoluble mineral phosphate in the soil. Phosphate-solubilizing microbial population constitutes around 20–40% of the culturable microbial population of soil. Among the phosphate-solubilizing microbial population, phosphate-solubilizing bacteria are the major ones. The majority of soils have been reported to have PSB strains, but the population in arid and semiarid soils is very less. Besides this, climatic conditions regulate PSB strains in soil. However, a mild and moist climate is more congenial for population buildup than dry and flooded conditions. The rhizosphere zone is the best habitat for PSB strain multiplication. These P-solubilizing bacteria produce phosphatase, phytases enzyme, and organic acids in both liquid and solid medium which leads to a drop. Nearly all P bacteria strain has the potential to solubilize Ca-P complexes, and only a few of them can solubilize Fe-P and Al-P complexes. Acid phosphatase and phytases are considered major phosphate-solubilizing substances. Besides this, P-solubilizing bacterial strains have other plant growth-promoting attributes like the production of siderophore, phytohormone, antibiotics, antimicrobial substances, vitamins, and HCN. Inoculation with PSB strains like *Pseudomonas fluorescens, Bacillus megaterium,* and *Bacillus polymyxa* (Bhatti and Yawar 2010; Chhabra et al. 2013; Demissie et al. 2013) were done on various crop plants by various researchers and recorded significant results. It was recorded that additional phosphate solubilization, 15–30 kg/ha, has been reported. A cheap source of phosphate in arid soils is rock phosphate but becomes inaccessible in normal and alkali soils. P-solubilizing bacterial strains have greater potential to release insoluble and fixed forms of phosphates when seed or soil inoculated.

7.3.3 Production of Plant Growth Regulating Substances

A tripartite (plant–soil–microbes) interaction takes place in the rhizosphere. Phytohormone is a growth regulator produced by either plants or microbes which assists plant for seed germination, root development, cell elongation, cell division, primordial formation, and other several morphological and physiological changes. Besides this, phytohormone increases plant resistance to environmental conditions, suppresses the expression of undesired genes, and assists in the biosynthesis of pigments, enzymes, and metabolites. The phytohormone is auxins, cytokinins, gibberellins, ethylene, and abscisic acids. For root initiation, auxins play a major role. In plants, auxins are produced only by a tryptophan-mediated pathway. The tryptophan is the precursor of auxin production by the crop plants means it is a limiting factor for auxin production in plants. In PGPRs, various known pathways of auxin production especially dominating indole-3-acetic acid (IAA) biosynthesis pathway have been reported. IAA biosynthesis pathways in PGPRs are indole-3-pyruvic acid, indole-3-acetamide, tryptophan conversion into indole-3-acetic aldehyde, and tryptophan conversion into the indole-3-acetonitrile pathway. The majority of PGPRs studied for IAA biosynthesis have IAA formation either indole-3-pyruvic acid pathway or tryptophan conversion into indole-3-acetic aldehyde pathway. Almost 80% of rhizobacteria isolated from rhizosphere soil have the potential to produce auxins during in vitro screening. The predominant bacterial population in the soil is *Pseudomonas, Bacillus, Rhizobium, Azotobacter, Azospirillum, Erwinia, Agrobacterium, Enterobacter*, and *Serratia.* Moreover, the rhizospheric microbial community of the plants has great potential in the conversion of tryptophan into IAA. Similarly, PGPRs are identified for cytokinins, gibberellins, and abscisic acid synthesis. *Pseudomonas fluorescens* is an efficient PGPR strain in synthesizing phytohormone, mainly IAA, cytokinins, and gibberellic acid in inoculated wheat crop. PGPR strains like *Bacillus pumilus, Herbaspirillum seropedicae, Acinetobacter calcoaceticus*, and *Promicromonospora* produce gibberellins. Pieces of literature are available that *Azospirillum brasilense* and *Bacillus megaterium* are involved in the production and enlargement of the root by the production of cytokinins (Bottini et al. 2004; Cohen et al. 2015; de Santi Ferrara et al. 2012). Excess production of ethylene is a negative factor for plant growth promotion. For ethylene production, 1-aminocyclopropane-1-carboxylate (ACC) plays a major role in conversion into ethylene but ACC is metabolized by PGPR strains by producing ACC deaminase enzyme. The ACC deaminase enzyme breaks ACC into α-ketobutyrate and ammonium resulting in reduced ethylene concentration. PGPR strains like *Bacillus subtilis, B. pumilus*, *Bacillus licheniformis*, *Achromobacter xylosoxidans*, *Lysinibacillus fusiformis*, and *P. putida* are ABA-producing bacteria. Thus, PGPRs play a vital role in the production of plant growth regulators, and phytohormones which influence plant growth and development (Cohen et al. 2015; de Santi Ferrara et al. 2012; Etesami et al. 2015).

7.3.4 Mycelia Growth Restriction of Soil-Borne Plant Pathogens by PGPRs

Biological control agents are a method whereby an undesirable population of plant pathogenic microbes is reduced by the application of beneficial microbial populations rather than a man. To date, several researchers have defined the biological control. Besides this, during cultivating agricultural crops, it is noticed that several economically important diseases are caused by the fungal population. The soil-inhabiting fungal pathogenic population is more and very difficult to manage. The application of fungicides is not always effective and eco-friendly besides the huge cost involved. So plant growth-promoting rhizobacteria are considered an alternatively green approach for soil-borne plant pathogen management. Sanford (1926) first time used antagonists as a biological control for managing potato scab disease. He emphasized that increasing the population density of certain saprophytic bacteria on decomposing crop residues reduces potato scab disease. For abiotic management, the ice-minus strain of *Pseudomonas syringae* is used to exclude ice nucleation strains of *P. syringae* from the foliage of frost-sensitive plants. In the cross-protection defense mechanism, inoculation of mild strain against a virulent strain of the pathogenic virus induces a defense pathway in the crop plants. Besides this, there are some classical examples of biocontrol agents where pruning wounds to provide protection against *Fomes* and *Armillaria* caused disease in plants. Prominent PGPRs used in biocontrol agents are as follows: (1) *Agrobacterium radiobacter* against *A. radiobacter pv. tumefaciens*, (2) *Bacillus subtilis*, *B. cereus,* and *Bacillus penetrans* against *Pythium, Phytophthora cinnamomi, Fusarium roseum,* and *Rhizoctonia solani*, (3) *Pseudomonas fluorescens, P. cepacia,* and *P. putida* against *Gaeumannomyces graminis, Fusarium oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Pythium*, etc., (4) *Erwinia herbicola* and *Erwinia uredovora* against *Erwinia amylovora*, (5) *Streptomyces griseus, Streptomyces praecox,* and *Streptomyces lavendulae* against *Phomopsis, Fusarium,* and *Gaeumannomyces* (Fravel 1988; Aliye et al. 2008; Beneduzi et al. 2012).

7.3.5 Production of Fungal Cell Wall Lysing Enzymes

The PGPRs besides enhancing growth of the crop are also potential biocontrol agents. These are usually isolated from the rhizospheric soils. Most of the PGPRs belong to the fluorescent *Pseudomonas* (*P. fluorescens* and *P. putida*), and also few are included in nonfluorescent *Pseudomonas* spp., like *Bacillus subtilis* and *Serratia* spp. These PGPR strains can parasitize fungi and kill them by secreting cell wall lytic enzymes like chitinase, B-1, 3-glucanases, proteases, and lipases. PGPR strain also produces low-molecular-weight fungi toxic compounds such as iturin and fengycin. For example, chitinase cell wall lytic enzyme is produced by *Serratia marcescens,* and RLOs have been associated with biocontrol of fungal kitinase of pea and bean. Besides this, chiA gene was cloned and expressed constitutively in PGPR culture *Pseudomonas putida*. This genetically engineered PGPR strain having

chiA + recombinant provided increased protection of radish against *Fusarium oxysporum* f. sp. *redolens*.

7.3.6 Plant Defense System Activation

The plant growth-promoting rhizobacteria can suppress plant disease caused by plant pathogens by triggering plant-mediated resistance mechanisms called induced systemic resistance (ISR). The induced systemic resistance is almost similar to plant pathogen-induced systemic-acquired resistance (SAR). The induced resistance works both locally and systemically. These both enhance resistance against challenging pathogens. The ISR and SAR differ in their only signaling pathways. This is evident when plant growth-promoting bacteria and plant pathogens are applied at specially separated locations on the plant. In ISR, jasmonic acid is a signaling molecule, while in SAR, it is salicylic acid. SAR is associated with the accumulation of novel pathogenesis-related proteins (PR proteins), some of them also reported to have antifungal activity. In ISR, the systemic infection is not linked with necrosis (without necrosis), while SAR works as with the necrosis system. For SAR, a transgenic plant with the Nah G gene was developed from *Pseudomonas putida*, which codes enzyme salicylate hydroxylase. It causes the conversion of salicylic acid to catechol, and as a result, no SAR is developed in plants (Ryals et al. 1996). Thus, Nah G transformed plants have been used to determine whether ISR-inducing PGPRs can trigger the SAR pathway. PGPR-mediated ISR signaling pathway does not initiate biosynthesis of salicylic acid or pathogenesis-related proteins. Rather, it synthesizes jasmonic acid (jasmonate) and ethylene and just like SAR depends on the regulatory protein NPR1 which contains ankyrin repeats. Thus, NPR1 regulatory protein differentially regulates ISR- and SAR-related gene expression depending on the pathway that is activated. The jasmonic acid and ethylene dependency of ISR are based on the enhanced sensitivity of these phytohormones, rather than an increase in their production. However, the extent of induced resistance attained is similar in both ISR and SAR signaling pathway (Bakker et al. 2013; Bent 2006). It is yet not clear whether ISR is broad-spectrum like SAR, but there is no evidence available that these PGPRs stimulate to produce antimicrobial compounds such as phytoalexins. However, some PGPRs which produce salicylic acid as siderophore under iron-limiting condition have been reported to induce SAR. Similarly, *Pseudomonas aeruginosa* strain 7NSK2 has been found to induce SAR in bean and tobacco plants against *Botrytis cinerea* and tobacco mosaic virus (TMV), respectively.

7.3.7 PGPR-Mediated Drought and Salt Stress Management

A worldwide drought and salt stress are a serious concern for soil quality and soil fertility. Due to poor soil quality and fertility, crop growth and crop productivity are adversely affected. Almost 12 million ha of cultivated land in India is salt-affected. A

major portion of salt-affected soil is found in semiarid and arid regions of the country. The pH of salt-affected soil is around 8.0 or more. In salt-affected soil, a mixtures of chlorides and sulfates of calcium, magnesium, and sodium are found. Among them, the concentration of sodium chlorides is dominant in nature. The ratio of sodium, calcium, and magnesium in most of the salt-affected soils has been recorded as 7:2:1. Due to the increased concentration of these mixtures of chlorides and sulfates, the availability of existed plant nutrients is affected. The reports are available that a large portion of applied inorganic phosphatic fertilizers in salt-affected soil usually precipitated in different forms such asmono-phosphates, di-phosphates, and tri-calcium phosphates. Precipitation of inorganic phosphates in the soil comes under soil salinization, where water-soluble salts accumulated in the soil. Similarly, it happens with other externally applied inorganic fertilizers. Due to deficiency of plant nutrients, crop growth and yield are badly affected. The reduction in crop growth and yield is due to a decrease in cell growth, leafsurface area, chlorophyll content, accelerated defoliation, and senescence. The soil salinity is measured by determining the conductivity of the saturation extract (dS/m). Among abiotic stresses, the drought stress is also the most destructive stress which causes a complete restriction on crop growth and yield. The severity of the damage depends on the drought period and crop stage. In drought conditions, the availability and transport of soil nutrients are affected. Besides, it induces free radicals which affect antioxidant defenses andreactive oxygen species mechanisms. Increased reactive oxygen species (ROS) causes various levels of plant physiological parameters. The decreased chlorophyll content is one of the major causes of drought stress. So in these prospects, a large group of plant growth-promoting rhizobacteria has been isolated, screened, characterized, and identified at the molecular level. The PGPR strain predominant in adverse climatic conditions is known to have a beneficial effect on drought and salt stress management in the cultivated crop. These PGPRs applied in drought and salt-affected soil performs different activities, such as phytohormone production (abscisic acid, gibberellic acid, cytokinins, indole—3 acetic acids), ACC deaminase production, induced systemic resistance, and exopolysaccharide production. Dimkpa et al. (2009) reported that IAA-producing *Azospirillum* strain enhances plant tolerance to drought stress. The effect of different PGPRs strain like *Azospirillum brasilense, A. lipoferum, Bacillus subtilis, Pseudomonas fluorescens,* and *Bacillus thuringiensis* has been reported on tomato, maize, wheat, soybean, etc., worldwide. Similarly, several researchers reported that the application of osmotolerance PGPR strain like *Pseudomonas fluorescens*, *Pseudomonas pseudoalcaligenes*, *B. subtilis*, and *Azospirillum brasilense* reduces osmotic pressure by the production of organic osmolytes (sugar and derivatives, amino acid and derivatives, polyols, betaines, and ectoines). Paul and Nair (2008) reported that *P. fluorescens* strain MSP-393 induces salt tolerance by the production of glycine betaine, alanine, glutamic acid, serine, threonine, osmolytes, and aspartic acid in their cytosol of wheat, rice, avocado, maize, chickpea, peanut, common bean, tomato, eggplant, cotton, radish, barley, lettuce, pea, groundnut, black pepper, kallar grass, etc.

7.4 Molecular Tools and Techniques for Identification of PGPRs

Rhizospheric soil is rich in microbial populations. A diverse group of beneficial plant growth-promoting microbes is reported worldwide. The beneficial plant growth-promoting microbes may be mainly bacteria and fungi. Few of them only 1–2% of the total soil microbial population are culturable in laboratory conditions. In laboratory conditions, phenotypic, biochemical, serological, and molecular-based identification can be done. Phenotypic, biochemical, and serological-based identification has certain limitations. So molecular-based identification provides correct identification, which can be correlated with a phenotypic-based identification system. The molecular-based identification of bacteria is done by the determination of base sequences of certain key nucleic acid genes such as 5S rRNA (120 bp), 16S rRNA (1600 bp), and 23S rRNA (2300 bp). In fungal identification, ITS region, TEF-α gene, ATP -6ATPase gene, and β-tubulin gene are targeted. This could be done due to the introduction of polymerase chain reaction, which allows for specific detection and investigation of even minor traces of genetic material. All these bases of nucleotide sequences of rRNA genes and their spacers can be used for phylogenetic analyses. Most commonly, 16S rRNA gene and ITS region are used for molecular identification of bacteria and fungi, respectively. After the sequencing of rRNA genes and spacer regions, sequencing data are blasted with GenBank-NCBI and deposited in public data banks (NCBI, EMBL, etc.). This approach provides information on isolated microbial culture and exact identification as well. Besides this, for the study of microbial diversity, community structure, and community response to environmental conditions in the soil, a meta-genomics approach can be used. Generally, the meta-genomics approach is adopted in the study of noncultural microbes. Further, soil meta-genomics study includes culture-dependent techniques (plate count, morphology analysis, community-level physiological profiling, CLPP) and culture-independent techniques—Microbial lipid-based (PLFA, FAME), non-PCR based (DNA reassociation, the G + C content of DNA, RSGP), PCR based (RAPD, RFLP, ARDRA, T-RFLP, RISA, ARISA, DGGE, TGGE, SSCP, HRP, qPCR, etc.), and sequencing based (clone library sequencing, amplicon sequencing, shotgun sequencing, etc.).

7.5 Bio-Formulation Development and Commercialization of PGPRs

In recent years, a number of entrepreneurs (small and medium levels) have entered commercial production of bio-formulations (biofertilizers and biocontrol agents) (Bhattacharjee and Dey 2014; Arora et al. 2016; Bashan and de Bashan 2015; Bashan et al. 2014). For bio-formulation development, it is a multistep process involving a wide range of activities. The first activity is to isolate potential microbial culture from the congenial natural or agro-ecosystems. In laboratory conditions, evaluation of isolated microbial culture is done for plant growth-promoting

attributes. The potential microbial strain is further screened at glass-house conditions. After getting significant results, the microbial culture is validated in field conditions, at farmers' fields, and/or by validating agencies. Besides this, microbial culture is also validated for shelf life, quality parameters, and population count in the eco-friendly and easily available multiplying substrate. After complete confirmation and validation, microbial culture is mass multiplied, formulations prepared, registration done, and released for application on farmer's field (Table 7.2).

7.6 Conclusion

The rhizospheric zone of the plant has been characterized by intense microbial activity. It is also an interface between plant roots and bulk soil, the soil which is not under influence of root exudates. Root exudate is a mixture of the released low and high metabolites by the plant. The metabolites are synthesized during the photosynthesis pathway. They are a good food substrate for microbial population and exist around the root vicinity. Due to rich in carbon and nitrogen-based compounds, it creates and develops selective microbial community and structure called rhizomicrobiome. The beneficial microbial population in the rhizosphere zone is called plant growth-promoting rhizobacteria (PGPRs). The beneficial microbes increase tremendous and perform a number of plant growth-promoting attributes that are benefitted directly to crop plants, known as biofertilizers, or indirectly by restricting the population of plant pathogenic microbes, called biocontrol agents. Besides this, some PGPR strains induce systemic-acquired resistance and provide tolerance to abiotic stresses like drought and salt conditions. Today, molecular approaches are need-based and used for the detection and identification of microbes, microbial community and structure, and their response to environmental conditions. To date, a number of microbial formulations are being developed and commercialized worldwide. The use of these microbial inoculants is eco-friendly and safe and also increases crop growth and yields besides reduction in the cost of cultivation. A lot of research has been done on PGPRs and has characterized the plant growth-promoting traits of PGPRs. Still, the study of microbial community and structure has great scope for studying system biology. Soil microbial activity and distribution are directly affected by soil organic matter content and environmental conditions as well. So better understanding of the composition and distribution of microbes in nature can maintain the diversity of beneficial microbes and improve plant growth and productivity without developing any harmful environmental issues.

Table 7.2 List of some bio-formulations developed worldwide

S. No.	Product name	Microbial culture used	Country
1.	AgriLife, Symbion-P	*B. megaterium, B. polymyxa*	India
2.	Ecofit, Bas derma, Tri-control	*Trichoderma viride*	India
3.	Kali sena	*Aspergillus niger*	India
4.	Symbion-N, CALOBIUM	*Rhizobium* sp.	India
5.	Symbion-K	*Frateuria aurantia*	India
6.	Symbion-S	*Thiobacillus thiooxidans*	India
7.	CALMONAS	*Pseudomonas* sp.	India
8.	CALSPIRAL	*Azospirillum* sp.	India
9.	CALZOTO	*Azotobacter* sp.	India
10.	Sardar Biofertilizers	Consortium-based formulation	India
11.	BioGrow	Consortium-based formulation	Vietnam
12.	Biosave 100, Biosave 110, Biosave 1000, Fastban A	*Pseudomonas syringae*	USA
13.	Conquer, Victus	*Pseudomonas fluorescens*	USA
14.	Blue circle	*Pseudomonas cepacia*	USA
15.	Soil gard	*Gliocladium virens*	USA
16.	System 3, Kodiak, Kodiak HB, Kodiak At, Epic	*Bacillus subtilis*	USA
17.	Rhizo-plus, Rhizo-plus Konz	*Bacillus subtilis*	Germany
18.	Bactophosphin	*Bacillus mucilaginosus*	Russia
19.	Rizotrophin	*Rhizobium* sp.	Russia
20.	Azotobacterin, Azotovit, Ekophit	*Azotobacter chroococcum*	Russia
21.	Mamezo, hyper-coating seeds, R-processing seeds	*Rhizobium* sp.	Japan
22.	Xin Sheng Li	*B. mucilaginosus, B. subtilis*	Japan
23.	Nitrogen Gold, TagTeam	*Rhizobium meliloti*	Japan
24.	Rizo Liq LLI, Rizo Liq TOP	*Rhizobium* sp.	Argentina
25.	TwinN	Consortium-based formulation	Australia
26.	FOSFORINA	*P. fluorescens*	Cuba
27.	EcoMic, Micofert	*Glomus* sp.	Cuba
28.	BuRIZE1	*Glomus* sp.	Mexico
29.	Dimargon1	*Azotobacter chroococcum*	Malaysia
30.	UPMB 10	*Bacillus sphaericus*	Malaysia
31.	Biophos	*Bacillus megaterium*	Sri Lanka
32.	BioPower	N-fixing consortium-based formulation	Pakistan
33.	Ferti-Bio	Consortium-based formulation	Pakistan

References

Adesemoye A, Torbert H, Kloepper J (2008) Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can J Microbiol 54:876–886

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Akpa E, Jacques P, Wathelet B (2001) Influence of culture conditions on lipopeptide production by *Bacillus subtilis*. App Biochem Biotech 91:551–561

Aliye N, Fininsa C, Hiskias Y (2008) Evaluation of rhizosphere bacterial antagonists for their potential to bioprotect potato (*Solanum tuberosum*) against bacterial wilt (*Ralstonia solanacearum*). Biol Control 47:282–288

Allen RS, Tilbrook K, Warden AC et al (2017) Expression of 16 nitrogenase proteins within the plant mitochondrial matrix. Front Plant Sci 8:287

Altomar C, Tringovska I (2011) Beneficial soil microorganisms, an ecological alternative for soil fertility management. In: Lichtfouse E (ed) Genetics, biofuels and local farming systems. Springer, Heidelberg, pp 161–214

Andreote FD, Pereira ESMC (2017) Microbial communities associated with plants: learning from nature to apply it in agriculture. Curr Opin Microbiol 37:29–34

Arora NK, Mehnaz S, Balestrini R (2016) Bioformulations: for sustainable agriculture. Springer, Berlin. https://doi.org/10.1016/j.mib.2017.03.011

Askeland RA, Morrison SM (1983) Cyanide production by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. Appl Environ Microbiol 45:1802–1807

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570. https://doi.org/10.1007/s10529-010-0347-0

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681

Badri DV, Chaparro JM, Zhang R et al (2013) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. J Biol Chem 2013(288):4502–4512

Baez-Rogelio A, Morales-García YE, Quintero-Hernández V et al (2017) Next generation of microbial inoculants for agriculture and bioremediation. Microb Biotechnol 10:19–21

Bais HP, Weir TL, Perry LG et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Bakker PAHM, Doornbos RF, Zamioudis C et al (2013) Induced systemic resistance and the rhizosphere microbiome. Plant Pathol 29:136–143

Baldani VLD, Baldani JI, Döbereiner J (2000) Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol Fertil Soils 30:485–491

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770

Bashan Y, de Bashan LE (2015) Inoculant preparation and formulations for *Azospirillum* spp. In: Cassán FD, Okon Y, Creus CM (eds) Handbook for *Azospirillum*. Springer, Berlin, pp 469–485

Bashan Y, de Bashan LE, Prabhu S et al (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378:1–33

Beattie GA (2015) Microbiomes: curating communities from plants. Nature 528:340–341

Bender CL, Scholz-Schroeder BK (2004) New insights into the biosynthesis, mode of action, and regulation of syringomycin, syringopeptin, and coronatine. In: Ramos JL (ed) Virulence and gene regulation. Springer, Boston, pp 125–158

Bender CL, Alarcon-Chaidez F, Gross DC (1999) *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiol Mol Biol Rev 63:266–292

Bender SF, Wagg C, Van Der Heijden MG (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol 31:440–452

Beneduzi A, Ambrosini A, Passaglia LM (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35(4):1044–1051
Benizri E, Baudoin E, Guckert A (2001) Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Sci Tech 11:557–574
Bent E (2006) Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). In: Tuzun S, Bent E (eds) Multigenic and induced systemic resistance in plants. Springer Science, New York, pp 225–259
Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486
Berg G, Grube M, Schloter M, Smalla K (2014) Unraveling the plant microbiome: looking back and future perspectives. Front Microbiol 5:148
Berg G, Rybakova D, Grube M et al (2016) The plant microbiome explored: implications for experimental botany. J Exp Bot 67:995–1002
Bhattacharjee R, Dey U (2014) Biofertilizer, a way towards organic agriculture: a review. Afr J Microbial Res 8:2332–2343
Bhattacharjee RB, Singh A, Mukhopadhyay SN (2008) Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. Appl Microbiol Biotechnol 80:199–209
Bhatti TM, Yawar W (2010) Bacterial solubilization of phosphorus from phosphate rock containing sulfur-mud. Hydrometallurgy 103:54–59
Bossio DA, Scow KM, Gunapala N et al (1998) Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb Ecol 36:1–12
Bottini R, Cassán F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503
Bottner P, Sallih Z, Billes G (1988) Root activity and carbon metabolism in soils. Biol Fertil Soils 7:71–78
Broeckling CD, Broz AK, Bergelson J et al (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738–744
Budzikiewicz H (1993) Secondary metabolites from fluorescent pseudomonads. FEMS Microbiol Rev 104:209–228
Bulgarelli D, Schlaeppi K, Spaepen S et al (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838
Bulgarelli D, Garrido-Oter R, Munch PC et al (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe 17:392–403
Cardinale M, Ratering S, Suarez C et al (2015) Paradox of plant growth promotion potential of rhizobacteria and their actual promotion effect on growth of barley (*Hordeum vulgare* L.) under salt stress. Microbiol Res 181:22–32
Chang CJ (1981) The biosynthesis of the antibiotic pyrrolnitrin by *Pseudomonas aureofaciens*. J Antibiot 24:555–566
Chaparro JM, Sheflin AM, Manter DK et al (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499
Chaparro JM, Badri DV, Vivanco JM (2014) Rhizosphere microbiome assemblage is affected by plant development. ISME J 8:790
Chauhan H, Bagyaraj D, Selvakumar G et al (2015) Novel plant growth promoting rhizobacteria—prospects and potential. Appl Soil Ecol 95:38–53
Chernin L, Chet I (2002) Microbial enzymes in biocontrol of plant pathogens and pests. In: Enzymes in the environment: activity, ecology, and applications. Marcel Dekker, New York, pp 171–225
Chhabra S, Brazil D, Morrissey J, Burke JI et al (2013) Characterisation of mineral phosphate solubilisation traits from a barley rhizosphere soil functional metagenome. Microbiology 2:717–724

Cohen AC, Bottini R, Pontin M et al (2015) *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. Physiol Plant 153:79–90

Compant S, Duffy B, Nowak J et al (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Dakora F, Matiru V, Kanu A (2015) Rhizosphere ecology of lumichrome and riboflavin, two bacterial signal molecules eliciting developmental changes in plants. Front Plant Sci 6:700

de Santi Ferrara FI, Oliveira ZM, Gonzales HHS et al (2012) Endophytic and rhizospheric enterobacteria isolated from sugar cane have different potentials for producing plant growth-promoting substances. Plant Soil 353:409–417

Delany I, Sheenan MM, Fenton A et al (2000) Regulation of production of the antifungal metabolite 2,4-diacetylphloroglucinol in *Pseudomonas fluorescens* F113: genetic analysis of phlF as a transcriptional repressor. Microbiology 146:537–543

Demissie S, Muleta D, Berecha G (2013) Effect of phosphate solubilizing bacteria on seed germination and seedling growth of faba bean (*Vicia faba* L.). Int J Agric Res 8:123–136

Desbrosses GJ, Stougaard J (2011) Root nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways. Cell Host Microbe 10:348–358

Dimkpa C, Weinand T, Asch F (2009) Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ 32:1682–1694

Dwivedi D, Johri BN (2003) Antifungal from fluorescent pseudomonads: biosynthesis and regulation. Curr Sci 85:1693–1703

Etesami H, Alikhani HA, Hosseini HM (2015) Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competentbacteria for rice growth-promoting agents. Methods X 2, 72–78

Etesami H, Mirseyed Hosseini H, Alikhani HA (2014) Bacterial biosynthesis of 1-aminocyclopropane-1-caboxylate (ACC) deaminase, a useful trait to elongation and endophytic colonization of the roots of rice under constant flooded conditions. Physiol Mol Biol Plants 20:425–434

Evangelou MW, Deram A (2014) Phytomanagement: a realistic approach to soil remediating phytotechnologies with new challenges for plant science. Int J Plant Biol Res 2:1023

Fahad S, Hussain S, Bano A et al (2015) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environ Sci Pollut Res 22:4907–4921

Fernando WDG, Nakkeeran S, Zhang Y (2005) Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilizer. Springer, Dordrecht, pp 67–109

Fravel DR (1988) Role of antibiosis in the biocontrol of plant-diseases. Annu Rev Phytopathol 26:75–91

Garcia-Pausas J, Paterson E (2011) Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biol Biochem 43:1705–1713

Gransee A, Wittenmayer L (2000) Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. J Plant Nutr Soil Sci 163:381–385

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412

Gray EJ, Di Falco M, Souleimanov A, Smith DL (2006) Proteomic analysis of the bacteriocin thuricin 17 produced by *Bacillus thuringiensis* NEB17. FEMS Microbiol Lett 255:27–32

Gupta G, Parihar SS, Ahirwar NK et al (2015) Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol 7:096–102

Heydarian Z, Yu M, Gruber M et al (2016) Inoculation of soil with plant growth promoting bacteria producing 1-aminocyclopropane-1-carboxylate deaminase or expression of the corresponding

acdS gene in transgenic plants increases salinity tolerance in Camelina sativa. Front Microbiol 7:1966
Hirsch PR, Mauchline TH (2012) Who's who in the plant root microbiome? Nat Biotechnol 30:961–962
Itelima JU, Bang WJ, Onyimba IA et al (2018) A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. J Microbiol Biotechnol Rep 2:22–28
Jha CK, Saraf M (2015) Plant growth promoting rhizobacteria (PGPR): a review. E J Agric Res Dev 5:108–119
Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480
Keel C, Voisard C, Berling C et al (1989) Iron sufficiency, a prerequisite for the suppression of tobacco black root rot by *Pseudomonas fluorescens* strain CHA 0 under gnotobiotic conditions. Phytopathology 79:584–589
Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–43
Kristin A, Miranda H (2013) The root microbiota—a fingerprint in the soil? Plant Soil 370:671–686
Leach JE, Triplett LR, Argueso CT et al (2017) Communication in the phytobiome. Cell 169:587–596
Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556
Matthijs S, Tehrani KA, Laus G et al (2007) Thioquinolobactin, a pseudomonas siderophore with antifungal and anti-Pythium activity. Environ Microbiol 9:425–434
Mitter B, Brader G, Afzal M et al (2013) Advances in elucidating beneficial interactions between plants, soil, and bacteria. Adv Agron 121:381–445
Nagrajkumar M, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath of blight pathogen. Microbiol Res 159:73–81
Nehra V, Choudhary M (2015) A review on plant growth promoting rhizobacteria acting as bioinoculants and their biological approach towards the production of sustainable agriculture. J Appl Nat Sci 7:540–556
Nelson LM (2004) Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. Crop Manag 3:301–305
Ortiz-Castro R, Contreras-Cornejo HA, Macias-Rodriguez L et al (2009) The role of microbial signals in plant growth and development. Plant Signal Behav 4:701–712
Oteino N, Lally RD, Kiwanuka S et al (2015) Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. Front Microbiol 6:745
Paul D, Nair S (2008) Stress adaptations in plant growth-promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. J BasicMicrobiol 48(5):378–384
Pessi G, Haas D (2000) Transcriptional control of the hydrogen cyanide biosynthetic genes hcnABC by the anaerobic regulator anr and the quorum-sensing regulators LasR and RhlR in Pseudomonas aeruginosa. J Bacteriol 182:6940–6949
Pieterse CM, Zamioudis C, Berendsen RL et al (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375
Pii Y, Mimmo T, Tomasi N et al (2015) Microbial interactions in the rhizosphere: beneficial influences of plant growth promoting rhizobacteria on nutrient acquisition process. A review. Biol Fertil Soils 51:403–415
Pleban S, Chernin L, Chet I (1997) Chitinolytic activity of an endophytic strain of *Bacillus cereus*. Lett App Microbiol 25:284–288
Rudrappa T, Splaine RE, Biedrzycki ML et al (2008) Cyanogenic pseudomonads influence multitrophic interactions in the rhizosphere. PLoS One 3:e2073
Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD (1996) Systemic acquired resistance. Plant Cell 8:1809–1819

Sivasakthi S, Usharani G, Saranraj P (2014) Biocontrol potentiality of plant growth promoting bacteria (PGPR)-Pseudomonas fluorescens and Bacillus subtilis: a review. Afr J Agric Res 9:1265–1277

Solomonson LP (1981) Cyanide as a metabolic inhibitor. In: Vennesland B, Conn EE, Knowles CJ, Westley J, Wissing F (eds) Cyanide in biology. Academic, London, pp 11–28

Stevenson FJ (1986) Cycle of Soil Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients. Wiley, New York

Subramanian S, Smith DL (2015) Bacteriocins from the rhizosphere microbiome - from an agriculture perspective. Front Plant Sci 6:909

Toju H, Peay KG, Yamamichi M, Narisawa K et al (2018) Core microbiomes for sustainable agroecosystems. Nat Plants 4:247–257

Turner TR, James EK, Poole PS (2013) The plant microbiome. Genome Biol 14:209

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

von Rohr MR, Furrer G, Brandl H (2009) Effect of iron and phosphate on bacterial cyanide formation determined by methemoglobin in two-dimensional gradient microcultivations. J Microbiol Methods 79:71–75

Wang QY, Dodd IC, Belimov AA et al (2016) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase growth and photosynthesis of pea plants under salt stress by limiting NaC accumulation. Funct Plant Biol 43:161–172

Wani SH, Kumar V, Shriram V et al (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J 4:162–176

Wituszynska W, Slesak I, Vanderauwera S et al (2013) Lesion simulating disease1, enhanced disease susceptibility1, and phytoalexin deficient4 conditionally regulate cellular signaling homeostasis, photosynthesis, water use efficiency, and seed yield in Arabidopsis. Plant Physiol 161:1795–1805

Wyciszkiewicz M, Saeid A, Chojnacka K (2017) In situ solubilization of phosphorus bearing raw materials by Bacillus megaterium. Eng Life Sci 17:749–758

Zhang R, Vivanco JM, Shen Q (2017) The unseen rhizosphere root-soil-microbe interactions for crop production. Curr Opin Microbiol 37:8–14

Flavonoid Infochemicals: Unravelling Insights of Rhizomicrobiome Interactions

8

Amit Verma, Harish Mudila, Parteek Prasher, and Shulbhi Verma

Abstract

Root exudation consists of several biochemicals which act as the modifier of rhizospheric ecosystem favouring plant growth and development. These biochemicals play a role of signals to call the beneficial microbes towards plant root and deterring the pathogenic species away from the rhizosphere due to which they are also described as "*Infochemicals*". Flavonoids are one of the most important infochemicals exudated from various plant roots that help in regulating rhizospheric nutrient status, microbial diversity and biotic and abiotic adaptation etc. owing to their importance and prominence in rhizospheric signalling, they have been studied for their chemistry and mode of action in exudation which resulted in collection of important information related to their synthesis and diversity in plant system as well as their actions in the rhizosphere which are helpful for plants to develop and adapt very stressful conditions due to their associations with PGPRs and other beneficial microorganism communities. Thus, this data collection enables presently what we term "*Rhizosphere engineering*" and in which flavonoids had an important role to play.

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Keywords

Flavonoids · Root exudate · Rhizosphere · Infochemicals · Rhizosphere engineering

8.1 Introduction

Phytometabolites of volatile nature are important infochemicals involved in diverse forms of rhizospheric signalling attracting beneficial microbes towards plant roots and repelling the pathogenic microbes. Actually, rhizosphere is an active ecosystem which is composed of various living entities which are in complex interactions facilitating plant growth and development. These chemical signals especially of plant origin keep the soil ecosystem in balance by modulating the root microbiome, facilitating nutrient bioavailability, promoting beneficial associations between microbes–microbes, and controlling the pathogenic microorganisms from the rhizosphere. These chemicals released from the roots are termed as root exudates and the process is termed as root exudation, which involves various classes of metabolites like alkaloids, glucosinolates, flavonoids, benzenoids, lignins, sterols, terpenoids, sugars, amino acids, organic acids, enzymes, etc. (Guerrieri et al. 2019). Flavonoids are one of the most important infochemicals which are found to be involved in various plant protection functions, viz. UV protection, antioxidative activity, hormone regulators, pathogen defence, symbiosis promotion, abiotic stress relievers, etc. (Hassan and Mathesius 2012). In the root exudate, different classes of flavonoid compounds are found and involved in the regulation of *nod* gene expression, rhizobial interaction with root, inhibition of pathogenic microbes in rhizosphere, promoting mycorrhizal interactions, allelopathic relationships, affecting the phenomenon of quorum sensing, and regulating the soil nutrient availability. Flavonoids are phenylpropanoid metabolites which are diverse in structure presenting various classes like flavonols, isoflavans, flavones, flavanones, isoflavonoids which are formed due to the modification of basic flavonoid skeleton by methylation, hydroxylation, acylation, glycosylation, prenylation, and polymerization (Li et al. 2014). Amino acid phenylalanine acts as precursor for phenylpropanoid biosynthesis via the synthesis of p-coumaroyl-CoA and malonyl-CoA (Pyne et al. 2019). Flavonoids thus synthesized are found in all plant parts with their specific function but they constitute a major portion of the root exudation (Canarini et al. 2019). Flavonoids exudation from roots involves both the active and passive process of transportation, sometimes in heavy amount due to the presence of elicitors. Thus, due to their wide variety of classes and diverse functions, these infochemicals are attracting the researchers to utilize these compounds for the structuring of rhizosphere for better plant growth and development which is termed as "*Rhizosphere Engineering*". Constructing plant beneficial conditions in soil requires a better understanding of the role of these infochemicals and their target by which they create the suitable condition supporting efficient plant development. This involves the investigation of root exudate flavonoids influencing microbial communities in rhizosphere, and

particular microbial community in the rhizosphere influenced and modulated by specific flavonoid class of compound having some beneficial effect on plant development and yield. Such investigations involve the study of interaction of large microbial communities in rhizosphere with a wide array of chemical signals and not limited up to traditional observation related to *nod* gene modulation.

8.2 Flavonoid Chemistry

Flavonoids are considered to be diverse group of biochemical (more specifically phytochemical produced by plants), which are profoundly present in nearly all food products, viz. fruits, vegetables, spices etc. These chemicals are responsible not only for the colour of vegan food articles but also for phytonutrition with thousands of its type. As discussed, flavonoids find their key role in various factors which are found to be beneficial to human health. Else, these flavonoids also play a crucial role in maintaining symbiotic relationship between the plant roots and beneficial microbes (by regulation of transcriptional mechanism) and fighting against the detrimental microbes (Singla and Garg 2017). Flavonoids owing to their *nod* gene generate a symbiotic relationship between roots and rhizobia by *Rhizobium*–legume (RL) and the arbuscular mycorrhiza (AM) interactions. These flavonoid types of phytonutrients are chemically related to the polyphenol class and are being employed in the regulation of human health for dermal protection, enhancing neurological functions, and the regulation of blood sugar (diabetes) and blood pressure (Kozłowska and Szostak-Wegierek 2018).

Not only in recent times but also in the past centuries, the chemistry behind natural products including flavonoids had been the epicentre for the organic chemists, and the biological activities of these were known to human society. As the various natural products are associated with health benefits, so are the flavonoids. Some of the benefits involve their use as antioxidants with certain anti-inflammatory and immune system welfares along with the protection from UV radiations (He et al. 2018). Apart from this, certain studies on flavonoids show their effectiveness in combating certain noxious health problems, viz. cancer/tumour, neurodegenerative, and cardiovascular disease along with their enzyme regulatory functions (Kozłowska and Szostak-Wegierek 2014; Kandaswami et al. 2005; Kozłowska and Szostak-Wegierek 2014; Panche et al. 2016). Presence of hydroxyl groups (–OH) facilitate the antioxidant effects through scavenging the free radicals and also by chelating the various metal ions that are present (Kumar and Pandey 2013). Presently, these flavonoids are being reflected as a valuable component in pharmaceutical, medicinal, nutraceutical, and various cosmetic applications (Panche et al. 2016). Though the actual mechanism of activity of these wonderful chemicals is still a mystery, research is being conducted to figure out this and advanced technologies, viz. bioinformatics, molecular docking etc. are being proved as an explicit tool for the same.

Evidence also shows that antioxidant flavonoids are found in the nucleus of mesophyll cells and in the centres of reactive oxygen species (ROS). Flavonoids

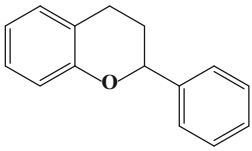

Fig. 8.1 General chemical structure of flavonoids

are classified into various classes, groups and subgroups. Flavanols, anthocyanidins, flavonols, flavones, flavonones and isoflavones are the various designated classes of flavonoids, which are based on the structure, degree of hydroxylation, degree of polymerization, substitutions, and conjugations. These flavonoids are generally polyphenolic compounds present with a benzo-γ-pyrone structure, these flavonoids are biochemically synthesized by phenylpropanoid pathway in rejoinder to various microbial activities (Kumar and Pandey 2013). Till date, 6×10^3 varieties had been identified and isolated, and chemically, flavonoid consists of a three-membered cyclic ring in which two benzene rings are attached to a central pyran ring (Fig. 8.1). Aglycones, glycosides, and methylated form are the most common ones for flavonoids (Table 8.1).

8.3 Flavonoids: Unique Rhizomicrobiome Regulators

Rhizomicrobiome is one of the complex systems which are inhabited by variety of organisms including bacteria, fungi, nematodes, insects, etc., which directly or indirectly favour the plant development. Thus, rhizomicrobiome is shaped under the influence of some selective pressure by plants. Therefore, as compared to the bulk soil, rhizomicrobiome constituted abundant microorganisms but with lowered diversity. Plant root-secreted metabolites play an important role in rhizomicrobiome constitution, which can be understood by the study of chemical interaction between plant species and the members of their rhizomicrobiome. Rhizomicrobiome shows interactions between microorganisms and plant in such a manner that supports the establishment of plant in its environment. The rhizomicrobiome assemblage not only favours the plant establishment but also deters the pathogenic species both below and above the ground; alleviates adverse effects of abiotic stress; increases plant yield, and soil nutrient availability, etc. (Szoboszlay et al. 2016). So, the study of these chemical signals which are the basis of rhizomicrobiome interactions helps in creating artificial rhizomicrobiome preventing crop failure due to various reasons. Rhizomicrobiome has a wide variety of intra- and inter-microorganism communications which facilitate the rhizospheric microorganisms to interact beneficially with each other as well as with plant. Studies of these chemical signals revealed that they are VOCs class of compounds which comprise much diversity, which depends upon the species of microorganisms under study. However, most important are the signalling compounds secreted in root exudation, as they modulate the composition of the rhizomicrobiome principally. Flavonoids are found to be one of the most important and are actively involved in various actions like suppression of

Table 8.1 Flavonoid classes: summary of different types of flavonoids, sources and benefits

Flavonoid class	Subgroups	Common sources	Benefits	References
Flavonols	Quercetin, Kaempferol, Myricetin, and Fisetin	Onions, Broccoli, tea, berries, apples, beans, etc.	Quercetin relieves from hay fever (allergic rhinitis) and hives (skin rash). Kaempferol acts as an anti-inflammatory and antioxidant, helps to relieve from chronic disease	Panche et al. (2016), Kim et al. (2016)
Anthocyanidins	Cyanidin, Malvidin, Pelargonidin and Peonidin	Coloured berries, red wine, plums, pomegranates, grapes, etc.	Antioxidant in nature, cardiac stimulator, combats against diabetes and obesity	Panche et al. (2016), Marunaka (2017)
Flavanols	Three forms: Monomeric (Catechins), dimeric and polymeric	Green tea, white tea (Catechins), black tea (dimers) apples, cocoa, grapes, berries, red wine, etc.	Catechins maintains cardiovascular and neurological health, dimers help in lowering cholesterol	Panche et al. (2016), Lopez et al. (2001)
Flavones	Apigenin and Luteolin	Parsley, celery, various kinds of herbs, peppers, etc.	Antioxidant, metabolize drugs	Panche et al. (2016), Kumar and Pandey (2013), Jiang et al. (2016)
Flavonones	Eriodictyol, Hesperetin and Naringenin	Citrus fruits	Antioxidant, anti-inflammatory, helps in maintaining cardiovascular health	Panche et al. (2016), Ruiz Cruz et al. (2017)
Isoflavones	Daidzein, Genistein and Glycitein	Legumes, soy products	Lowers the risk of cancers (viz. breast, prostate, hormonal and endometrial), antioxidants, treatment of symptoms of menopause	Panche et al. (2016), Kumar and Pandey (2013), Ballard and Maróstica Junior (2019)

quorum sensing, which is one of the important factors for the virulence of pathogenic strains (Kirwa et al. 2018).

Flavonoids find their ecological role in the interaction between the roots and the microbes, they entice the compatible microbes towards roots, impede the pathogenic ones, coordinate the nutrients present in the soil, and affect *nod* gene expression, etc. The fortune of flavonoid in a rhizosphere depends upon various biotic and abiotic factors along with the microbe of the soil and the type of flavonoids secreted by the plant. Various nitrogen-fixing bacteria (rhizobia) are attached to specific legumes of their own, at the root tip, and there takes place the preliminary host (plant)-bacteria interaction (endosymbiosis). The legume plant roots (more specifically legumes) are invaded by the respective compatible bacteria, then the bacteria get segregated into bacteroids and fixed nitrogen, and both the partners are equally benefitted (Singla and Garg 2017). Several non-leguminous plants, known as actinorhizal plants, also symbioses with nitrogen-fixing actinomycetes to fulfil their need for nitrogen with the help of rhizomes while the bacteria in turn get their needful amount of carbon.

Formononetin (Isoflavonoid) present in the nodule primordia (roots) was found to enhance auxin breakdown due to peroxidase (Mathesius 2001). It was revealed from the studies that the variation in flavonoid concentration results in auxin level accumulation during the process of nodule formation. The results are consistent with previous observations on the localization of auxin during nodule organogenesis (Mathesius 2001). Peer and Murphy (2007) described the auxin (plant hormone) modulation by flavonoids by regulating P-glycoproteins and phosphatases and kinases (Peer and Murphy 2007). Taylor and Grotewold (2005) described that the aglycone (flavonoid) is diffused into the rhizobial bacteria through porins. A prompt transcriptional instigation of the bacterial *nod* genes gets initiated with molecular interaction of the bacterial nodulating D (NodD) protein with flavonoids, which starts the curling of root hairs and is further involved in root nodule formation (Taylor and Grotewold 2005). The flavonoids behave as cell division signalling agents. It was suggested that the plants can select over a number of microbes, for a variety of functions, depending upon its developmental stage. The plants were found to secrete selective phytochemicals, depending upon the developmental stage so as to assemble rhizosphere microbiome. The expression of a particular phytochemical can be enhanced and regulated by the plant itself. Flavonoids secreted by plants are one of the chief phytochemicals secreted which coordinates between the plant and the rhizomicrobiome (Chaparro et al. 2013).

According to studies, flavonoids act as a chemoattractant and induce the *nod* genes in the synthesis of lipochitin–oligosaccharide signalling molecules. The presence of rhizobial nod D proteins is the chief way of recognition of flavonoids from the plant roots, and then the root hair cell and nodule generation takes place. Certain flavonoids, isoflavonoids (viz. daidzein and genistein) and flavone (viz. luteolin) produce chemotaxis and growth in bacteria along with the expression of *nod* gene, which in turn assist the rhizobium to the correct plant and to get attached to its root hairs (Ma et al. 2016). According to studies, the legume-containing plants secrete phytochemical flavonoids, which enhance the assembly/generation of nod factors which are like chitin in their structure and contain N-acetylglucosamine. Some of the

rhizobium bacteria are thought to control certain fungi which are phytopathogenic by degrading chitin, but sometimes, the symbiotic relation can also get disrupted by this control (Mabood et al. 2014). Broughton et al. (2003) describe the action of flavonoids which act as nodding molecules/chemical to attract nitrogen-fixing bacteria. Along with a variety of phytochemicals generated by plant root exudates, flavonoids are one of the components which may attract pathogens or may acts as symbiotic agents with rhizobium, their composition and concentration may change and depend upon the environment of the plant, its age and soil type (Broughton et al. 2003).

The flavonoids appear in copious amounts in the photosynthesizing plant cells, and participate in light-dependent phase of photosynthesis. By catalysing the electron transport, the flavonoids regulate ion channels associated with photophosphoregulation (Kumar and Pandey 2013). The death of photosynthesizing cells releases flavonoids in plant-based resins . The flavonoids do not occur naturally in animal cells, but they appear in animals via ingesting plants and vegetables. Essentially, the flavonoids do not exert toxicity to either plant or animal cells, and no residual flavonoids reportedly accumulate in the body (Agati et al. 2012). The flavonoids reportedly exhibit pharmacophore properties in designing novel therapeutics for targeting various diseases. The flavonoids' role in the inhibition of several essential cellular processes including enzyme inhibition, and their properties in scavenging the free radicals and reactive oxygen species further validate their candidature in modern-day therapeutics (Zhao and Dixon 2010). The members of the flavonoid family play important roles in plant physiology such as transport of phytohormones, including auxins and indole-3-acetic acid. Kaempferol and quercetin inhibit IAA oxidases, and scavenge the ROS generated during the metabolism of IAA (Ferdinando et al. 2012). Reportedly, the flavonoids filter the UV radiation, to prevent the photocatalytic DNA damage induced by UV-B radiation, which also potentiates the degradation of photosystem II reaction centre (Mullineaux and Karpinski 2002). The presence of flavonoids in the plasma membrane effectively attenuates multidrug-resistant glycoproteins that hinder the intercellular movement of phytohormones such as auxins. The glycosylation of hydroxyl group of flavonoids improves their physiological solubility in the aqueous cellular environment and provides shielding to the reactive hydroxyl groups from autooxidation (Verma and Pratap 2010). This enables the transport of flavonoids from the endoplasmic reticulum to other cellular compartment, secreting them to the cell wall and plasma membrane. In addition to free radicals, the flavonoids effectively scavenge cellular H_2O_2, hydroxyl ions, and singlet oxygen species (Zhang et al. 2013). The flavonoids-mediated movement of phytohormones manifests stress-triggered morphogenic response in plants. The presence of flavonoids reportedly regulates the plant phenotypes in an ecosystem. As such, the shady plant species rich in apigenin possess long internodes, reduced leaf thickness and large leaf lamina (Erlejman et al. 2004). However, the plant rich in dihydroxy flavonoids primarily resides in sunny environment (Jansen 2002) and possess dwarf bushy phenotypes with smaller, thicker leaves to irradiate direct sunlight, and to protect against the light-induced perturbations in cellular homeostasis (Kuhn et al. 2011).

8.4 Flavonoids Rhizospheric Engineering

Rhizospheric zone is the crucial zone for the plant and microbes interaction. This zone is maintained by several organic and inorganic compounds excreted from the plant in the form of root exudates and microbes exist in the soil. Their combination participates in the soil fertility, enhances the soil microbe communication and increases the interaction of plant and microbes for more support in bioremediation, defence, signalling and allelopathy, etc. (Fig. 8.2).

There are several categories of microorganisms that exist beneath the plant surface in the soil. Each microorganism behaves in a different way with the root excaudate. Among several molecules, the most promising molecules known as flavonoids are secreted from the plant root. Flavonoids are in the category of secondary metabolites and are produced from the phenylpropanoid and shikimate pathways. These flavonoids vary according to the stage of the tissue and organ. There are different classes of flavonoids such as flavones, flavonols, flavanones, flavanonol, isoflavones and flavan-3-ols. Several enzymes are involved in the flavonoids biosynthesis like chalcone synthase, chalcone isomerise, isoflavone reductase, flavonoid hydroxylase, dihydroflavonol 4 reductase, flavonol synthase. Phenylpropanoids pathway in plants contains a wide range of secondary metabolites; among those, flavonoids are also produced in the enzymatic biosynthesis, which assists in plant growth and development, enhances the immunity to cope the stress

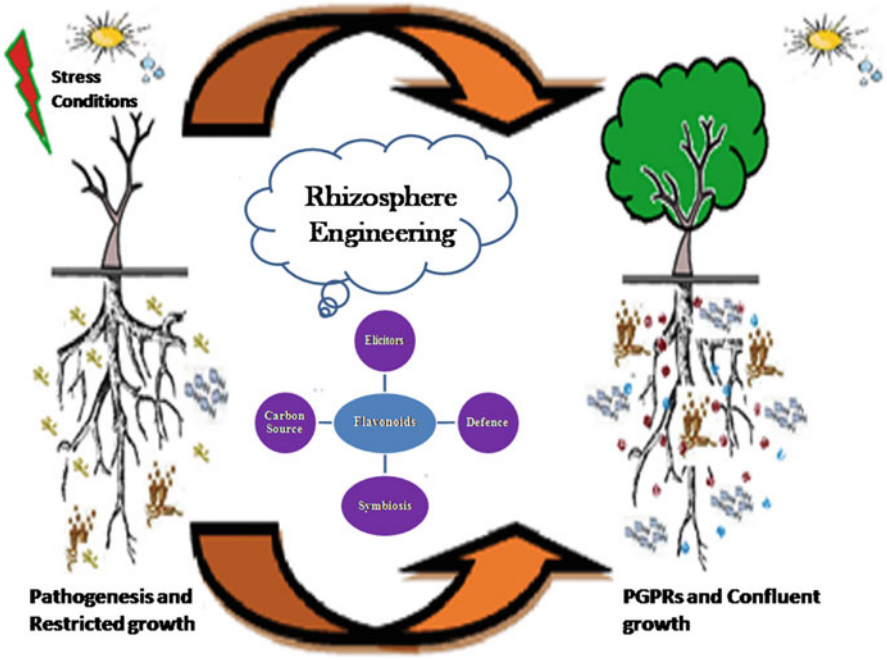

Fig. 8.2 Role of flavonoids in rhizosphere

situations in plants, protects the plants from the pathogenic activity and enhances plant–microbes interactions (Table 8.2). Flavonoids are present in the cytosol, loosely bound with the endoplasmic reticulum and accumulate in the vacuoles or cell wall. In fact, different types of transporters are involved in different mechanisms such as in anthocyanin transporter from ER to vacuole is multidrug resistance-associated protein type transporter on vacuolar membranes these MRP proteins are glutathione S-X pumps whereas vesicles mediated transporter involved the glutathione transferase and two multidrug and toxic extrusion type transporter. Biosynthesis of flavonoid genes is regulated through different transcription factors of different families, such as basic helix loop helix, R2R3 MYB transcription factors, and WD40 proteins are differentially regulated in the different pathways. Likewise, when plants have symbiosis with bacteria or other microorganism, they produce different types of signalling molecules which induce the microorganism. Here, flavonoids stimulate the *nod* gene of rhizobia. Particularly, lipochitooligosaccharides are nod factor for the symbiosis, whereas arbuscular mycorrhizal fungi with plants produce strigolactones as signal for the stimulation. Symbiosis enhances the plant and rhizospheric microorganism growth, stress, and defence-related mechanism (Lateif et al. 2012). Flavonoids in the rhizospheric zone exudate through plant roots which can be increased through microbial strains present below the ground such as *Pseudomonas*, *Bacillus*, Rhizobial strains and fungi species. In fact, flavonoids have the capacity to influence the soil diversity and various microbial enzyme activities. Enzymes such as dehydrogenases, protease, phosphomonoesterase and organic acids such as oxalic acid, carboxylic acid possess the capacity to change the metabolic activity of microbes; in result, they act as the defender against the pathogenic microbes (Del Valle et al. 2020). These soil microbes enhance the plant growth, stimulate the nitrogen content through symbiosis process through the interaction with flavonoids. These flavonoids act as a carbon source for the soil microbes. Microbial activity produces dehydrogenase, protease, acid phosphatase, urease, which correlate with the flavonoids and other secondary metabolites production. *Lupinus albus* root excretes high amount of flavonoids in the soil which increases the quorum sensing, induces *nod* gene, decreases the soil respiration and inhibits the pathogenic activity. Flavonoids possess the antimicrobial property related to plant defence which may be the essential component of rhizosphere. To enhance the defence activity in the soil, biological metabolic engineering of the flavonoid pathways is the main interest (Yechun Wang et al. 2011).

In plant, crop engineering relies on more production of flavonoids, whereas in microbes, engineering is in the flavonoid biosynthesis pathway for producing more flavonoid molecules. There are several enzymes participating in the synthesis of flavonoids, as said in the above text; among them, key steps which have more impact in the production, enhanced through other molecules such as stilbene synthase, have similarity with chalcone synthase. Metabolic engineering in plant and microbes can be done at structural gene over expression through the introduction of rate-limiting enzymes, preferably for those enzymes which do not have feedback inhibition, transcriptional/translational regulation with identification of metabolite-specific transcription factor over expression, over expression of target genes or suppression of

Table 8.2 Flavonoids in action: summary of different types of flavonoids identified from different plant rhizosphere having important role for sustainable plant growth and development

S. No.	Plant	Flavonoid component	Role/Action	References
1	*Psidium guajava* L.	Quercetin, quercetin-3-O-arabinoside	Anti-quorum activity	Vasavi et al. (2014)
2	*Allium cepa*	Quercetin aglycone and quercetin 3-β-D-glucoside	Anti-quorum activity	Quecan et al. (2019)
3	Several plant sources	Fisetin, phloretin, and curcumin	Antibiofilm and Antivirulence effects	Raorane et al. (2019)
4	*Medicago sativa*	4′-dihydroxyflavone and naringenin	Shaping bacterial community structure	Szoboszlay et al. (2016)
5	*Zea mays* L.	Genistein	Enhancing nodulation and N2 fixation	Li et al. (2016)
6	*Quercus ilex*	Acacetin	Abiotic stress adaptation	Gargallo-Garriga et al. (2018)
7	*Sonchus oleraceus*	Flavonoid aglycones and flavonoid glycosides	Allelopathic effect	Gomaa et al. (2015)
8	*Hordeum vulgare* L	Saponarin	Allelopathic effect	Bouhaouel et al. (2019)
9	*Ludwigia hexapetala*	Quercitrin, prunin, myricitrin	Allelopathic effect	Thiébaut et al. (2018)
10	*Echinochloa crus-galli* (L.) Beauv	Flavonoids	Allelopathic effect	Zhang et al. (2019)
11	*Medicago sativa*	Naringenin	Plant-microbe communication	Del Valle et al. (2020)
12	Temperate forest tree species	Flavanols	Soil microbial respiration	Zwetsloot et al. (2018)
13	*Sesbania virgata*	Flavonoids	Myccorhizal association	Coelho et al. (2019)
14	Apple seedlings	Phloretin-2′-O-β-D-glucoside	Host signalling	Hofmann et al. (2009)
15	*Desmodium uncinatum*	Isoschaftoside, a C-glycosylflavonoid	As allelochemical	Hooper et al. (2010)
16	Tomato	Quercetin and luteolin	Disease control	Kirwa et al. (2018)
17	Maize	Flavonoids	Favours association with phytostimulant endophytic Aspergillus fumigatus	Mehmood et al. (2018)
18	Maize	Naringenin	*H. seropedicae* root colonization	Tadra-Sfeir et al. (2011)
19	Rice	Naringenin	Promotion of nitrogen fixing association in the form of a biofilm	Shamala et al. (2018)

(continued)

Table 8.2 (continued)

S. No.	Plant	Flavonoid component	Role/Action	References
20	Faba bean	Genistein, hesperetin, and naringenin	Promotion of nodulation	Liu et al. (2019)
21	*Abelmoschus esculentus*	Quercetin	Chemoattractants of endophytic *Alcaligenes faecalis*	Ray et al. (2018)
22	*Zea mays* L.	Catechin and quercetin	Amelioration of aluminium toxicity	Kidd et al. (2001)
23	*Haplopappus multifolius*	Quercetin and rhamnetin	Oxidative stress alleviation	Torres et al. (2006)
24	Maize and Faba bean	Kaempferol, luteolin, and quercetin	Root growth stimulation	Li et al. (2012)
25	*Myracrodruon urundeuva*	Quercetin rhamnoside	Antihelminthic activity	Soares et al. (2018)

competitive metabolic pathways related to flux control and accumulation of product in cell and sub-cell through transporter which assist in sequestration and in equilibrium without toxicity. Bacterial TAL (tyrosine ammonia lyase) gene over expression in *Arabidopsis*, *UGT72E2* and *UGT72E3* gene over expression in *Arabidopsis* enhanced the flavonoid accumulation. Generally, in microbes such as in bacteria, heterologous production is used for more flavonoid production. Enzymes such as PAL, CHS and a 4CL from *Streptomyces coelicolor* introduced in *E. coli.* Likewise, cloned TAL, 4CL, CHS from *Rhodobacter sphaeroides* in *E. coli.* Several genes such as *accBG* and *dtsR1* for more malonyl-CoA, acetyl-CoA levels which directly affect the flavonoids production, combination of malonyl-CoA synthetase (*matB*) and malonate carrier protein (*matC*) increases the flavonoids production. Knockout genes *ackA*, *pta* and *adhE* from competing pathway assist in more flavonoid production; transcription factors such as Lc, C1, Cmyb, helix loop helix and WD40 engaged in flavonoid production, modification can be done on the basic skeleton enzyme of flavonoid pathways such as glycosyl transferases, methyl transferases and acyl transferases and other structural genes involved in the modification of flavonoid pathways for more production. High-throughput technologies such as transcriptomics, genomics, proteomics and metabolomics assist in the analysis of genetic-engineered pathways at the genome level for more improvement (Yechun Wang et al. 2011). Biotechnology approaches are implemented to enhance the production of flavonoids through callus and cell suspension culture, hairy root culture for more production elicitors, and nanoparticle application. Elicitors are capable of inducing the production of secondary metabolites like flavonoids and nano particles act as novel elicitors which assist in the enhancement of flavonoids production because nanoparticle has the capacity to increase the surface area and total energy for stimulation (Amer 2018).

The structure, quantity, and function of a flavonoid compound are analysed by liquid chromatography and FTIR spectroscopy. These activities regarding flavonoids assist in genetic engineering in the pathway molecules. Metabolite profiling is another tool to analyse the engineered pathway of flavonoids in respect to their composition and function. So, metabolomics tool provides us a more clear view of the newly introduced genes for more flavonoids (Żuk et al. 2011).

8.5 Conclusions and Future Prospects

Plant–microbe associations are the key aspects in plant development and understanding its key features only can enable us for sustainable cropping system. Many measures are taken to curtail the use of chemical pesticides and fertilizers, but they have gone in vain due to the lack of significant knowledge about the rhizospheric associations and association controlling biomolecule factors. Flavonoids are presently found to be the principal compound in these signals for establishing beneficial relationships in rhizosphere and thus can be exploited for antimicrobial effects, as allochemicals, biocontrol actions, improving soil texture, and nutrient availability. Thus, its wide variety of action also proves its utility for rhizosphere engineering, which is a new technique presenting the hope of economical, eco-friendly and efficient (*EEE*) agriculture. However, more efforts are needed to simplify the flavonoid biosynthesis and its signalling mechanisms undergoing in the rhizosphere which support its industrial production and field applications.

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References

Agati G, Azzarello E, Pollastri S, Tattini M (2012) Flavonoids as antioxidants in plants: location and functional significance. Plant Sci 196:67–76

Amer A (2018) Biotechnology approaches for in vitro production of flavonoids. J Microbiol Biotechnol Food Sci 7(5):457–468

Ballard CR, Maróstica Junior MR (2019) Health benefits of flavonoids. Bioactive compounds. Health benefits and potential applications, pp 185–201

Bouhaouel I, Gaëtan R, Marie-Laure F, Marc O, Laurent F, Aurélie G, Hajer SA, Patrick du J (2019) Identification of Barley (*Hordeum vulgare* L. subsp. vulgare) root exudates allelochemicals, their autoallelopathic activity and against *Bromus diandrus* Roth. germination. Agronomy 9:345

Broughton WJ, Zhang F, Perret X, Staehelin C (2003) Signals exchanged between legumes and Rhizobium: agricultural uses and perspectives. Plant Soil 252:129–137

Canarini A, Kaiser C, Merchant A, Richter A, Wanek W (2019) Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front Plant Sci 10:157

Chaparro JM, Badri DV, Vivanco JM (2013) Rhizosphere microbiome assemblage is affected by plant development. ISME J 8(4):790–803

Coelho LCS, Mignoni DSB, Silva FSB, Braga MR (2019) Seed exudates of Sesbania virgata (Cav.) Pers. stimulate the asymbiotic phase of the arbuscular mycorrhizal fungus *Gigaspora albida* Becker & Hall. Hoehnea 46(1):e272018

Del Valle I, Webster TM, Cheng HY, Thies JE, Kessler A, Miller MK et al (2020) Soil organic matter attenuates the efficacy of flavonoid-based plant-microbe communication. Sci Adv 6:8254

Erlejman AG, Verstraeten SV, Fraga CG, Oteiza PI (2004) The interaction of flavonoids with membranes: potential determinant of flavonoid antioxidant effects. Free Radic Res 38:1311–1320

Ferdinando MD, Brunetti C, Fini A, Tattini M (2012) Flavonoids as antioxidants in plants under abiotic stresses. In: Ahmad P, Prasad MNV (eds) Abiotic stress responses in plants: metabolism, productivity and sustainability. Springer, New York, pp 159–179

Gargallo-Garriga A, Preece C, Sardans J et al (2018) Root exudate metabolomes change under drought and show limited capacity for recovery. Sci Rep 8:12696

Gomaa NH, Hassan MO, Fahmy GM, González L, Hammouda O, Atteya AM (2015) Flavonoid profiling and nodulation of some legumes in response to the allelopathic stress of *Sonchus oleraceus* L. Acta Botanica Brasilica 29(4):553–560

Guerrieri A, Dong L, Bouwmeester HJ (2019) Role and exploitation of underground chemical signaling in plants. Pest Manag Sci 75:2455–2463

Hassan S, Mathesius U (2012) The role of flavonoids in root-rhizosphere signalling: opportunities and challenges for improving plant-microbe interactions. J Exp Bot 63(9):3429–3444

He JW, Yang L, Mu ZQ, Zhu YY, Zhong GY, Liu ZY, Zhou QG, Cheng F (2018) Anti-inflammatory and antioxidant activities of flavonoids from the flowers of Hosta plantaginea. RSC Adv 8:18175–18179

Hofmann A, Wittenmayer L, Arnold G, Schieber A, Merbach W (2009) Root exudation of phloridzin by apple seedlings (Malus x domestica Borkh.) with symptoms of apple replant disease. J Appl Bot Food Qual 82:193–198

Hooper AM, Tsanuo MK, Chamberlain K, Tittcomb K, Scholes J, Hassanali A, Khan ZR, Pickett JA (2010) Isoschaftoside, a C -glycosylflavonoid from *Desmodium uncinatum* root exudate, is an allelochemical against the development of *Striga*. Phytochemistry 71:904–908

Jansen MAK (2002) Ultraviolet-B radiation effects on plants: induction of morphogenic responses. Physiol Plant 116:423–429

Jiang N, Doseff AI, Grotewold E (2016) Flavones: from biosynthesis to health benefits. Plants (Basel, Switzerland) 5(2):1–25

Kandaswami C, Lee LT, Lee PP, Hwang JJ, Ke FC, Huang YT, Lee MT (2005) The antitumor activities of flavonoids. In Vivo 19(5):895–909

Kidd PS, Llugany M, Poschenrieder C, Gunsé B, Barceló J (2001) The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). J Exp Bot 52(359):1339–1352

Kim K, Vance TM, Chun OK (2016) Estimated intake and major food sources of flavonoids among US adults: changes between 1999–2002 and 2007–2010 in NHANES. Eur J Nutr 55:833–843

Kirwa HK, Murungi LK, Beck JJ, Torto B (2018) Elicitation of differential responses in the root-knot nematode *Meloidogyne incognita* to tomato root exudate cytokinin, flavonoids, and alkaloids. J Agric Food Chem 66(43):11291–11300

Kozłowska A, Szostak-Wegierek D (2014) Flavonoids--food sources and health benefits. RoczPanstwZaklHig 65(2):79–85

Kozłowska A, Szostak-Węgierek D (2018) Flavonoids – food sources, health benefits, and mechanisms involved. In: Mérillon JM, Ramawat K (eds) Bioactive molecules in food, reference series in phytochemistry. Springer, Cham, pp 1–27

Kuhn BM, Geisler M, Bigler L, Ringli C (2011) Flavonols accumulate asymmetrically and affect auxin transport in Arabidopsis. Plant Physiol 156:585–595

Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Sci World J 162750:1–16

Lateif KA, Bogusz D, Hocher V (2012) The role of flavonoids in the establishment of plant roots endosymbioses with arbuscular mycorrhiza fungi, rhizobia and Frankia bacteria. Plant Signal Behav 7(6):636–641

Li B, Krumbein A, Neugart S, Li L, Schreiner M (2012) Mixed cropping with maize combined with moderate UV-B radiations lead to enhanced flavonoid production and root growth in faba bean. J Plant Interact 7(4):333–340

Li SS, Wu J, Chen LG, Du H, Xu YJ, Wang LJ et al (2014) Biogenesis of *C*-glycosyl flavones and profiling of flavonoid glycosides in lotus (*Nelumbo nucifera*). PLoS One 9(10):e108860

Li B, Li Y-Y, Hua-Mao W, Zhang F-F, Li C-J, Li X-X, Lambers H, Li L (2016) Root exudates drive interspecific facilitation by enhancing nodulation and N_2 fixation. PNAS 113(23):6496–6501

Liu Y, Yin X, Xiao J et al (2019) Interactive influences of intercropping by nitrogen on flavonoid exudation and nodulation in faba bean. Sci Rep 9:4818

Lopez M, Martinez F, Del Valle C, Orte C, Miro M (2001) Analysis of phenolic constituents of biological interest in red wines by high-performance liquid chromatography. J Chromatogr A 922(1–2):359–363

Ma Y, Oliveira RS, Freitas H, Zhang C (2016) Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. Front Plant Sci 7:918

Mabood F, Zhou X, Smith DL (2014) Microbial signaling and plant growth promotion. Can J Plant Sci 94(6):1051–1063

Marunaka Y (2017) Actions of quercetin, a flavonoid, on ion transporters: its physiological roles. Ann N Y Acad Sci 1398:142–151

Mathesius U (2001) Flavonoids induced in cells undergoing nodule organogenesis in white clover are regulators of auxin breakdown by peroxidase. J Exp Bot 52.(Spec Issue:419–426

Mehmood A, Hussain A, Irshad M, Khan N, Hamayun M, Ismail SGA, In-Jung L (2018) IAA and flavonoids modulates the association between maize roots and phytostimulant endophytic *Aspergillus fumigatus* greenish. J Plant Interact 13(1):532–542

Mullineaux PM, Karpinski S (2002) Signal transduction in response to excess light: getting out of the chloroplast. Curr Opin Plant Biol 5:43–48

Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5:e47

Peer WA, Murphy AS (2007) Flavonoids and auxin transport: modulators or regulators? Trends Plant Sci 12(12):556–563

Pyne ME, Narcross L, Martin VJJ (2019) Engineering plant secondary metabolism in microbial systems. Plant Physiol 179:844–861

Quecan BXV, Santos JTC, Rivera MLC, Hassimotto NMA, Almeida FA, Pinto UM (2019) Effect of quercetin rich onion extracts on bacterial quorum sensing. Front Microbiol 10:867

Raorane CJ, Lee JH, Kim YG, Rajasekharan SK, García-Contreras R, Lee J (2019) Antibiofilm and antivirulence efficacies of flavonoids and curcumin against *Acinetobacter baumannii*. Front Microbiol 10:990

Ray S, Mishra S, Bisen K, Singh S, Sarma BK, Singh HB (2018) Modulation in phenolic root exudate profile of Abelmoschus esculentus expressing activation of defense pathway. Microbiol Res 207:100–107

RuizCruz S, Chaparro Hernández S, Hernández Ruiz KL, Cira Chávez LA, Estrada Alvarado MI, Ortega LEG, Ornelas Paz JJ, Lopez Mata MA (2017) Flavonoids: important biocompounds in food, flavonoids - from biosynthesis to human health, Goncalo C. Justino. IntechOpen. https://doi.org/10.5772/67864

Shamala T, Thilini AP, Anuradha S, Kulasooriya SA, Seneviratne G (2018) The effect of flavonoid naringenin coupled with the developed biofilm *Azorhizobium caulinodans-Aspergillus* Spp. on increase in rice yields in conventionally and organically grown rice. Int J Plant Stu 1(1):1–6

Singla P, Garg N (2017) Plant flavonoids: key players in signaling, establishment, and regulation of rhizobial and mycorrhizal endosymbioses. Mycorrhiza - function, diversity, state of the art, pp 133–176

Soares AMS, Oliveira JTA, Rocha CQ, Ferreira ATS, Perales J, Zanatta AC et al (2018) *Myracrodruon urundeuva* seed exudates proteome and anthelmintic activity against *Haemonchus contortus*. PLoS One 13(7):e0200848

Szoboszlay M, White-Monsant A, Moe LA (2016) The effect of root exudate 7, 4′-dihydroxyflavone and naringenin on soil bacterial community structure. PLoS One 11(1):e0146555

Tadra-Sfeir MZ, Souza EM, Faoro H, Müller-Santos M, Baura VA, Tuleski TR, Rigo LU et al (2011) Naringenin regulates expression of genes involved in cell wall synthesis in *Herbaspirillum seropedicae*. Appl Environ Microbiol 77(6):2180–2183

Taylor LP, Grotewold E (2005) Flavonoids as developmental regulators. Curr Opin Plant Biol 8 (3):317–323

Thiébaut G, Thouvenot L, Rodríguez-Pérez H (2018) Allelopathic effect of the invasive *Ludwigia hexapetala* on growth of three macrophyte species. Front Plant Sci 9:1835

Torres R, Faini F, Modak B, Urbina F, Labbé C, Guerrero J (2006) Antioxidant activity of coumarins and flavonols from the resinous exudate of *Haplopappus multifolius*. Phytochemistry 67:984–987

Vasavi HS, Arun AB, Rekha PD (2014) Anti-quorum sensing activity of *Psidium guajava* L. flavonoids against *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1. Microbiol Immunol 58(5):286–293

Verma AK, Pratap R (2010) The biological potential of flavones. Nat Prod Rep 27:1571–1593

Wang Y, Chen S, Yu O (2011) Metabolic engineering of flavonoids in plants and microorganisms. Appl Microbiol Biotechnol 91:949–956

Zhang Q, Zhao X, Qiu H (2013) Flavones and flavonols: phytochemistry and biochemistry. In: Ramawat K, Mérillon JM (eds) Natural products. Springer, Berlin, pp 1821–1847

Zhang Q, Zheng X, Lin S et al (2019) Transcriptome analysis reveals that barnyard grass exudates increase the allelopathic potential of allelopathic and non-allelopathic rice (*Oryza sativa*) accessions. Rice 12:30

Zhao J, Dixon RA (2010) The "ins" and "outs" of flavonoid transport. Trends Plant Sci 15:72–80

Żuk M, Kulma A, Dymińska L, Szołtysek K, Prescha A, Hanuza J, Szopa J (2011) Flavonoid engineering of flax potentiate its biotechnological application. BMC Biotechnol 11:10

Zwetsloot MJ, Andre K, Taryn LB (2018) Phenolic root exudate and tissue compounds vary widely among temperate forest tree species and have contrasting effects on soil microbial respiration. New Phytol 218:530–541

Augmenting the Abiotic Stress Tolerance in Plants Through Microbial Association

9

Ankur Singh and Aryadeep Roychoudhury

Abstract

Recurring climate change due to irresponsible behaviour of human leads to unfavourable climatic condition such as drought, salt stress, extreme temperature and metal toxicity, which significantly decrease the quality and yield of various plants. Several physiological, biochemical and molecular parameters are hampered due to abiotic stress conditions. To enhance the fertility of soil, chemical fertilizers are used which causes soil pollution. Hence, it is necessary to develop a method which is safe and can increase the productivity of the plants by inducing their tolerance capability against abiotic stress. Application of soil dwelling microorganisms is a promising method which can be effectively applied in the field as a bio-fertilizer to induce the crop yield and overcome other symptoms of abiotic stress. Microbes enhance the tolerance mechanism in plants by increasing the mobilization of major elements present in soil, thus facilitating their uptake by plants. In addition, they also induce the formation of hormones, siderophores, osmolytes and antioxidants, which can combat the effect of unfavourable conditions. The interaction between plants and microbes is essential as it is a biological process and in the near future, it can replace the conventional methods of farming which decrease the fertility of lands. In this chapter, our aim is to review various mechanisms adopted by the soil microbes to abrogate the negative effects of abiotic stresses in plants for their better growth and productivity.

Keywords

Abiotic stress · Microbes · Siderophores · Rhizobacteria · Exopolysaccharides · Plant–microbe interaction

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9.1 Introduction

Adverse change in the climate due to human activities is the major cause for decline in growth and productivity of plants (Grayson 2013). With growing population, the demand for higher productive crops is continuously increasing, but factors such as reducing areas of field for cultivation and unfavourable conditions decrease the yield of the plants. Earlier, The Food and Agriculture Organization (FAO) of the United Nations (2007) reported that only 3.5% of total land area is unaffected by any abiotic stress condition. Major abiotic stress influencing the agricultural land and growth and development of plants include water scarcity, variation in temperature, salinity, light intensity, submergence and nutrition deficiency (Hirel et al. 2007; Agarwal and Grover 2006). Of the agricultural land, 64% is affected by drought stress, whereas 13% of the land is affected by flood or excess water stress. Cold stress affects 57% of the crops and salinity stress affects 6% of the land; 9% of the land shows lower productivity of the crop due to mineral deficiency (Cramer et al. 2011; Mittler 2006). Abiotic stress significantly affects the agricultural land which ultimately reduces the quantity and quality of the crops, but it is not possible to accurately estimate the total agricultural loss caused due to harsh and unpredictable environmental conditions (Cramer et al. 2011).

Abiotic stress stimulates the initiation of various pathways which produce reactive oxygen species (ROS). ROS damage the internal organelles and cause membrane damage of cells. Along with this, due to their high reactive nature, they also degrade essential biomolecules like lipids, proteins, carbohydrate and DNA molecules (Toivonen 2005). Membrane damage caused due to ROS also produces several toxic metabolites such as methylglyoxal and malondialdehyde (MDA) (Sharma et al. 2012). Long-term exposure of the plants to stress condition may cause cell death, which will affect the quality of the plants and reduce their yield. To cope with this condition, plants have their internal protective machineries, which can scavenge ROS and ultimately reduce the production of other toxic substances in the cells. Reduced levels of ROS in cells also lower membrane damage and protect the internal organelles of the cells (Singh et al. 2020; Banerjee and Roychoudhury 2018).

In addition to the internal protective machineries, the association between plants and the microbiome inhabiting the surrounding soil also helps to decrease the burden of abiotic stress on the plants (Ngumbi and Kloepper 2014). Microorganisms are the microscopic living system found on the earth and they positively regulate the growth and yield of the plants during unfavourable conditions. With the germination and growth of the seeds, microorganisms continuously multiply and form a symbiotic association on the surface of the plant tissues or endophytic interactions within the plant tissues such as leaves, stems or roots (Turner et al. 2013). Major microbes which help the plants to survive under abiotic stress include mycorrhizal fungi, cyanobacteria which take part in nitrogen fixation and bacteria/rhizobacteria involved in promotion of plant growth and actinomycetes (Elhindi et al. 2017; Singh et al. 2013; Kaushal and Wani 2016; Grover et al. 2016). Along with the abrogation of abiotic stress conditions, association of microbiome also enhances the

production of hormones involved in maintaining the growth of the plants, production of secondary metabolite, availability of minerals from the surrounding and their uptake and protection of plants against diseases, pests or other parasites. Farrar et al. (2014) reported that the association between plants and microbes greatly influences the biochemical, molecular and physical response of the plants against abiotic stress. Thus, the development of multi-omics technology has provided new ways for dissecting and understanding one of the most dynamic and complex associations between plants and the microbiome, which consequently helps the plant system to overcome several harsh conditions of the environment, thereby maintaining their growth, development and yield (Meena et al. 2017). Our aim in this chapter is to overview the effects of abiotic stress on plants and response of the plants in terms of molecular and biochemical processes during these stress conditions when they are associated with different microorganisms. We begin this chapter by providing an overview of different groups of beneficial microbes found in the environment followed by the mechanism adopted by these microbes to enhance the tolerance ability of the plants against various stresses. We will finally conclude this chapter by providing a brief overview about microbial action under various stress conditions such as salt, water deficiency, excess temperature, nutrition deficiency and metal toxicity.

9.2 Types of Beneficial Microbes

Probiotic microbes are microorganisms which co-evolved with the plants and benefited them by forming a free living or symbiotic association with them. Plants produce organic carbons which are released from the roots as root exudates and form a positive association between beneficial microbes, enhancing the growth of the plants under abiotic stress (Gomez et al. 2012). In addition to positive interactions, several root exudates also form a negative association between pathogenic microbes, invertebrate herbivores and parasitic plants (Philippot et al. 2013). Soil microbes can be of several types such as cyanobacteria involved in nitrogen fixation, disease-suppressive microbes, rhizobacteria promoting the growth of plants, microorganisms which detoxify soil toxicants and actinomycetes (Singh et al. 2011) (Fig. 9.1).

9.2.1 Rhizobacteria-Enhancing Growth of Plants

Rhizobacteria dwelling on the surface or around the root of the plants help in the uptake of micro and macro elements from the soil and also enhance the level of plant hormones. They reduce the negative effect of pathogens which hamper the physiology of plants. Hayat et al. (2010) observed that rhizobacteria can efficiently solubilize and transport the minerals from the soil to the plants. Rhizobacteria residing between the space of root cortical cells or in the rhizospheres are regarded as extracellular rhizobacteria which include microorganisms such as *Pseudomonas, Azospirillum, Caulobacter*, *Flavobacterium*, *Erwinia*, *Serratia, Agrobacterium*,

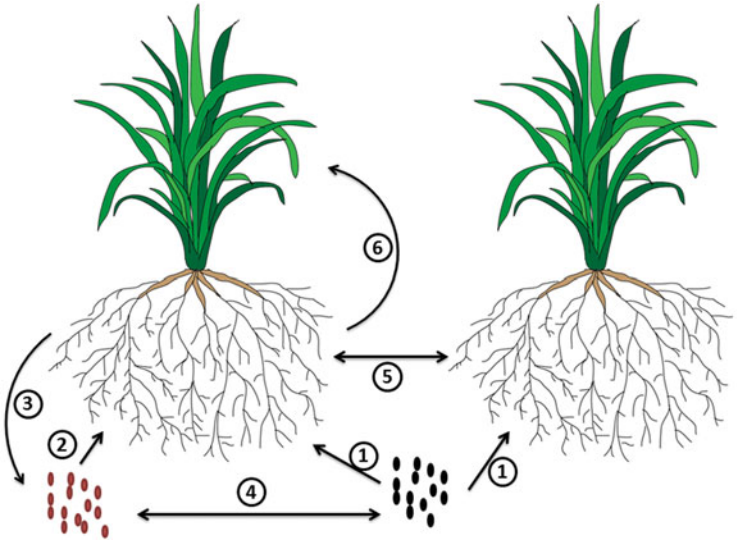

Fig. 9.1 The interaction between plants and microbes. Black dots represent beneficial microbes, whereas brown dots represent pathogens. The numbers represent various types of interaction: (1) positive interaction between plant root and beneficial microbes, (2) attack of pathogenic microbes on plant roots, (3) negative interaction between pathogens and roots, (4) interaction between beneficial and pathogenic microbes, (5) inter-plant interaction and (6) interaction between roots and leaves of plants (Mhlongo et al. 2018)

Bacillus, *Arthrobacter*, *Azotobacter*, etc. (Bhattacharyya and Jha 2012). Along with this, another group of rhizobacteria resides within the root cells and promotes the formation of nodules in roots; these microbes are known as intracellular rhizobacteria, which include *Mesorhizobium, Allorhizobium*, *Rhizobium, Bradyrhizobium* and *Azorhizobium* (Figueiredo et al. 2011). Association between actinomycetes and rhizosphere microbes enhance the plant growth trait significantly (Bhattacharyya and Jha 2012). Several groups of researchers have demonstrated the action of various other traits of rhizobacteria such as degradation and enhanced tolerance level against pesticides, detoxification of heavy metals, salt tolerance capability and resistance to the growth of insects and phytopathogens during their interaction with plants (Ahemad and Khan 2012a; Ma et al. 2011; Tank and Saraf 2009; Russo et al. 2008). Rhizobacteria also induce the growth of plants by stimulating the action of phytohormones, increasing mineralization of phosphate and production of ammonia by increasing nitrogenase activity (Ahemad and Khan 2012b; Glick 2012).

9.2.2 Vesicular-Arbuscular Mycorrhiza (VAM)

The interaction between fungi and plant roots is known as mycorrhiza. Fungi obtain their nutrients from plant roots and in return provide elements such as calcium (Ca), nitrogen (N), phosphorus (P), potassium (K), sulfur (S) and zinc (Zn) to the plants. Vesicular-Arbuscular Mycorrhiza (VAM) is formed due to symbiotic interaction between the roots of angiosperm and phycomycetous fungi. VAM is widely used as a bio-fertilizer and it has wide and significant effects on the growth and yield of the plants. Arbuscules and vesicles are noted on the mycelial network of the fungi, which forms colonies within the root cortex. Vesicles act as a storage unit, whereas arbuscules help the plants to absorb the nutrients from the soil (Benjamin 1979). All the angiosperms and the roots of some aquatic plants are dwelled by the VAM fungi. Fungi produce enzymes, which efficiently mineralize the nutrients from the soil and they also retain soil water, which is absorbed by the plant roots, thus ensuring superior growth and enhancement of tolerance capacity of plants under harsh conditions (Sreenivasa and Bagyaraj 1989).

9.2.3 Other Soil-Dwelling Microbes

Various habitats are colonized by fungi and their interaction with plants controls the plant health and the activities of pathogenic microorganisms (Smith and Read 2008). Suppression of disease-forming pathogens is one of the major functions of actinobacteria, firmicutes and proteobacteria. Competition for resources, enhancing resistance level of plants and production of organic compounds such as antibiosis are some of the mechanisms adopted by the microbes to inhibit formation of diseases in host plants. Cyanobacteria in association with brown and red algae like *Anabaena* sp. and *Nostoc* sp. can fix nitrogen from the atmosphere and thus can be used as a potential bio-fertilizer in paddy fields (Blaak et al. 1993). Crawford et al. (1993) showed that actinomycetes can grow by utilizing the exudates of roots and can act as a strong inhibitor of fungal pathogenic infection. Streptomycetaceae and Actinomycetaceae are important for agricultural industry for their promising effects on plant growth and protection against pathogens.

9.3 Regulation of Tolerance Mechanism in Plants by Microbes

Association between microbes and plants leads to the activation of various signalling cascades of the plants, which enhance the function of defence machineries of the plants such as enhanced expression of genes involved in the synthesis of protective metabolites or inducing certain traits, which controls several metabolic pathways such as hormone, protein and carbohydrate metabolism (Singh 2014). Moreover, the microbes can also change the root and shoot morphology, which stimulates the adaptability of plants under abiotic stress conditions by increasing the absorption of the nutrients, biosynthesis of osmoprotectants and enhanced activity of enzymes

which are involved in reducing the content of ROS and altering the content of various phytohormones of the plants (Van Oosten et al. 2017).

Phytohormones regulate the growth and development of the plants (Farooq et al. 2009). Contesto et al. (2010) reported that rhizobacteria associated with the growth of the plant induces the development and structure of the root by enhancing the endogenous content of indole acetic acid (IAA). Marulanda et al. (2009) observed that the interaction between roots of the clover plant and rhizobacteria enhances the relative water content of the plants via higher production of IAA. Application of *Pseudomonas* spp. having 1-Aminocyclopropane-1-carboxylate (ACC) deaminase which hydrolyses ACC to α-ketoglutaric acid and ammonia help to overcome water deficit conditions in pea plants (Arshad et al. 2008). ACC is the precursor of ethylene; thus, reducing the level of ACC in plants by ACC deaminase also decreases the endogenous content of ethylene, which regulates the development of plants (Siddikee et al. 2011). The endogenous content of abscisic acid (ABA) is also controlled by the microbes during unfavourable environments. A higher ABA content in *Arabidopsis* and lettuce was noted when they were treated by rhizobacteria (Cohen et al. 2008; Arkhipova et al. 2007). Rhizobacteria regulate the morphology of roots like root topology and increase the formation of lateral roots and root hairs and induce the water and mineral uptake of the plants, which increase the tolerance capacity of the plants under unfavourable conditions. This mechanism of stress tolerance was reported in maize plants associated with several strains of rhizobacteria, under water-deficit conditions (Naveed et al. 2014) (Fig. 9.2).

Maize plants when exposed to a water-deficit environment and inoculated with *Azospirillum brasilense* BR11005 spp. showed higher water content due to the stomatal closure induced by the bacterial ABA which increased the tolerance capacity of the plants (Casanovas et al. 2002). Rhizobacteria also helps to scavenge ROS produced during stress conditions by improving the activity of anti-oxidative enzymes observed in several crops such as cucumber, wheat, lettuce and maize (Wang et al. 2012, Kasim et al. 2013; Kohler et al. 2010; Sarma and Saikia 2014). Treatment of several crops such as potato, mung bean and cucumber with rhizobacteria also enhanced the level of proline, which acts as a major osmoprotectant by reducing the level of ROS and regulates the activity of enzymes (Gururani et al. 2013; Sarma and Saikia 2014; Wang et al. 2012)

9.4 Role of Microorganisms in Abrogating the Effects of Abiotic Stresses

Plants require optimal conditions for their normal growth and yield. The deviations from optimal conditions are sensed by the plants, which lead to the appropriate response so that the plants can cope with the condition for better tolerance level (Jiang et al. 2016). Several factors such as salinity, drought, excess temperature, metal contamination and nutrient deficiency can negatively affect the growth of the plants, which reduces the yield of the plants.

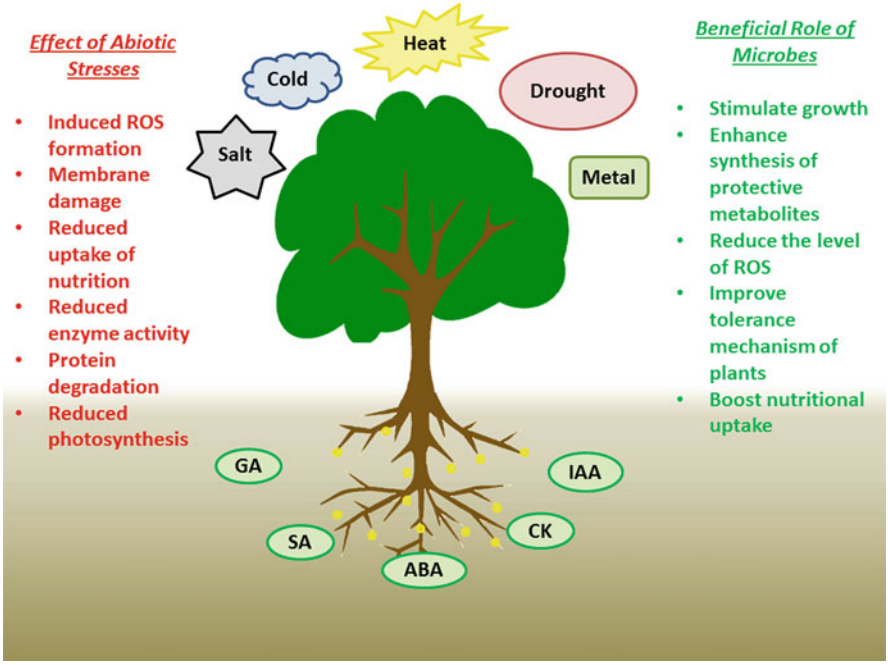

Fig. 9.2 Synthesis of hormones such as abscisic acid (ABA), gibberellin (GA), indole acetic acid (IAA), salicylic acid (SA) and cytokinin (CK) due to the association of microbes with the roots of plants (represented by yellow dots) improves the tolerance mechanism of the plants by increasing growth of plants, uptake of nutrition and production of protective metabolites which lower the accumulation of toxic species such as ROS formed due to abiotic stress such as salinity, cold, heat, drought and heavy metals (Egamberdieva et al. 2017)

9.4.1 Salt Stress

High salt level in agricultural soil is one of the most common problems faced by the farmers. Salinity stress reduces the level of water in soil, which leads to osmotic stress in plants due to reduced translocation of water and nutrients from the soil (Shrivastava and Kumar 2015). Salt stress negatively affects nodulation in roots, nitrogen fixation, seed germination, uptake of water and nutrient from the soil and lower yield of the plants. The activity of nitrogenase enzymes is hampered due to salinity stress, which reduces the nitrogen content of the soil by decreasing the nitrogen fixation process (Tejera et al. 2004). During uptake of water from the soil, salt is also transported through the roots of the plants, which is accumulated in the cells and thus causes ionic toxicity within the cells. Ion toxicity and osmotic stress mainly contribute to salinity stress in both plants and microbes.

Various groups of researchers have shown that salt stress can be mitigated by rhizobacteria or endophytic microbes. Microbial activity reduces the symptoms of salt stress in various economic crops such as chili pepper, lettuce and barley (Bacilio et al. 2016; Barassi et al. 2006; Kasim et al. 2016). Hayat et al. (2010) observed that

microbes reduce the toxicity of salt stress through the generation of siderophores, nutrient transportation, production of phytohormones such as ethylene, auxin, gibberellins and cytokinins and fixation of nitrogen in the soil, which ultimately affects the morphology of the plants in ways such as enhanced root growth and higher surface area of roots. Microbes can reduce the effect of salt stress in the host plant cells by accumulating excess salt in their cytoplasm, which balances the osmotic condition of cells. In addition to this, the exopolysaccharides secreted by the microbes quench the cations formed during salt stress (Vardharajula et al. 2011). Damodaran et al. (2013) isolated the two strains of rhizospheric bacteria, *Bacillus subtilis* and *Bacillus pumilus*, from saline soil and showed that they contain traits such as production of IAA, hydrogen cyanide and ammonia and can also solubilize the phosphate of the soil, which helps in the mitigation of salt stress. Inoculation of rice plants *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes* induces the production of glycine betaine, which reduces the effects of salt stress in plants (Jha et al. 2011). Bal et al. (2013) and Tank and Saraf (2010) isolated two rhizobacterial strains *P. stutzeri* and *P. fluorescens* from the rhizosphere of tomato plants grown under high salt concentrations and showed that these strains have the ability to produce phytohormones and higher ACC deaminase activity for higher survival ability of the host plant under salt-stressed environment. The hormones produced by the endophytic bacteria also help to reduce the toxic effect of salt stress in plants. Shahzad et al. (2017) reported that inoculation of rice plants with *Bacillus amyloliquefaciens* reduces the symptoms of salt toxicity in plants by the production of auxin and ABA. Microbes thus reduce the effects of salt stress in plants by the production of phytohormones, antioxidants and osmolytes, which enhances the adaptability of plants and increases their production capacity.

9.4.2 Drought Stress

The crisis of water is a serious concern for the developing countries. Most of the water sources are either at the verge of extinction or are heavily contaminated with pollutants. Water deficiency mostly results in drought, which severely retards the growth and productivity of the plants. Drought stress mostly reduces the cell size, hampers the integrity of membranes, leads to the formation of ROS, induces leaf senescence and reduces the yield of the plants (Tiwari et al. 2016). The ROS produced during drought stress negatively affects the function of lipid membranes, changes the conformation of protein and leads to lipid peroxidation. Under severe water-deficit conditions, the cells may even die. In addition, the photosynthetic apparatus of the plants are damaged, which results in lower chlorophyll content (Lata and Prasad 2011).

Various groups of microorganisms can effectively escape water-deficit conditions. Several tolerance mechanisms such as formation of thick cell wall, secretions of exopolysaccharides or osmolytes and entering into a dormant phase are triggered by the microbes, which protects them from desiccation. Several direct and indirect mechanisms such as formation of phytohormones, enhanced activity of

ACC deaminase, secretion of exopolysaccharide and inducing the function of internal machineries operate in plants due to their interaction with microbes, which help the host plants to escape a water-deficit environment (Porcel et al. 2014; Farooq et al. 2009). Goswami et al. (2015) reported that rhizobacteria have the ability to synthesize plant hormones such as IAA, which stimulates formation of lateral and adventitious roots, tissue differentiation and cell division, which stimulates the growth of the shoot during unfavourable conditions. ABA is regarded as the main hormone responsible for survival of the plants under drought stress. Inoculation of *Arabidopsis thaliana* with *Azospirillum brasilense* reduces the symptoms of drought stress by inducing a level of endogenous ABA (Cohen et al. 2015). Drought stress reduces the chlorophyll content and hampers the photosynthesis of soybean plants, which was shown to be alleviated by inoculating the plants with *Pseudomonas putida* H-2-3 (Kang et al. 2014). Treatment of maize plants with three different bacterial strains, *Alcaligenes faecalis*, *Proteus penneri* and *Pseudomonas aeruginosa*, induced the formation of proline, which increased the water content of plants along with higher production of other protective metabolites under water-deficit conditions (Naseem and Bano 2014). During water shortage, bacteria secrete exopolysaccharides, which make plants associated with such bacteria tolerant to stress conditions (Sandhya et al. 2009). Synthesis of solutes such as trehalose, proline and glycine betaine is also a protective mechanism adopted by bacteria, which maintain the cellular structures, activity of major enzymes and permeability of membrane, thus allowing survival under water-deficit environments (Chithrashree et al. 2011).

9.4.3 Temperature Stress

An optimum temperature is required for maximum growth and productivity of plants. Due to global warming, the climate is changing drastically, which negatively affects the physiological and morphological conditions of the plants. Both high and low temperatures significantly lower the yield of the plants. Temperature stress mostly affects the fluidity of the cell membrane, reduces the water content by enhancing the water loss through transpiration and hinders enzyme activity and cell division. Tropical and sub-tropical regions are mostly affected by climate change (Rodell et al. 2009). To protect themselves from heat stress, plants have various internal machineries such as heat shock proteins and antioxidative enzymes, which can scavenge ROS and osmolytes to maintain the osmotic balance of the cells (Kotak et al. 2007; Qu et al. 2013). Several crops still fail to survive under harsh temperature and thus various external protective measures are taken to induce the survival ability of the plants.

The microbes effectively protect the cell membrane and nucleic acid by expressing the enzymes which are resistant against temperature stress. Based on the survival temperature, the microbes can be divided into psychrotrophic microorganisms which can survive at or above 15 °C and psychrophilic microorganisms, which show maximum growth below 15 °C. Induced expression

of heat shock proteins and molecular chaperons is highly regulated in microbes which protect them from heat stress. In addition, several metabolites such as trehalose have been reported to be accumulated in the microorganisms when exposed to heat or cold stress, which protect cells from injury (Li et al. 2009). Meena et al. (2015) showed that rhizobacteria isolated from the root nodules of pea plants can be effectively used as a bio-fertilizer in low-temperature zones. Javani et al. (2015) observed that psychrophilic bacteria have antimicrobial activities. Application of phosphate-dissolving thermotolerant microbes as a bio-fertilizer enhances the phosphorus content of the plants by breaking the insoluble phosphorus of the soil to the soluble form, which induces the tolerance of the plants under temperature stress (Chang and Yang 2009).

9.4.4 Nutrition Deficiency

Availability of nutrients from the soil is an important factor which controls the yield of the crops. Binding of a major element to the soil or inability of the plants to uptake nutrients from soil are two major causes of nutrition stress in plants. Inorganic phosphate, N and iron (Fe) are mostly essential for the growth of the plants. Plants lacking adequate amounts of nutrition are reduced in size with lower grain quality and quantity.

Microbes can efficiently mobilize the nutrient from the soil and supply them to the host plants. Compant et al. (2005) reported that bacteria associated with plant roots form siderophores which sequester ferric ions from the surrounding and transport them to the host plants. In leguminous plants, nitrogen-fixing bacteria form nodules, which later help in the fixation of nitrogen, which plants can absorb from soil and utilize them further to form amino acids. Another important element in the soil is phosphorus, which can be effectively solubilized by various genera of bacteria and fungi. Rivas et al. (2006) reported the association between *Cicer arietinum* and two phosphorus-solubilizing microbes *Mesorhizobium mediterraneum* and *Mesorhizobium ciceri*. Uptake of Zn was enhanced in wheat plants when a symbiotic association of roots with *Azotobacter chroococcum* and *Pseudomonas indica* was established (Abadi and Sepehri 2016). Mycorrhiza solubilizes and enhances the uptake of several major elements such as P, copper (Cu), Zn, magnesium (Mg), N and K in the plants (Smith and Read 2008). Lehmann and Rillig (2015) showed that inoculation of *Miscanthus sacchariflorus* with the fungi *Gigaspora margarita* enhances the uptake of nutrients such as N, P, Fe, Cu, Zn, K and Mg from the soil. Mycorrhiza secretes a glycoprotein known as glomalin, which stabilizes the soil by forming an aggregate in between the soil particles, thus controlling the soil characteristics (Gadkar and Rillig 2006).

9.4.5 Heavy Metal Toxicity

With the growth of human civilization, the number of industries has increased due to higher demand for products, which leads to an enhanced contamination of soil with heavy metals. The soil quality is also deteriorated due to higher application of chemical fertilizers, pesticides, insecticides and other chemical products, decreasing the fertility of soil, which significantly affects the growth and yield of the plants (Table 9.1).

Application of microbes in the agricultural field is an effective method to combat the metal toxicity of soil. Several mechanisms such as chelation of heavy metals, detoxification of absorbed metal through various enzymatic activities, reducing the uptake of heavy metal and sequestering them in the exopolysaccharide layer are adopted by the microorganisms to decrease the toxicity of heavy metals (Kumar and Verma 2018). Microbes release chemicals which can chelate heavy metals such as Fe, Zn and Cu, which thus reduce the uptake of these elements by the roots of the host plants (Dimkpa et al. 2009). Pishchik et al. (2002) reported that barley plants grown in soil contaminated with cadmium in association with *Klebsiella mobilis* CIAM 880 showed better growth and lowered cadmium due to the release of chemicals which chelated cadmium ions and thus reduced their availability to the host plant. Hashem et al. (2016) reported that induced levels of MDA and hydrogen peroxide in the *Solanum lycopersicum* were lowered when inoculated with fungi, which reduced the effect of cadmium (Cd) toxicity in plants. Siderophores released from the microbes associated with plants have higher affinity toward Fe^{3+} but can also effectively chelate other heavy metals, which ultimately reduced the uptake and toxicity of these metals (Saha et al. 2015). Złoch et al. (2016) reported that rhizospheric bacteria are more effective in the production of siderophores as compared to that of endophytic bacteria; thus, endophytic bacteria adapt different mechanisms such as providing nutrients to the host plant and synthesis of enzymes and compounds which are able to promote plant growth to reduce the toxic effects of heavy metals. The synthesis of phytochelatins by some fungi also helps to sequester heavy metals present in soil, thus decreasing their mobilization through the plant transport system (Gadd 2010). Bolan et al. (2014) reported that methylation of heavy metals such as lead (Pb), selenium (Se), mercury (Hg), tin (Sn) and arsenic (As) by transferring a methyl group through bacterial activity also helps in mobilisation of these metals. Endophytic bacteria reduce pathogenic infection and induce the resistance of plants towards heavy metal stress (Ma et al. 2016). Thus, microbes reduce the effects of metal toxicity by forming siderophores, biosurfactants and organic acid or by various processes such as reduction or bio-methylation of heavy metals (Ullah et al. 2015).

Table 9.1 Interaction between plants and microbes leads to the development of various protective mechanisms, which enhance the survival of plants against various heavy metal stress

Host plants	Microbes	Metal Stress	Mechanism of stress amelioration	References
Oryza sativa	*Bacillus thuringiensis*, *Paenibacillus glucanolyticus*	As	Reduces the accumulation of As in plant tissue, enhances the activity of enzymatic antioxidants, phenolics and flavonoids which scavenge the ROS	Banerjee et al. (2020)
Sorghum bicolor	*Bacillus cereus*, *Providencia rettgeri*, *Myroides odoratimimus*	Cr	Reduces Cr^{6+} to Cr^{3+}, induces the production of siderophores and IAA; induces the activity of antioxidative enzymes and their gene expression	Bruno et al. (2020)
Triticum aestivum	*Bacillus subtilis*	Cr	Enhances the content of chlorophyll, ABA and proteins, converts Cr^{6+} to Cr^{3+} and also reduces the uptake of Cr^{6+} by roots	Seleiman et al. (2020)
Zea mays	*Enterobacter asburiae*	Cd	Decreased transpiration rate; down-regulation of iron transporter gene	Zhou et al. (2019)
Zea mays	*Glomus intraradices*	Cd	Increased translocation of P, S and Cu, up-regulation of antioxidative genes and plant hormones	Gu et al. (2019)
Brassica napus	*Microbacterium oxydans*, *Burkholderia cepacia*, *Pseudomonas thivervalensis*	Cu	Reduces the translocation of Cu; enhances the activity of enzymatic antioxidants along with higher production of glutathione and ABA	Ren et al. (2019)
Vigna radiata	*Acinetobacter lwoffii*	As	Production of IAA, exopolysaccharides and siderophores, which helps to maintain the growth of plants and also reduces the accumulation of As in plant tissues	Das and Sarkar (2018)
Arabidopsis	*Bacillus subtilis*, *Azospirillum brasilense*	Cd	Increases the endogenous content of ABA; reduces the expression of iron-regulated transporter 1 and accumulation of Cd	Xu et al. (2018)
Brassica juncea	*Brevibacterium frigoritolerans*, *Bacillus paralicheniformis*	Pb	Decrease in metal uptake by plants; increases the biomass of plants, induces	Yahaghi et al. (2018)

(continued)

Table 9.1 (continued)

Host plants	Microbes	Metal Stress	Mechanism of stress amelioration	References
			the production of IAA and siderophores	
Cicer arietinum	*Trichoderma* sp.	As	More toxic inorganic As species converted to less toxic organic As; enhances the nutrient content of plants, reduces the expression of gene responsible for abiotic stress	Tripathi et al. (2017)
Lens culinaris	*Providencia vermicola*	Cu	Production of plant growth hormone such as IAA, siderophores, higher activity of ACC deaminase, solubilization of P	Islam et al. (2016)
Miscanthus sinensis	*Pseudomonas koreensis*	Pb	Sequestration of heavy metal on the outer surface of bacteria, reduction in ROS due to higher activity of catalase and superoxide dismutase, increases biomass, chlorophyll and protein content	Babu et al. (2015)
Lens culinaris	*Pseudomonas* sp., *Rahnella aquatilis*, *Agrobacterium tumefaciens*	Pb	Higher production of IAA and siderophores, induces the activity of enzymatic antioxidants such as superoxide dismutase and peroxidase	Jebara et al. (2015)
Glycine max	*Penicillium funiculosum*	Cu	Synthesis of proline and glutamate, higher nutritional uptake to maintain carbon, hydrogen and nitrogen contents of shoot	Khan and Lee (2013)
Sedum plumbizincicola	*Phyllobacterium myrsinacearum*	Pb	Synthesis of ACC deaminase, IAA, siderophores, solubilization of phosphate and higher uptake of essential metals	Ma et al. (2013)
Cicer arietinum	*Paenibacillus lentimorbus*	Cr	Reduces the uptake of Cr by roots	Khan et al. (2012)
Commelina communis	*Bacillus* sp., *Acinetobacter* sp.	Pb	Higher ACC deaminase activity and production of IAA, increase in the dry weight of plants and root length	Zhang et al. (2011)

9.5 Conclusion and Future Perspectives

Unfavourable environmental conditions such as water, salt, temperature stress, low availability of minerals and contamination of soil with heavy metals hamper growth, productivity and survival of plants. Abiotic stress mainly causes hormonal imbalance, protein distortion, production of ROS and affects the cell organelles and cell membranes, which may lead to plant death or can lower their yield and quality, causing a serious problem for global food security. Application of chemical fertilizers can increase the crop yield for short periods of time, but it accumulates large amounts of toxic chemicals in the field, which later decrease the productivity of land and also cause serious health-related problems due to accumulation of chemicals in the grains. Thus, the only solution to this problem is the application of microbes which are naturally present in soil and can induce quality without causing any environmental issues. Various earlier researches have helped us to understand that interaction between beneficial microbes and host plants have enhanced the tolerance mechanism of the plants under various abiotic stresses. Association between microbes and plants leads to the production of siderophores, regulates the hormonal balance of plants, improves the uptake of nutrition from the soil and enhances the defensive mechanism of the plants, thereby enhancing the tolerance capability of plants along with their growth and yield. Based on these potentialities, it can be concluded that application of microbial-assisted plant interaction in the agricultural field can bring a new revolution in the near future. In coming times, more researches must be conducted to identify more potential yet unidentified microbes which can play a major role in improving the quality and quantity of yield of plants and implementing sound policies to realize their beneficial effects in the agricultural sector.

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References

Abadi VAJM, Sepehri M (2016) Effect of *Piriformospora indica* and *Azotobacter chroococcum* on mitigation of zinc deficiency stress in wheat (*Triticum aestivum* L.). Symbiosis 69:9–19

Agarwal S, Grover A (2006) Molecular biology, biotechnology and genomics of flooding-associated low O_2 stress response in plants. Crit Rev Plant Sci 25:1–21

Ahemad M, Khan MS (2012a) Ecological assessment of biotoxicity of pesticides towards plant growth promoting activities of pea (*Pisum sativum*) specific *Rhizobium* sp. strain MRP1. Emir J Food Agric 24:334–343

Ahemad M, Khan MS (2012b) Evaluation of plant growth promoting activities of rhizobacterium *Pseudomonas putida* under herbicide-stress. Ann Microbiol 62:1531–1540

Arkhipova TN, Prinsen E, Veselov SU, Martinenko EV, Melentiev AI, Kudoyarova GR (2007) Cytokinin producing bacteria enhance plant growth in drying soil. Plant Soil 292:305–315

Arshad M, Shaharoona B, Mahmood T (2008) Inoculation with *Pseudomonas* spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.). Pedosphere 18:611–620

Babu AG, Shea PJ, Sudhakar D, Jung I-B, Oh B-T (2015) Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal(loid)-contaminated mining site soil. J Environ Manag 151:160–166

Bacilio M, Moreno M, Bashan Y (2016) Mitigation of negative effects of progressive soil salinity gradients by application of humic acids and inoculation with *Pseudomonas stutzeri* in a salt-tolerant and a salt-susceptible pepper. Appl Soil Ecol 107:394–404

Bal HB, Nayak L, Das S, Adhya TK (2013) Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil 366:93–105

Banerjee A, Roychoudhury A (2018) Abiotic stress, generation of reactive oxygen species, and their consequences: an overview. In: Singh VP, Singh S, Tripathi D, Mohan-Prasad S, Chauhan DK (eds) Revisiting the role of reactive oxygen species (ROS) in plants: ROS Boon or bane for plants? Wiley, Hoboken, NJ, pp 23–50

Banerjee A, Hazra A, Das S, Sengupta C (2020) Groundwater inhabited *Bacillus* and *Paenibacillus* strains alleviate arsenic-induced phytotoxicity of rice plant. Int J Phytoremediation 16:1–11

Barassi CA, Ayrault G, Creus CM, Sueldo RJ, Sobrero MT (2006) Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce. Sci Hort 109:8–14

Benjamin RK (1979) Zygomycetes and their spores. In: Kendrick B (ed) The whole fungus: the sexual asexual synthesis. Ottawa, National Museums of Natural Sciences, pp 573–622

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Blaak H, Schnellmann J, Walter S, Henrissat B, Schrempf H (1993) Characteristics of an exochitinase from *Streptomyces olivaceoviridis*, its corresponding gene, putative protein domains and relationship to other chitinases. Eur J Biochem 214:659–669

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014) Remediation of heavy metal(loid)s contaminated soils – to mobilize or to immobilize? J Hazard Mater 266:141–166

Bruno LB, Karthik C, Ma Y, Kadirvelu K, Freitas H, Rajkumar M (2020) Amelioration of chromium and heat stresses in *Sorghum bicolor* by Cr^{6+} reducing-thermotolerant plant growth promoting bacteria. Chemosphere 244:125521

Casanovas EM, Barassi CA, Sueldo RJ (2002) *Azospirillum* inoculation mitigates water stress effects in maize seedlings. Cereal Res Commun 30:343–350

Chang CH, Yang SS (2009) Thermo-tolerant phosphate-solubilizing microbes for multifunctional biofertilizer preparation. Bioresour Technol 100:1648–1658

Chithrashree UAC, Nayaka SC, Reddy MS, Srinivas C (2011) Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. oryzae. Biol Control 59:114–122

Cohen AC, Bottini R, Piccoli PN (2008) *Azospirillum brasilense* Sp 245 produces ABA in chemically defined culture medium and increases ABA content in *Arabidopsis* plants. Plant Growth Regul 54:97–103

Cohen AC, Bottini R, Pontin M, Berli FJ, Moreno D, Boccanlandro H, Travaglia CN, Piccoli PN (2015) *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. Physiol Plant 153:79–90

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Contesto C, Milesi S, Mantelin S, Zancarini A, Desbrosses G, Varoquaux F, Bellini C, Kowalczyk M, Touraine B (2010) The auxin-signaling pathway is required for the lateral root response of *Arabidopsis* to the rhizobacterium *Phyllobacterium brassicacearum*. Planta 232:1455–1470

Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol 11:163

Crawford DL, Lynch JM, Whipps JM, Ousley MA (1993) Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Appl Environ Microbiol 59:3899–3905

Damodaran T, Sah V, Rai RB, Sharma DK, Mishra VK, Jha SK, Kannan R (2013) Isolation of salt tolerant endophytic and rhizospheric bacteria by natural selection and screening for promising plant growth-promoting rhizobacteria(PGPR) and growth vigour in tomato under sodic environment. Afr J Microbiol Res 7:5082–5089

Das J, Sarkar P (2018) Remediation of arsenic in mung bean (*Vigna radiata*) with growth enhancement by unique arsenic-resistant bacterium *Acinetobacter lwoffii*. Sci Total Environ 624:1106–1118

Dimkpa CO, Merten D, Svatoš A, Büchel G, Kothe E (2009) Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696

Egamberdieva D, Wirt SJ, Alqarawi AA, Abd_Allah EF, Hashem A (2017) Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Front Microbiol 8:2104

Elhindi KM, El-Din AS, Elgorban AM (2017) The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.). Saudi J Biol Sci 24:170–179

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212

Farrar K, Bryant D, Cope-Selby N (2014) Understanding and engineering beneficial plant–microbe interactions: plant growth promotion in energy crops. Plant Biotechnol J 12:1193–1206

Figueiredo MVB, Seldin L, Araujo FF, Mariano RLR (2011) Plant growth promoting rhizobacteria: fundamentals and applications. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Berlin, pp 21–43

Gadd GM (2010) Metals, minerals, and microbes: geo-microbiology and bioremediation. Microbiology 156:609–643

Gadkar V, Rillig MC (2006) The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. FEMS Microbiol Lett 263:93–101

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:963401

Gomez CG, Valero NV, Brigard RC (2012) Halotolerant/alkalophilic bacteria associated with the cyanobacterium *Arthrospira platensis* (Nordstedt) Gomont that promote early growth in *Sorghum bicolor* (L.). Agron Colomb 30:111–115

Goswami D, Thakker JN, Dhandhukia PC (2015) Simultaneous detection and quantification of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) produced by rhizobacteria from l-tryptophan (Trp) using HPTLC. J Microbiol Method 110:7–14

Grayson M (2013) Agriculture and drought. Nature 501:S1–S1

Grover M, Bodhankar S, Maheswari M, Srinivasarao C (2016) Actinomycetes as mitigators of climate change and abiotic stress. In: Subramaniam G, Arumugam S, Rajendran V (eds) Growth promoting actinobacteria. Springer, Singapore, pp 203–212

Gu L, Zhao M, Ge M, Zhu S, Cheng B, Li X (2019) Transcriptome analysis reveals comprehensive responses to cadmium stress in maize inoculated with arbuscular mycorrhizal fungi. Ecotoxicol Environ Safe 186:109744

Gururani MA, Upadhyaya CP, Strasser RJ, Yu JW, Park SW (2013) Evaluation of abiotic stress tolerance in transgenic potato plants with reduced expression of PSII manganese stabilizing protein. Plant Sci 198:7–16

Hashem A, Abd_Allah EF, Alqarawi AA, Al Huqail AA, Egamberdieva D, Wirth S (2016) Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. Saudi J Biol Sci 23:272–281

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369–2387

Islam F, Yasmeen T, Ali Q, Mubin M, Ali S, Arif MS, Hussain S, Riaz M, Abbas F (2016) Copper-resistant bacteria reduces oxidative stress and uptake of copper in lentil plants: potential for bacterial bioremediation. Environ Sci Pollut Res Int 23:220–233

Javani S, Marín I, Amils R, Abad JP (2015) Four psychrophilic bacteria from Antarctica extracellularly biosynthesize at low temperature highly stable silver nanoparticles with outstanding antimicrobial activity. Colloids Surf A Physicochem Eng Asp 483:60–69

Jebara SH, Saadani O, Fatnassi IC, Chiboub M, Abdelkrim S, Jebara M (2015) Inoculation of *Lens culinaris* with Pb-resistant bacteria shows potential for phytostabilization. Environ Sci Pollut Res Int 22:2537–2545

Jha Y, Subramanian RB, Patel S (2011) The combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. Acta Physiol Plant 33:797–802

Jiang Q-Y, Zhuo F, Long S-H, Zhao H-D, Yang D-J, Ye Z-H, Li S-S, Jing Y-X (2016) Can arbuscular mycorrhizal fungi reduce Cd uptake and alleviate Cd toxicity of *Lonicera japonica* grown in Cd-added soils? Sci Rep 6:21805

Kang SM, Radhakrishnan R, Khan AL, Kim MJ, Park JM, Kim BR, Shin DH, Lee IJ (2014) Gibberellin secreting rhizobacterium, *Pseudomonas putida* H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. Plant Physiol Biochem 84:115–124

Kasim W, Osman M, Omar M, Abd El-Daim I, Bejai S, Meijer J (2013) Control of drought stress in wheat using plant-growth promoting rhizobacteria. J Plant Growth Regul 32:122–130

Kasim WA, Gaafar RM, Abou-Ali RM, Omar MN, Hewait HM (2016) Effect of biofilm forming plant growth promoting rhizobacteria on salinity tolerance in barley. Ann Agric Sci 61:217–227

Kaushal M, Wani SP (2016) Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. Ann Microbiol 66:35–42

Khan AL, Lee IJ (2013) Endophytic *Penicillium funiculosum* LHL06 secretes gibberellin that reprograms *Glycine max* L. growth during copper stress. BMC Plant Biol 13:86

Khan N, Mishra A, Chauhan PS, Sharma YK, Nautiyal CS (2012) *Paenibacillus lentimorbus* enhances growth of chickpea (*Cicer arietinum* L.) in chromium-amended soil. Antonie Van Leeuwenhoek 101:453–459

Kohler J, Caravaca F, Roldàn A (2010) An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of *Lactuca sativa*. Soil Biol Biochem 42:429–434

Kotak S, Larkindale J, Lee U, von Koskull-Döring P, Vierling E, Scharf K-D (2007) Complexity of the heat stress response in plants. Curr Opin Plant Biol 10:310–316

Kumar A, Verma JP (2018) Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52

Lata C, Prasad M (2011) The role of DREBs in the regulation of abiotic stress responses in plants. J Exp Bot 62:4731–4748

Lehmann A, Rillig MC (2015) Arbuscular mycorrhizal contribution to copper, manganese and iron nutrient concentrations in crops—a meta-analysis. Soil Biol Biochem 81:147–158

Li L, Ye Y, Pan L, Zhu Y, Zheng S, Lin Y (2009) The induction of trehalose and glycerol in *Saccharomyces cerevisiae* in response to various stresses. Biochem Biophys Res Commun 387:778–783

Ma Y, Rajkumar M, Luo Y, Freitas H (2011) Inoculation of endophytic bacteria on host and non-host plants effects on plant growth and Ni uptake. J Hazard Mater 195:230–237

Ma Y, Rajkumar M, Luo Y, Freitas H (2013) Phytoextraction of heavy metal polluted soils using *Sedum plumbizincicola* inoculated with metal mobilizing *Phyllobacterium myrsinacearum* RC6b. Chemosphere 93:1386–1392

Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Inoculation of *Brassica oxyrrhina* with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. J Hazard Mater 320:36–44

Marulanda A, Barea JM, Azcón R (2009) Stimulation of plant growth and drought tolerance by native microorganisms (AM Fungi and Bacteria) from dry environments: Mechanisms related to bacterial effectiveness. J Plant Growth Regul 28:115–124

Meena RK, Singh RK, Singh NP, Meena SK, Meena VS (2015) Isolation of low temperature surviving plant growth–promoting rhizobacteria (PGPR) from pea (*Pisum sativum* L.) and documentation of their plant growth promoting traits. Biocatal Agric Biotechnol 4:806–811

Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A, Singh DP, Prabha R, Sahu PK, Gupta VK, Singh HB, Krishanani KK, Minhas PS (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:172

Mhlongo MI, Piater LA, Madala NE, Labuschagne N, Dubery IA (2018) The chemistry of plant–microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front Plant Sci 9:112

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19

Naseem H, Bano A (2014) Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance in maize. J Plant Interact 9:689–701

Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A (2014) Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD 17. Environ Exp Bot 97:30–39

Ngumbi E, Kloepper J (2014) Bacterial-mediated drought tolerance: current and future prospects. Appl Soil Ecol 105:109–125

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799

Pishchik VN, Vorobyev NI, Chernyaeva II, Timofeeva SV, Kozhemyakov AP, Alexeev YV, Lukin SM (2002) Experimental and mathematical simulation of plant growth promoting rhizobacteria and plant interaction under cadmium stress. Plant Soil 243:173–186

Porcel R, Zamarreño Á, García-Mina J, Aroca R (2014) Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants. BMC Plant Biol 14:36

Qu A-L, Ding Y-F, Jiang Q, Zhu C (2013) Molecular mechanisms of the plant heat stress response. Biochem Biophys Res Commun 432:203–207

Ren XM, Guo SJ, Tian W, Chen Y, Han H, Chen E, Li BL, Li YY, Chen ZJ (2019) Effects of plant growth-promoting bacteria (PGPB) inoculation on the growth, antioxidant activity, Cu uptake, and bacterial community structure of rape (*Brassica napus* L.) grown in Cu-contaminated agricultural soil. Front Microbiol 10:1455

Rivas R, Peix A, Mateos PF, Trujillo ME, Martinez-Molina E, Velazquez E (2006) Biodiversity of populations of phosphate solubilizing rhizobia that nodulates chickpea in different Spanish soils. Plant Soil 287:23–33

Rodell M, Velicogna I, Famiglietti JS (2009) Satellite-based estimates of groundwater depletion in India. Nature 460:999–1002

Russo A, Vettori L, Felici C, Fiaschi G, Morini S, Toffanin A (2008) Enhanced micropropagation response and biocontrol effect of *Azospirillum brasilense* Sp245 on *Prunus cerasifera* L. clone Mr.S 2/5 plants. J Biotechnol 134:312–319

Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P (2015) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res 23:3984–3999

Sandhya V, Sk ZA, Grover M, Reddy G, Venkateswarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. Biol Fert Soils 46:17–26

Sarma RK, Saikia R (2014) Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. Plant Soil 377:111–126

Seleiman MF, Ali S, Refay Y, Rizwan M, Alhammad BA, El-Hendawy SE (2020) Chromium resistant microbes and melatonin reduced Cr uptake and toxicity, improved physio-biochemical traits and yield of wheat in contaminated soil. Chemosphere 250:126239

Shahzad R, Khan AL, Bilal S, Waqas M, Kang SM, Lee IJ (2017) Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in *Oryza sativa*. Environ Exp Bot 136:68–77

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012:1–26

Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123–131

Siddikee MA, Glick BR, Chauhan PS, Yim WJ, Sa T (2011) Enhancement of growth and salt tolerance of red pepper seedlings (*Capsicum annuum* L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1- aminocyclopropane-1-carboxylic acid deaminase activity. Plant Physiol Biochem 49:427–434

Singh S (2014) A review on possible elicitor molecules of cyanobacteria: their role in improving plant growth and providing tolerance against biotic or abiotic stress. J Appl Microbiol 117:1221–1244

Singh LP, Gill SS, Tuteja N (2011) Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signal Behav 6:175–191

Singh P, Tiwari A, Singh SP, Asthana RK (2013) Desiccation induced changes in osmolytes production and the antioxidative defence in the cyanobacterium *Anabaena* sp. PCC 7120. Physiol Mol Biol Plants 19:61–68

Singh A, Banerjee A, Roychoudhury A (2020) Seed priming with calcium compounds abrogate fluoride-induced oxidative stress by upregulating defence pathways in an indica rice variety. Protoplasma 257:767–782

Smith SE, Read DJ (2008) In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis. Academic, London, pp 1–800

Sreenivasa MN, Bagyaraj DJ (1989) Use of pesticides for mass production of vesicular-arbuscular mycorrhizal inoculum. Plant Soil 119:127–132

Tank N, Saraf M (2009) Enhancement of plant growth and decontamination of nickel-spiked soil using PGPR. J Basic Microbiol 49:195–204

Tank N, Saraf M (2010) Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. J Plant Interact 5:51–58

Tejera NA, Campos R, Sanjuan J, Lluch C (2004) Nitrogenase and antioxidant enzyme activities in *Phaseolus vulgaris* nodules formed by *Rhizobium tropici* isogenic strains with varying tolerance to salt stress. J Plant Physiol 161:329–338

Tiwari S, Lata C, Chauhan PS, Nautiyal CS (2016) *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. Plant Physiol Biochem 99:108–117

Toivonen PMA (2005) Post harvest storage procedures and oxidative stress. Hort Sci 39:938–942

Tripathi P, Singh PC, Mishra A, Srivastava S, Chauhan R, Awasthi S, Mishra S, Dwivedi S, Tripathi P, Kalra A, Tripathi RD, Nautiyal CS (2017) Arsenic tolerant *Trichoderma* sp. reduces arsenic induced stress in chickpea (*Cicer arietinum*). Environ Pollut 223:137–145

Turner TR, James EK, Poole PS (2013) The plant microbiome. Genome Biol 14:209

Ullah A, Heng S, Munis MFH, Fahad S, Yang X (2015) Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ Exp Bot 117:28–40

Van Oosten MJ, Pepe O, De Pascale S, Silletti S, Maggio A (2017) The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chem Biol Technol Agric 4:5

Vardharajula S, Ali ZS, Grover M, Reddy G, Bandi V (2011) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6:1–14

Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX, Wang YP, Guo JH (2012) Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS One 7:e52565

Xu Q, Pan W, Zhang R, Lu Q, Xue W, Wu C, Song B, Du S (2018) Inoculation with *Bacillus subtilis* and *Azospirillum brasilense* produces abscisic acid that reduces Irt1-mediated cadmium uptake of roots. J Agric Food Chem 66:5229–5236

Yahaghi Z, Shirvani M, Nourbakhsh F, de la Peña TC, Pueyo JJ, Talebi M (2018) Isolation and characterization of Pb-solubilizing bacteria and their effects on Pb uptake by *Brassica juncea*: implications for microbe-assisted phytoremediation. J Microbiol Biotechnol 28:1156–1167

Zhang YF, He LY, Chen ZJ, Zhang WH, Wang QY, Qian M, Sheng XF (2011) Characterization of lead-resistant and ACC deaminase-producing endophytic bacteria and their potential in promoting lead accumulation of rape. J Hazard Mater 186:1720–1725

Zhou C, Ge N, Guo J, Zhu L, Ma Z, Cheng S, Wang J (2019) *Enterobacter asburiae* reduces cadmium toxicity in maize plants by repressing iron uptake-associated pathways. J Agric Food Chem 67:10126–10136

Złoch M, Thiem D, Gadzała-Kopciuch R, Hrynkiewicz K (2016) Synthesis of siderophores by plant-associated metallotolerant bacteria under exposure to Cd^{2+}. Chemosphere 156:312–325

Role of Functional Defence Signalling Molecules in Plant–Microbe Interactions

10

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Abstract

The structural basis of effector protein disease promotion will open new insights of how microbes modulate hosts for their own advantage providing new tools for further research. Structural and functional studies of host resistance genes mediate activation of HR and SAR host defence and provide knowledge that can be used to engineer future crops which will be resistant to a broad range of pathogens. These studies will focus on how effectors are recognized by plant immune system. Research applied on model species demonstrates that the interactions between plant defence and development under abiotic and biotic stresses are mostly mediated by hormone cross-signalling. In this chapter, we discuss briefly the signalling molecules including transcription factors, volatile compounds and their role in plant defence response.

Keywords

Biotic stress · Effector proteins · Hypersensitive response · Plant immune system · Systemic acquired resistance

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10.1 Introduction

Scientific advancements; particularly in the field of plant molecular biology have played a pivotal role in unravelling the complex process of plant–pathogen interaction. Plants are surrounded by diverse pathogenic invaders and thus serve as a host for several infectious diseases caused by a variety of bacteria, viruses, fungi and nematodes leading to the impairment of plant developmental processes. Plants are generally resistant to most pathogens; however, few successful pathogens are able to cause disease because of their ability to evade recognition or suppression of the host defence mechanism. Plant immune response depends on numerous basal events happening inside the cell and has striking similarity with the vertebrate innate immunity, but plants also have remarkable and expanded recognition ability to compensate for their lack of mobility and an adaptive immune system (Ausubel 2005). Some of the plant defences, such as secondary metabolites, are constitutive and restricted to specific cellular compartment (Bottger et al. 2018). However, more specialized responses are generally pathogen-specific and are elicited upon recognition of molecular cues from the potential pathogens (Corwin and Kliebenstein 2017). Exposure of plant to the virulent elicitors leads to a series of highly localized events known as hypersensitive response, which prevents the progression of pathogen to the adjoining healthy tissues (Balint-Kurti 2019; Camagna and Takemoto 2018). The HR response not only restricts the pathogen spread, but also invokes the systemic acquired resistance (SAR), which safeguards from the future invasions at a site distant from the site of invasion (Klessig et al. 2018). The entire process of signal perception to the defence response is highly specialized and extremely well-coordinated. Emerging findings suggest plant phytohormones as the key player modulating the plant defence responses. Although in the last two decades significant insight is obtained into the complex process of plant immune response, the complete mechanism of regulation of defence response is yet to be elucidated. Recent findings, particularly the involvement of small RNA in the establishment of defence response, have opened new avenues for understanding the process of defence signalling in plants. In this chapter, we have tried to provide an overview of important components of defence signalling in plants, highlighting the recent findings and addressing the gaps in our understanding of these processes. We discuss briefly the signalling molecules including transcription factors and volatile compounds with their role in plant defence response.

10.2 The Plant Immune System

The innate immunity of plants is capable of recognizing potential pathogens and preventing their progression by eliciting sophisticated defences through the naturally occurring phytomolecules and specific structures. The outermost covering of plants, the epidermis and the cuticle, are composed mainly of fatty acids and lipids, which provide the first line of defence against invading pathogens (Xia et al. 2012). Phytomolecules such as flavonoids, alkaloids, coumarins and antimicrobial proteins

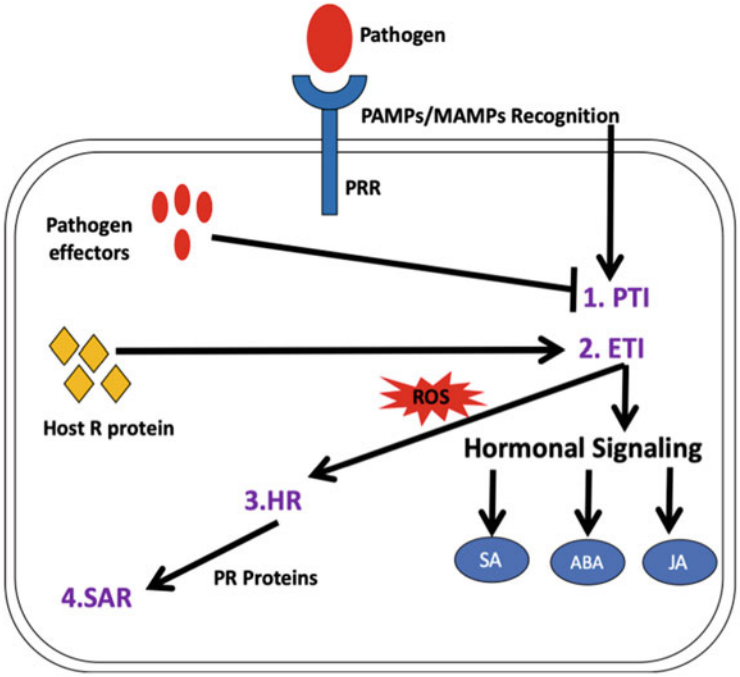

Fig. 10.1 Schematic representation of plant–pathogen interaction and its response

like defensins, phytoalexins, chitinase and peroxidises are categorically involved in the antimicrobial activities (Savoia 2012). Microbes or pathogen produced elicitors called as microbes/pathogen-associated molecular pattern (MAMP/PAMP) which are recognized by the pattern recognition receptors (PRRs) present on the host surfaces (Dangal and Jones 2001). Recognition of virulent elicitors leads to HR categorized by the programmed cell death at the site of pathogen attack. Generally, HR is effective to contain the biotrophic and hemibiotrophic pathogens and is less or ineffective against the necrotrophs (Mayer et al. 2001). The HR response prepares the plants for a more robust defence response to subsequent infection from specific and nonspecific pathogens known as systemic acquired resistance (SAR). The process involves rapid generation of signals at the primary site of infection, which is further transported to the systemic parts of the plant presumably via the phloem and lasts for days as shown in Fig. 10.1.

The SAR and HR-mediated responses are an outcome of incompatible interaction between the resistance gene product (R gene) and the phytopathogens virulence gene product (*Avr* gene) (Flor and Comstock 1971).

The protein encoded from different classes of *R* genes provides defence against diverse biotrophic pathogens. These R genes consist of a nucleotide-binding (NB) domain and a leucine-rich repeat (LRR) domain (Meyers et al. 2005). *R* gene-encoded proteins or R proteins indirectly recognize the pathogen effectors

by monitoring host cellular response against the virulent elicitors. Several R genes have been cloned from different plant species. For example, in *Solanum demissum*, 20 R gene of CC–NB–LRR (discussed in detail in the next section) class has been identified (Kim et al. 2012). Some bacteria have evolved several mechanisms to exploit *R* gene strategy, as in case of *Xanthomonas oryzae*, which targets susceptibility gene of the plant through TALEs (transcription activator-like effectors) resulting in enhanced multiplication of bacterial colony. However, in such case, plants also evolve the strategies to counteract the virulence by diversifying the TALE binding element in the susceptibility gene resulting in recessive R genes. Therefore, the plant immune system recognizes pathogen effectors either by direct physical contact or indirectly through accessory proteins. Interaction between MAMP or PAMP with their respective PRRs activates the downstream genes resulting in nonspecific hypersensitive response called as PAMP-triggered immunity or PTI sometimes referred as non-host resistance (Dodds and Rathjen 2010). The classical example is of bacterial flagellin, which triggers defence responses in various plants. Pathogen evading the host PTI responses is detected at next level by host intracellular receptors. This recognition induces effector-triggered immunity (ETI). However, these relationships are not exclusive and depend on the elicitor molecules present in each infection. Transcriptomics studies show that genes induced by the PTI and ETI are substantially overlapped and similar classes of genes are activated during an incompatible reaction; however, the magnitude of gene induction is different suggesting the convergence of defence signalling in PTI and ETI (Peng et al. 2018).

10.3 Components of Plant Defence

Plants have evolved various strategies to combat the different kinds of pathogen either through immediate encounter or through receptors that induce the process of defence signalling. Several proteins, transcription factors and signalling molecules play a pivotal role in the defence mechanism. A detailed discussion is provided in the following sub-headings.

10.3.1 ROS and Plant Defence Signalling

It has been long established that reactive oxygen species (ROS) plays a central role in plant immune responses. Reactive oxygen species are oxygen-containing molecules showing higher chemical reactivity than O_2. The major forms of ROS in plants include singlet oxygen ($^1O_2$), superoxide anion (O_2.-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) (Karuppanapandian et al. 2011). ROS plays an important role in mediating defence signals activation; however, the regulatory role of ROS occurs in conjunction with various plant signalling molecules, such as nitric oxide and salicylic acid (Torres et al. 2006). During pathogen attack, the ROS is produced in different cellular compartment including cell wall, plasma membrane, chloroplast and peroxisomes/glyoxysomes. The plasma membrane-localized

NADPH oxidase (also called as respiratory burst oxidase homologs, RBOHs) cell wall peroxidases and amine oxidase catalyse the production of apoplastic ROS, whereas the intracellular ROS is mainly produced in chloroplast and peroxisomes, and to a lesser extent in mitochondria (Torres and Dangl 2005). ROS are toxic to plant cell and therefore the production of ROS is effectively counterbalanced by ROS scavenging enzymes including catalases, superoxide dismutase and ascorbate peroxidases. Frequent alterations in redox balance and ROS homeostasis in the cell are the initial symptoms of changes in environmental conditions. Recognition of pathogen molecular pattern by plant cell increases the intracellular calcium level which in turn leads to activation of RBOHs and generation of apoplastic ROS. This pattern-triggered ROS production through RBOHDs regulates stomatal closure and thus restricts the microbial invasion through stomata (Munemasa et al. 2015). Moreover, the pattern-triggered ROS also known to regulate localized callose deposition at the cell wall, which increases host resistance to fungal penetration (Daudi et al. 2012). Our insight into ROS biogenesis during immunity has significantly increased during the last decade; however, how the plant sense ROS signal is yet to be deciphered. Emerging cues suggest the involvement of receptor-like kinases in ROS signalling; however, it remains unknown whether they directly or indirectly sense the ROS. A detailed insight into the signalling of ROS in plants will shed new light on pattern-triggered immune responses in plants, and will undoubtedly equip us with genetic tools for engineering durable and sustainable resistance in plants.

10.3.2 Resistance Gene (*R* Gene): Molecular Switch of Plant Defence

Plant defence against specific race of pathogens is governed by specific class of genes called resistance genes (R genes). According to the Flor and Comstock (1971) gene for gene hypothesis, ETI depends upon the interaction between dominant/semi-dominant resistance gene product (*R gene*) found in the plant and phytopathogen virulence dominant gene product (*Avr gene*). The resistance from pathogens conferred only if both the *R* gene and corresponding *Avr* gene are present in the same interaction (Balint-Kurti 2019). The R protein is expected to activate signalling pathways coordinating with the initial plant defence actions to impair pathogen's further ingress. Several studies on the structure and function of *R* gene provide a detailed understanding of their role in plant immune system. The breakthrough was cloning and characterization of the *Hm1* gene of maize revealed that it controls disease resistance against *Cochliobolus carbonum* by encoding the NADPH-dependent reductase which inactivates the fungus-produced toxic compounds (Johal and Briggs 1992). Since then, several classes of R gene have been cloned and characterized.

Structurally, there are several different classes of *R* gene; however, the major class encodes proteins containing a nucleotide-binding site (NBS) and C-terminal leucine-rich repeats (Meyers et al. 2005). The NBS domains contain conserved motifs to bind and hydrolyse ATP and GTP, and the LRR motif is typically involved

in protein–protein interactions. These NBS–LRR domain-containing proteins play an important role in the recognition and resistance to diverse pathogens ranging from viruses, bacteria, and fungi to insects and nematodes (Dangl and Jones 2001). Depending upon the amino-terminal structure, the NBS–LRR proteins are classified into two sub-categories: proteins having domain for intracellular signalling like Drosophila and mammalian interleukin (IL)-1 receptors (Toll/interleukin receptor) also known as TNL or TIR–NBS–LRR proteins and the proteins having coiled-coil domain called as CNL or CC–NBS–LRR having varying sizes and location of coiled coil (Ellis et al. 2000). Computational analysis reveals that R protein specificity resided in LRRs. Pathogen effectors of various kingdoms are recognized by NBS–LRR leading to the defence response in plants. For example, the NBS–LRR genes encoded from diverse species like *Arabidopsis RPS2* gene, the tobacco *N* gene, tomato *Cf9* gene and *L6* gene of flax share identical mechanism for combating diverse pathogens (Staskawicz 2001; Staskawicz et al. 1995). However, the *R* gene-mediated resistance is often subjugated by the rapidly evolving virulent effectors (Ellis et al. 2000). Therefore, R gene must rapidly evolve to resist microbial isolates through a potent mechanism which requires rapid evolution creating diverse classes of R gene. Recombination events, gene duplication, mutations and crossing over are mainly responsible for the much-needed diversity of R genes in the land plants (Meyers et al. 2005). Since NBS–LRR is the most important region of R gene-encoded protein, the modification in the length of LRR region leads to an important contributor in the diversification of R gene. For example, the diverse recognition specificities of tomato resistance gene *Cf4* and *Cf9* are attributed to the presence of *Cf* gene cluster varying for number of LRRs and amino acid substitution in the LRR motifs (Dixon et al. 1998). Mutation in *R* gene also results in expansion and diversification of LRR regions as studied in *Arabidopsis* (Parker et al. 1997). The presence of enormous diversity in R gene has been exploited extensively by breeders for breeding durable resistance in agronomically important crops.

Hence, the diverse role of R gene attracts the scientists to exploit its benefit by applying in the area of agriculture through genetic engineering to combat disease resistance. The recognition of resistant gene in agronomically important wild crops and their introgression into commercial cultivars attracts the major focus of several plant breeders for the development of engineered crops. Most resistance genes are inherited as single genetic loci which allow plant biologists to assess genetic strategies for cloning R genes. Thus, molecular mechanism exerted by disease resistance gene possesses enough detail to predict about the genetically engineered plants which can identify the pathogenic effector molecules to restrict pathogenicity in important agricultural crops.

10.3.3 Hormonal Crosstalk in Defence Signalling

Phytohormones play crucial role in orchestrating the defence trade-off in plants. Hormonal crosstalk enables the plant with a potent regulatory mechanism and allows

it to alter its defence response against the pathogenic invaders (Kunkel and Brooks 2002). The salicylic acid (SA) and ethylene (ET)/jasmonic acid (JA)-mediated defence signalling pathways are the backbone of plant immune responses against biotrophic and necrotrophic invaders, respectively, whereas abscisic acid (ABA), brassinosteroids, gibberellic acid (GA) and cytokinin (CK) are considered to be involved in the plant defence response through modulating the SA and ET/JA defence signalling pathways (Li and Wang 2020). Since the SA- and JA/ET-mediated defence signalling pathways are extensively studied, we will restrict ourselves discussing the hormonal crosstalk involving these pathways.

SA is synthesized from chorismite via two different enzymatic pathways, the phenylalanine ammonia lyase (PAL) and the isochorismate synthase pathway. Endogenous SA levels influence a number of physiological processes during the plant immune response. Studies from plant immune responses clearly indicate that SA plays a critical role in HR and cell death (Alvarez 2000). Another important role attributed to SA is the establishment of systemic acquired resistance, which prime the plant against any further infection at distant sites. Moreover, SA is not only crucial for plant defence but is also a key player involved in maintaining the trade-off between growth and immunity in plants (Rojo et al. 2003). The exogenous application of SA also triggers immunity against necrotrophs.

The another phytohormone critical for plant defences, JA, is synthesized from α-linolenic acid (α-LA) and is immediately converted to methyl jasmonate (MeJ) (Pieterse et al. 2012). The plant hormones JA and MeJ and their derivative compounds are synthesized from octadecanoid pathway and collectively called as jasmonates (Avanci et al. 2010). These compounds are the member of plant's crucial metabolic processes including plant immunity, growth and secondary metabolism (Arimura et al. 2005; Gfeller et al. 2006; Wasternack 2007). Our understanding about the role of JA in defence signalling is largely derived from studies in model eudicot Arabidopsis JA biosynthesis and signalling mutants. In Arabidopsis and several other angiosperms, endogenous JA regulates resistance against necrotrophic pathogen (Antico et al. 2012). In rice, overexpression of allene oxidases, an important enzyme involved in JA biosynthesis, leads to enhanced resistance to pathogenic fungi with an immediate induction of several pathogen-related genes, including *PR3, PR1a* and *PR5* (Mei et al. 2006). Although JA is reported to act antagonistically to SA, the several emerging evidences from Arabidopsis and rice pathosystem indicate that both JA and SA positively contribute to immunity against both hemibiotrophic and necrotrophic pathogens (Tsuda et al. 2009). The third primary defence hormone, ethylene, works in close coordination with JA, and regulates plant immunity depending on the pathogen, environmental condition, and plant species (Hoffman et al. 1999; Helliwell et al. 2013). Other phytohormones such as auxin, abscisic acid, gibberellin, cytokinin, and brassinosteroids are also module defence responses either alone or through interaction with the primary defence hormones (Robert-Seilaniantz et al. 2011; Pieterse et al. 2012).

Crosstalk between the defence signalling pathways strongly influences the magnitude and amplification of the defence response sensing an attempted invasion. Since defence response is a costly investment plant needs to balance the potential

benefits and the cost of the investment to fine tune its overall fitness (Cipollini et al. 2018). Although it is difficult to completely unravel the intricate hormonal crosstalk, interesting insights have emerged from the scientific studies during the last two decades. The JA and SA crosstalk are the most studied and are known to antagonize each other. The enhanced resistance to biotrophs imparted by the increase in SA levels is often correlated with compromised JA-dependent defences with increased susceptibility to necrotrophs, and vice versa (Jonas and Dangl 2006). In *Arabidopsis thaliana*, the increase in levels of SA leads to a steep decline in the expression of JA-responsive genes. The protein NPR-1 plays a pivotal role in regulating the SA–JA crosstalk. SA induces changes in the cellular redox potential which is sensed by NPR-1 and acts as an important transducer of SA signal (Pieterse et al. 2012). NPR-1 also functions as an important transcriptional activator of the PR genes (Pieterse and Van Loon 1999). The dual role of NPR1 is governed by its spatial localization in cytosol and nucleus. The nuclear localization of NPR-1 is not required for SA-mediated suppression of JA signalling, indicating the regulation of JA–SA crosstalk by the novel function of cytosolic NPR-1 (Spoel et al. 2003; Yuan et al. 2007). Mitogen-activated protein kinase (MAPK), WRKY and TGA transcription factors, and glutaredoxins (GRXs) are also important regulators affecting SA/JA crosstalk. MAP kinase 4 acts early in the SA signalling pathways and regulates the SA- and JA-dependent response via EDS1 (enhanced disease susceptibility 1) and PAD4 (phytoalexin-deficient 4) proteins (Brodersen et al. 2006). Downstream to JA perception by JAZ receptor, JA response pathway is regulated with two antagonistic branches governed by MYC- and ERF-type transcription factors (Caarls et al. 2017). The activation of JA/ET pathway antagonizes the SA response.

In Arabidopsis, activation of JA and ET pathways is required to regulate plant defensin gene PDF1.2. Ethylene and jasmonate signalling pathways transcriptionally activate the two members of apetala2/ethylene response factor (AP2/ERF) superfamily: the ethylene response factor 1 (ERF1) and octadecanoid-responsive Arabidopsis 59 (ORA59) that regulate the expression of pathogen-responsive genes, thus preventing disease progression (Lorenzo et al. 2003). The transcription factor MYC2 (like ERF1 and ORA59) also plays an important role in regulating JA-responsive genes where it acts as a negative regulator of PDF1.2 gene which is activated by ERFs. Therefore, MYC-responsive genes are activated in the absence of ET signalling (Pieterse et al. 2009). Within the JA-responsive pathway, the MYC and ERF branches are mutually antagonistic as shown in Fig. 10.2.

10.3.4 Small RNA and Plant Immunity

In the recent years, small RNAs have been identified as the master regulator of different biological processes in most of the eukaryotes.

As we know, the first line of defence in plants comprises recognition of pathogen-associated molecular patterns (PAMPs) leading to the activation of PAMP-triggered immunity (PTI). However, PTI can be suppressed by the pathogen effector proteins.

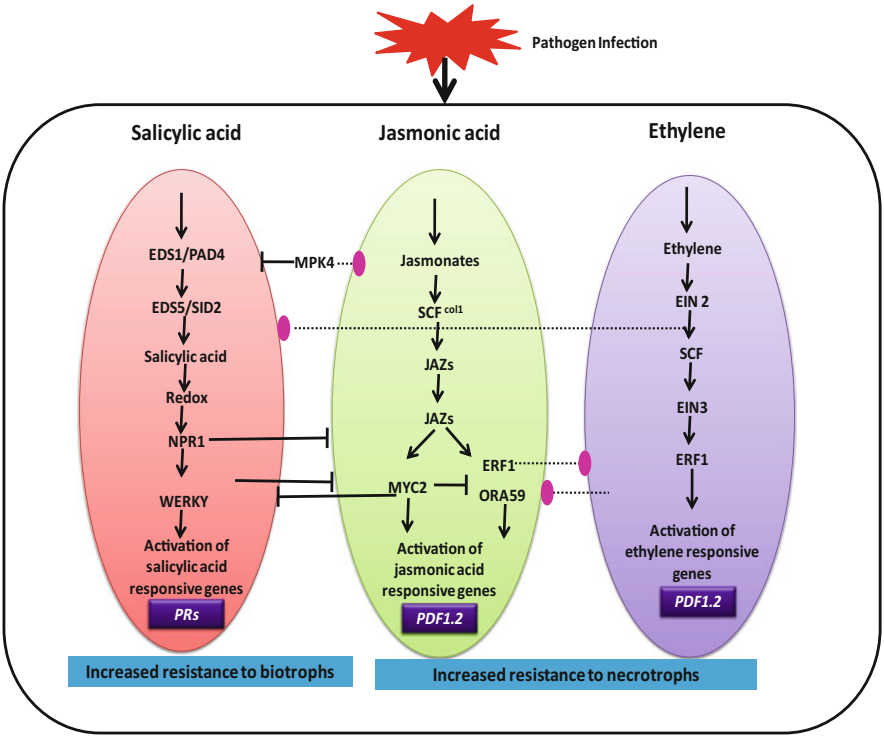

Fig. 10.2 Schematic representation of crosstalk between salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signalling pathways in plant defence upon pathogen attack. SA regulates defence response against biotrophs, whereas jasmonic acid and ethylene pathways are mainly responsible for activating defence signalling against the necrotrophs. T: negative effect; pink circle: positive effect

The resistant gene-encoded proteins having NB–LRR domains act as the second line of defence as discussed in the above section. Recent evidence has shown that the plant innate immune system has evolved the small RNA (sRNA)-mediated gene silencing which is an inbred and evolved characteristic feature of plants against pathogen attack and occurs at both transcriptional and post-transcriptional stages (Ding and Voinnet 2007).

The eukaryotic genome is enriched with two types of endogenous small RNA; the small interfering RNAs (siRNAs) having 20–24-nt double-stranded and the microRNAs (miRNAs 20–22-nt long produced from primary miRNAs). The processing of both siRNA and miRNA is performed inside the cell through RNase III-like endonucleases called as Drosha/Dicer, bound to Argonaute (AGO) proteins and inserted into RNA-induced silencing complex (RISC) (Carthew and Sontheimer 2009). Reports state that these small RNAs (sRNAs) including miRNA and siRNA are transferred within tissues of individual organisms as well as across various eukaryotic species, acting as a connection between the eukaryotes and prokaryotes

(Zeng et al. 2019). The novel communication type revealed that sRNA molecules are transmitted between distantly correlated organisms called as cross-kingdom RNAi (Knip et al. 2014). For example, upon infection with fungus *Verticillium dahliae*, the cotton plant accumulates and exports miR159 and miR166 which target the fungal encoded protein Clp-1 and HiC-15, respectively, essential for virulence. This defence strategy of cotton plant by exporting the specific miRNA is called as cross-kingdom gene silencing in fungal pathogen to prevent disease (Zhang et al. 2016). Thus, miRNA exerts cross-kingdom regulation of gene expression in host–pathogen interactions in which plant-derived miRNA is transmitted to the pathogen to downregulate the gene essential for virulence. Thus, plants might have adapted RNA interference through cross-kingdom mechanisms to deliver immune responses against pathogen infection (Bundo et al. 2020).

Besides this broad-spectrum immunity of plants, microbes evolve their own strategy to exploit the host immunity and spread colonization. Some examples are discussed here to understand the pathogen's own defence strategy. As we know, sRNAs can act as effectors through a mechanism which silences the host genes in order to repress plant immunity and spread infection. The sRNAs accumulated by *B. cinerea* hijack the RNAi machinery of plant by binding to the *Arabidopsis* AGO proteins, which in response silence the host immunity gene (Weiberg et al. 2013a, b). Therefore, the fungal pathogen delivers "virulent" sRNA as effectors molecule into the host plant to suppress its immunity and spread infection demonstrating a naturally occurring mechanism of cross-kingdom RNAi as an advanced process. Thus, these findings implement that with the development of potent genomics and sRNA sequencing technologies; other cross-kingdom sRNAs and their effector molecules will be discovered to combat RNAi defence signalling.

Plant not only interacts with pathogenic bacteria but also shows symbiotic association as in case of leguminous plants. The nitrogen fixating bacteria form nodule in leguminous plants which require the suppression of host plant defence to prevent immune responses against these bacteria. In a study, the subset of plant miRNAs superfamily (miR482/2118, miR1507, miR2109) target the NB–LRR genes and regulate plant immunity (Deng et al. 2018; Su et al. 2018; Zhai et al. 2011). These miRNAs repress NB–LRR expression and upregulated in the developing nodules of *Medicago truncatula* upon symbiotic interactions. These results suggest that the repression of NB–LRR resistance genes in the emerging nodule produces a favourable niche for nitrogen fixating bacterial colonization to synthesize nodule (Sos-Hegedus et al. 2020).

10.3.5 G Proteins Cascade

Heterotrimeric G proteins play as a connecting point in plant defence signalling activated by multiple receptors to exert plant growth and stress signalling (Liu et al. 2013; Morillo and Tax 2006). G proteins are commonly known as guanine nucleotide-binding proteins, a family of proteins that regulate the molecular switches. Their activity is stimulated by the guanosine triphosphate (GTP). Heterotrimeric G proteins are present in eukaryotes universally, it may be due to their conserved nature throughout the evolution. In animals, Gproteins relay signals from 7-transmembrane spanning G-protein-coupled receptors (GPCRs) to intracellular downstream effectors; however, the existence of GPCRs in plants is controversial. The heterotrimeric G protein complex contains Gα, β and γ subunits involved in transducing extracellular signal received by the G-protein-coupled receptors. The signal is transmitted through the GPCR with their cognate G proteins leading to the release of GDP and subsequent binding of GTP to the Gα subunit (Temple and Jones 2007). This exchange of guanine nucleotide leads to the activation of Gα subunit and get dissociated from the Gβ and Gγ dimer. These moieties further interact with several downstream effector molecules to initiate unique intracellular signalling responses (Tuteja and Sopory 2008). In plants, G proteins are mainly involved in the plant defence mechanism. The main protein involved in the plant defence during the pathogenic attack is called receptor-like kinases (RLKs) (Aranda-Sicilia et al. 2015).

Various microbial strains cause pathogenicity in the plants. Plants respond to these pathogens using the molecular signature known as pathogen-associated molecular patterns (MAMPs/PAMPs), that are detected by cell-surface-localized receptor-like kinases (RLKs) (Macho and Zipfel 2014). The N-terminal of the RLK consisted of the ligand-binding site, whereas the C-terminal of these kinases occurring on the internal side of the membrane. The ligand-binding site of the plant MAMPs consisted of the leucine-rich repeats. This leucine-rich repeat showed affinity towards the bacterial flagellin and with transcription factor such as EF-Tu (Zipfel et al. 2006). In addition to this, these binding pockets can also bind to the cell membrane component viz., chitin and peptidoglycan (Willmann et al. 2011). It was noticed that plant genomes contain many sets of the RLK genes but only a few of them have been characterized well (Sakamoto et al. 2012). Bacterial flagellin, elongation factors and chitin receptor kinase have been characterized for the plant defence mechanism (Macho and Zipfel 2014).

Gα subunit is an important component in initiating signalling response showed highly reduce hypersensitivity response against rice blast disease due to the impairments in the single copy of Gα protein subunit suggesting that this protein is essential for resistance against blast disease (Suharsono et al. 2002). Arabidopsis Ga subunit (GPA1) plays a key role in its immune signalling pathway activated through bacterial flagellin epitope flg22 in which GPA1 interacts with NADPH oxidase, RbohD to accelerate flg22-induced ROS burst solely of the central cytoplasmic kinase BIK (Xu et al. 2019). Besides GPCR, receptor-like kinases (RLKs) initiate signal transduction at the cell surface. However, it is unclear how the RLK

and G proteins physically interact, for which Aranda-Sicilia et al. (2015) have demonstrated the physical interaction between the G protein subunits and the defence-associated RD-type receptor-like kinases CERK1, BAK1 and BIR1, which showed the signal transduction. However, no interaction was found with the non-RD RLK-like FLS2 confirming that signal transduction proceeds downstream of pathogenesis-associated RLKs. While performing the defence response, G proteins function in various physiological responses and one of them is the closure of stomata on pathogen attack. Genetic evidence states that the stomatal responses triggered by PAMP are governed by similar PAMP receptors and the heterotrimeric G proteins (Zhang et al. 2012; Liu et al. 2013; Yu et al. 2018). Silencing of G protein subunits in *N. benthamiana* extensively impaired stomatal closure in guard cells (Zhang et al. 2012). These results suggest that heterotrimeric G proteins play a crucial role in regulating defence response through modulating stomatal aperture.

To date, G protein function has been investigated very less in plants especially during plant–microbe interaction. Plant–microbe interaction can be negative with pathogenic strains and positive with beneficial strains. So, we need to focus on the specific roles of the plant G proteins with pathogenic microbial strains. More research on this aspect will elucidate more about the control mechanisms of the key molecular signature in pathogenicity.

10.3.6 Transcription Factor

Transcription factors (TFs) play a key role in controlling various plant development processes and response against various external biotic and abiotic threats. They are main component of plant defence signalling and adaptation mechanism. TF is a protein that controls the rate of transcription by interacting at specific promoter sequence on cis-regulatory DNA element of the genes having binding sites for TFs and regulates the gene expression. Having a DNA-binding domain is a specific feature of TFs which allows it to bind to a specific sequence of the DNA adjacent to the target gene. On the basis of gene, they either activate or repress the expression of target gene (Gordan et al. 2011). The function of TFs is to regulate turn on and off the genes and change in TFs binding site causes variation in the level of gene expression. Thus, the presence and availability of binding site and conservation of binding domain affect the binding of TFs to its specific binding site (Dai and Dai 2011). In plants, TFs are encoded by different gene families like AREB, MYB, WRKY, bZIP and ZFP based on their structure of their DNA-binding domain. Some of them are discussed here.

Expression of defence gene and its regulation is conciliated by modulated activity of TFs either by up- or downregulation. Likewise, upon *Ustilago maydis* infection, the expression of bZIP TF is found to be increased in maize (Wei et al. 2012).

Zinc finger protein (ZFPs) TFs perform crucial role in plant stress tolerance, apoptosis, transcriptional regulation and protein–protein interactions. In transgenic tobacco plant overexpression of PF1 TFs, that is, CaPF1 interacts with promoter cis-element of gene which enhanced immunity against pathogens. CaPF1 interacted

with promoter cis-elements of gene and induced the expression of defence-related genes, that is, PR-2, PR-3, PR-4 and PR-5 (Yi et al. 2004). The combination of genomic data with the functional studies shows novel insights in regulating plant defence mechanism especially for crops with improved resistance against pathogens. The functions of ZFPs as positive or negative regulator arbitrate resistance to the pathogens and make basis for understanding associated genes and TFs regulating different pathways. Furthermore, these TFs may offer a complete transgenic tool for developing disease resistance in plant genetics and breeding programs. Structural analysis of R gene proteins was analysed in which zinc finger domain and nucleotide binding domain have been elucidated (Gupta et al. 2012).

The WRKY family has highly conserved WRKY-DBD sequence of 60 amino acids, which contains the almost unvaried sequence motif, that is, WRKYGQK, at the N-terminal and a zinc-binding motif. Amongst the different classes of transcription factor identified to modulate the expression of defence-related genes, WRKY proteins that bind to W-box element are extensively studied primarily because of their involvement in a variety of biotic and abiotic stress responses in plants (Jiang et al. 2015). WRKY family of the TFs is one of the largest family of TFs in plants, with at least 74 members in *Arabidopsis* and more than 100 members in rice, soybean and popular (Dong et al. 2003).

In rice, the expression of WRKY genes is induced by pathogen attack. OsWRKY70 has been reported to be involved in defence against biotic stress, as its expression increases in the presence of SA/JA and also acts downstream of NPR1 which leads to the activation of *PR* genes involved in defence response (Pieterse et al. 2012; Li et al. 2004). Rice line overexpressing OsWRKY71 is reported to have enhanced resistance against bacterial pathogen *Xanthomonasoryzae* (Liu et al. 2007). Similarly, in Arabidopsis, the expression of AtWRKY6 is largely influenced with the senescence or defence response trigger (Robatzek and Somssich 2001).

WERKY family explains the role of *Paeonia lactiflora* PlWRKY13 which had been shown to be induced by four types of abiotic stress, heat stress, low-temperature, water logging and salt stress. Its expression tended to first decrease and then increase after infection with *Alternaria tenuissima* (Wang et al. 2019). WRKY proteins are also involved in beneficial plant–microbe interactions, for example, Trichoderma treatment enhances the positive regulators AtWRKY70 and AtWRKY54 in salicylic acid pathway and (AtWRKY8, AtWRKY33, AtWRKY38, AtWRKY42 and AtWRKY60) in jasmonic acid-mediated pathway (Sáenz-Mata et al. 2014).

Plant structures cuticle, which is physiologically involved in defence response against biotic and abiotic stresses, is made up of cuticular wax. It has been reported that *Wolly* (WO) transcription factor in tomato involved in the expression of wax transporter gene SlLTP and wax synthesizing genes *SlKCR1, SlCER6* and *SlPAS2* promoting cuticular wax accumulation (Xiong et al. 2020).

Other R2R3-MYB-type transcription factors govern the transcriptional regulation of the stilbenes which is the key indicators of grapevine innate immunity and the stilbene synthase promoters are activated by MYB14 and MYB15 (Chang et al. 2011; Holl et al. 2013). GATA-family transcription factors of fungus act as universal

Table 10.1 Transcription factors involved in plant defence

Transcription factor	Function	Plant species	References
Teosinte branched1/ Cycloidea/proliferating cell factor (TCP)	Regulation of cell growth and proliferation	Potato	Bao et al. (2019)
AP/ERF and WRKY	Regulation of the salicylic acid	*Arabidopsis thaliana*	Zhou et al. (2018)
OsERF71	Expression of xylanase inhibitor	*Oryza sativa*	Zhan et al. (2017)
Ethylene response factor (ERF)	Stress signalling with the activation of wound repair mechanisms	*Arabidopsis*	Heyman et al. (2018)
WRKY18 and WRKY40	Negative regulators of flg22	*Arabidopsis*	Birkenbihl et al. (2017)
AP2/ERF, bHLH, MYB and WRKY	Regulating plant secondary metabolism	*Arabidopsis*	Zhou and Memelink (2016)
SlSHINE3	Cuticle production	tomato	Buxdorf et al. (2014)

nitrogen regulators playing a crucial role in the utilization of host nitrogen resources (Fernandez et al. 2014). Thus, these studies demonstrate that the involvement of transcription factor by activating or repressing the target gene is essential for regulation defence response in plants. Some transcription factors and their role are listed in Table 10.1.

10.4 Plant–Pathogen Arms Race

Frequent co-evolution of plants and microbe occurs at different timescales. The rapid evolution of interacting entities largely depends upon their environment and genome pattern. The ability of pathogen to acquire a new host depends on the genetic compatibility between the two groups. Pathogenic bacteria and fungi constantly effort to adopt their evolved virulence mechanism and to sustain themselves in various adverse conditions (Rodriguez-Melcon et al. 2018). The host–parasite interaction contributes to the horizontal gene transfer leading to the transfer of various molecules as genetic material, proteins and metabolites showing a great impact on the evolutionary changes in interacting species. Horizontal gene transfer also leads to the transfer of transposable elements, for example, LINE-1 which is the most frequently found transposable element occurs in mammalian and plant species *Crassostrea gigas* and *Fraxinus excelsior* respectively (Ivancevic et al. 2018). Therefore, the active transposable elements lead to genetic modifications and positive selection of the plant–host interaction (Faino et al. 2016). These transfers of genetic material into the trans-kingdoms impart a driving force for host parasites'

evolutionary arm races (Zhao and Guo 2019). For example, the trans-kingdom approach is seen in cotton plants which exports its miRNAs into the *V. dahlia* to inhibit the pathogenic genes and restricting fungal pathogenesis (Zhang et al. 2016). Further interaction is based upon the protein level through which pathogen's effector protein (Avr-protein) encounters their host plant protein (R-protein) which have a unique structure to recognize their respective effector proteins (Dangl and Jones 2001). These proteins can be the molecular player of plant–pathogen interaction and evolution. Several pathogenic effector proteins from the primary pathogen including their virulence activity, subcellular localization and PDB structure are identified in their respective hosts (Mukhi et al. 2020). Every specific protein and/or molecule/metabolites involved in plant growth and survival are being synthesized from different biosynthetic pathways which are current evolving weapon for emerging plant–pathogen interaction (Frantzeskakis et al. 2019). While microbial attack, plants secrete antimicrobial compounds to combat the pathogen; however, the pathogens are able to detoxify these compounds using various enzymes which are specifically encoded by pathogens' genome as, for example, a fungal pathogen *Fusarium pseudograminearum* can degrade benzene derivative xazolinones (Kettle et al. 2015). Moreover, the individual metabolic occurrence is restricted to individual pathogens explaining the evolutionary concept of biosynthetic pathways as the metabolic diversification may occur due to mutations or genetic rearrangements. With these evolutionary changes, the microbes get benefited and can escape from the plant defence while the plants have evolved more potent immune system to combat other pathogens. The understanding of evolutionary ecology of host–pathogen interactions in different contexts can improve novel epidemics and their hosts' eventual efforts for biological control may provide various opportunities to increase our understanding on the biology of plant–pathogen interactions (Parker and Gilbert 2004). An invariable theory of the never-ending molecular arms race between microbes and their hosts is the ability to avoid or suppress the host defence pathways (Pumplin and Voinnet 2013).

10.5 Conclusion

Since the last decade, tremendous progress has been done to unravel the molecular basis of plant–microbe interactions. Several reports are available on the mechanisms of the pathogenic infection of plants, which lead to either resistance or disease. Significant advancement has suggested that plant–pathogen effector protein function in the host cells. All plant pathogenic interactions lead to cross-kingdom communication in which plant defence mechanisms employ various strategies to bypass their innate immunity. The co-evolution along with their hosts prompts microbes to effectively respond to the modifications of host immunity temporally. The microbes continuously adapt their niche of virulence strategies to keep their arms race effective.

References

Alvarez ME (2000) Salicylic acid in the machinery of hypersensitive cell death and disease resistance. In: Programmed Cell Death in Higher Plants, vol 44. pp 185–198

Antico CJ, Colon C, Banks T, Ramonell KM (2012) Insights into the role of jasmonic acid-mediated defenses against necrotrophic and biotrophic fungal pathogens. Front Biol 7:48–56

Aranda-Sicilia MN, Trusov Y, Maruta N, Chakravorty D, Zhang Y, Botella JR (2015) Heterotrimeric G proteins interact with defense-related receptor-like kinases in Arabidopsis. J Plant Physiol 188:44–48

Arimura GI, Kost C, Boland W (2005) Herbivore-induced, indirect plant defences. Biochimica et Biophysica Acta (BBA)-Mol Cell Biol Lipids 1734:91–111

Ausubel FM (2005) Are innate immune signaling pathways in plants and animals conserved? Nat Immunol 6:973–979

Avanci NC, Luche DD, Goldman GH, Goldman MH (2010) Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. Genet Mol Res 9:484–505

Balint-Kurti P (2019) The plant hypersensitive response: concepts, control and consequences. Mol Plant Pathol 8:1163–1178

Bao S, Owens RA, Sun Q, Song H, Liu Y, Eamens AL, Feng H, Tian H, Wang MB, Zhang R (2019) Silencing of transcription factor encoding gene StTCP23 by small RNAs derived from the virulence modulating region of potato spindle tuber viroid is associated with symptom development in potato. PLoS Pathog 15:1–29

Birkenbihl RP, Kracher B, Roccaro M, Somssich IE (2017) Induced genome-wide binding of three Arabidopsis WRKY transcription factors during early MAMP-triggered immunity. Plant Cell 29:20–38

Bottger A, Vothknecht U, Bolle C, Wolf A (2018) Plant secondary metabolites and their general function in plants. In: Lessons on Caffeine, Cannabis & Co, 3–17

Brodersen P, Petersen M, Bjørn Nielsen H, Zhu S, Newman MA, Shokat KM, Rietz S, Parker J, Mundy J (2006) Arabidopsis MAP kinase 4 regulates salicylic acid-and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. Plant J 47:532–546

Bundo M, Campo S, San Segundo B (2020) Role of microRNAs in Plant–Fungus Interactions. In: Plant Micro RNAs, pp 199–220

Buxdorf K, Rubinsky G, Barda O, Burdman S, Aharoni A, Levy M (2014) The transcription factor SlSHINE3 modulates defense responses in tomato plants. Plant Mol Biol 84:37–47

Caarls L, Elberse J, Awwanah M, Ludwig NR, de Vries M, Zeilmaker T, Van Wees SC, Schuurink RC, Van den Ackerveken G (2017) Arabidopsis jasmonate-induced oxygenases down-regulate plant immunity by hydroxylation and inactivation of the hormone jasmonic acid. Proc Natl Acad Sci 114:6388–6393

Camagna M, Takemoto D (2018) Hypersensitive response in plants. 1–7

Carthew RW, Sontheimer EJ (2009) Origins and mechanisms of miRNAs and siRNAs. Cell 136:642–655

Chang X, Heene E, Qiao F, Nick P (2011) The phytoalexin resveratrol regulates the initiation of hypersensitive cell death in Vitis cell. PLoS One 6:1–12

Cipollini D, Walters D, Voelckel C (2018) Costs of resistance in plants: from theory to evidence. Annu Plant Rev Online:263–307

Corwin JA, Kliebenstein DJ (2017) Quantitative resistance: more than just perception of a pathogen. Plant Cell 29:655–665

Dai Z, Dai X (2011) Gene expression divergence is coupled to evolution of DNA structure in coding regions. PLoS Comput Biol 7:1–13

Dangl JL, Jones JD (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Daudi A, Cheng Z, O'Brien JA, Mammarella N, Khan S, Ausubel FM, Bolwell GP (2012) The apoplastic oxidative burst peroxidase in Arabidopsis is a major component of patterntriggered immunity. Plant Cell 24:275–287

Deng Y, Wang J, Tung J, Liu D, Zhou Y, He S, Du Y, Baker B, Li F (2018) A role for small RNA in regulating innate immunity during plant growth. PLoS Pathog 14:e1006756
Ding SW, Voinnet O (2007) Antiviral immunity directed by small RNAs. Cell 10:413–426
Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JD (1998) The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell 10:1915–1925
Dodds PN, Rathjen JP (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet 11:539–548
Dong J, Chen C, Chen Z (2003) Expression profiles of the *Arabidopsis* WRKY gene superfamily during plant defense response. Plant Mol Biol 51:21–37
Ellis J, Dodds P, Pryor T (2000) Structure, function and evolution of plant disease resistance genes. Curr Opin Plant Biol 3:278–284
Faino L, Seidl MF, Shi-Kunne X, Pauper M, van den Berg GC, Wittenberg AH, Thomma BP (2016) Transposons passively and actively contribute to evolution of the two-speed genome of a fungal pathogen. Genome Res 26:1091–1100
Fernandez J, Marroquin-Guzman M, Wilson RA (2014) Mechanisms of nutrient acquisition and utilization during fungal infections of leaves. Annu Rev Phytopathol 52:155–174
Flor HH, Comstock VE (1971) Flax cultivars with multiple rust-conditioning genes 1. Crop Sci 11:64–66
Frantzeskakis L, Kusch S, Panstruga R (2019) The need for speed: compartmentalized genome evolution in filamentous phytopathogens. Mol Plant Pathol 20:3–7
Gfeller A, Liechti R, Farmer EE (2006) *Arabidopsis* jasmonate signaling pathway. Sci STKE 3:322
Gordan R, Murphy K, McCord RP, Zhu C, Vedenko A, Bulyk ML (2011) Curated collection of yeast transcription factor DNA binding specificity data reveals novel structural and gene regulatory insights. Genome Biol 12:125
Gupta SK, Rai AK, Kanwar SS, Sharma TR (2012) Comparative analysis of zinc finger proteins involved in plant disease resistance. PLoS One 7:e42578
Helliwell EE, Wang Q, Yang Y (2013) Transgenic rice with inducible ethylene production exhibits broad-spectrum disease resistance to the fungal pathogens *Magnaportheoryzae* and *Rhizoctoniasolani*. Plant Biotechnol J 11:33–42
Heyman J, Canher B, Bisht A, Christiaens F, De Veylder L (2018) Emerging role of the plant ERF transcription factors in coordinating wound defense responses and repair. J Cell Sci 15:131
Hoffman T, Schmidt JS, Zheng X, Bent AF (1999) Isolation of ethylene-insensitive soybean mutants that are altered in pathogen susceptibility and gene-for-gene disease resistance. Plant Physiol 119:935–950
Höll J, Vannozzi A, Czemmel S et al (2013) The *R2R3-MYB* transcription factors *MYB14* and *MYB15* regulate stilbene biosynthesis in *Vitis vinifera*. Plant Cell 25:4135–4149
Ivancevic AM, Kortschak RD, Bertozzi T, Adelson DL (2018) Horizontal transfer of BovB and L1 retrotransposons in eukaryotes. Genome Biol 19:85
Jiang W, Wu J, Zhang Y, Yin L, Lu J (2015) Isolation of a WRKY30 gene from *Muscadiniarotundifolia* (Michx) and validation of its function under biotic and abiotic stresses. Protoplasma 252:1361–1374
Johal GS, Briggs SP (1992) Reductase activity encoded by the HM1 disease resistance gene in maize. Science 258:985–987
Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329
Karuppanapandian T, Moon JC, Kim C, Manoharan K, Kim W (2011) Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Aust J Crop Sci 5:709
Kettle AJ, Carere J, Batley J, Benfield AH, Manners JM, Kazan K, Gardiner DM (2015) A γ-lactamase from cereal infecting Fusarium spp. catalyses the first step in the degradation of the benzoxazolinone class of phytoalexins. Fungal Genet Biol 83:1–9
Kim HJ, Lee HR, Jo KR, Mortazavian SM, Huigen DJ, Evenhuis B, Kessel G, Visser RG, Jacobsen E, Vossen JH (2012) Broad spectrum late blight resistance in potato differential set

plants MaR8 and MaR9 is conferred by multiple stacked R genes. Theor Appl Genet 124:923–935
Klessig DF, Choi HW, Dempsey DM (2018) Systemic acquired resistance and salicylic acid: past, present, and future. Mol Plant-Microbe Interact 31:871–888
Knip M, Constantin ME, Thordal-Christensen H (2014) Trans-kingdom cross-talk: small RNAs on the move. PLoS Genet 4:10
Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 5:325–331
Li F, Wang Y (2020) Plant micro RNAs regulate innate immunity through diverse mechanisms. In: Plant micro RNAs, pp 221–238
Li J, Brader G, Palva ET (2004) The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell 16:319–331
Liu X, Bai X, Wang X, Chu C (2007) OsWRKY71, a rice transcription factor, is involved in rice defense response. J Plant Physiol 164:969–979
Liu J, Ding P, Sun T, Nitta Y, Dong O, Huang X, Yang W, Li X, Botella JR, Zhang Y (2013) Heterotrimeric G proteins serve as a converging point in plant defense signaling activated by multiple receptor-like kinases. Plant Physiol 161:2146–2158
Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R (2003) Ethylene response factor 1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15:165–178
Macho AP, Zipfel C (2014) Plant PRRs and the activation of innate immune signaling. Mol Cell 54:263–272
Mayer AM, Staples RC, Gil-ad NL (2001) Mechanisms of survival of necrotrophic fungal plant pathogens in hosts expressing the hypersensitive response. Phytochemistry 58:33–41
Mei C, Qi M, Sheng G, Yang Y (2006) Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, PR gene expression, and host resistance to fungal infection. Mol Plant-Microbe Interact 19:1127–1137
Meyers BC, Kaushik S, Nandety RS (2005) Evolving disease resistance genes. Curr Opin Plant Biol 8:129–134
Morillo SA, Tax FE (2006) Functional analysis of receptor-like kinases in monocots and dicots. Curr Opin Plant Biol 9:460–469
Mukhi N, Gorenkin D, Banfield MJ (2020) Exploring folds, evolution and host interactions: understanding effector structure/function in disease and immunity. New Phytol
Munemasa S, Hauser F, Park J, Waadt R, Brandt B, Schroeder JI (2015) Mechanisms of abscisic acid-mediated control of stomatal aperture. Curr Opin Plant Biol 28:154–162
Parker IM, Gilbert GS (2004) The evolutionary ecology of novel plant-pathogen interactions. Annu Rev Ecol Evol Syst 35:675–700
Parker JE, Coleman MJ, Szabò V, Frost LN, Schmidt R, van der Biezen EA, Moores T, Dean C, Daniels MJ, Jones JD (1997) The Arabidopsis downy mildew resistance gene RPP5 shares similarity to the toll and interleukin-1 receptors with N and L6. Plant Cell 9:879–894
Peng Y, van Wersch R, Zhang Y (2018) Convergent and divergent signaling in PAMP-triggered immunity and effector-triggered immunity. Mol Plant-Microbe Interact 14:403–409
Pieterse CM, Van Loon LC (1999) Salicylic acid-independent plant defence pathways. Trends Plant Sci 4:52–58
Pieterse CM, Leon-Reyes A, Van der Ent S, Van Wees SC (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5:308–316
Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521
Pumplin N, Voinnet O (2013) RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. Nat Rev Microbiol 11:745–760
Robatzek S, Somssich IE (2001) A new member of the Arabidopsis WRKY transcription factor family, AtWRKY6, is associated with both senescence-and defence-related processes. Plant J 28:123–133

Robert-Seilaniantz A, Grant M, Jones JD (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 49:317–343

Rodriguez-Melcon C, Riesco-Pelaez F, Carballo J, Garcia-Fernandez C, Capita R, Alonso-Calleja C (2018) Structure and viability of 24-and 72-h-old biofilms formed by four pathogenic bacteria on polystyrene and glass contact surfaces. Food Microbiol 76:513–517

Rojo E, Solano R, Sánchez-Serrano JJ (2003) Interactions between signaling compounds involved in plant defense. J Plant Growth Regul 22:82–98

Sáenz-Mata J, Salazar-Badillo FB, Jiménez-Bremont JF (2014) Transcriptional regulation of *Arabidopsis thaliana* WRKY genes under interaction with beneficial fungus *Trichoderma atroviride*. Acta Physiol Plant 36:1085–1093

Sakamoto T, Deguchi M, Brustolini OJ, Santos AA, Silva FF, Fontes EP (2012) The tomato RLK superfamily: phylogeny and functional predictions about the role of the LRRII-RLK subfamily in antiviral defense. BMC Plant Biol 12:229

Savoia D (2012) Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol 7:979–990

Sós-Hegedűs A, Domonkos Á, Tóth T, Gyula P, Kaló P, Szittya G (2020) Suppression of NB-LRR genes by miRNAs promotes nitrogen-fixing nodule development in Medicago truncatula. Plant Cell Environ 43:1117–1129

Spoel SH, Koornneef A, Claessens SM, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Métraux JP, Brown R, Kazan K, Van Loon LC (2003) NPR1 modulates cross-talk between salicylate-and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15:760–770

Staskawicz BJ (2001) Genetics of plant-pathogen interactions specifying plant disease resistance. Plant Physiol 125:73–76

Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JD (1995) Molecular genetics of plant disease resistance. Science 268:661–667

Su Y, Li HG, Wang Y, Li S, Wang HL, Yu L, He F, Yang Y, Feng CH, Shuai P, Liu C (2018) Poplar miR472a targeting NBS-LRRs is involved in effective defence against the necrotrophic fungus *Cytospora chrysosperma*. J Exp Bot 69:5519–5530

Suharsono U, Fujisawa Y, Kawasaki T, Iwasaki Y, Satoh H, Shimamoto K (2002) The heterotrimeric G protein α subunit acts upstream of the small GTPase Rac in disease resistance of rice. Proc Natl Acad Sci 99:13307–13312

Temple BR, Jones AM (2007) The plant heterotrimeric G-protein complex. Annu Rev Plant Biol 58:249–266

Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8:397–403

Torres MA, Jones JD, Dangl JL (2006) Reactive oxygen species signaling in response to pathogens. Plant Physiol 41:373–378

Tsuda K, Sato M, Stoddard T, Glazebrook J, Katagiri F (2009) Network properties of robust immunity in plants. PLoS Genet 5

Tuteja N, Sopory SK (2008) Plant signaling in stress: G-protein coupled receptors, heterotrimeric G-proteins and signal coupling via phospholipases. Plant Signal Behav 3:79–86

Wang X, Li J, Guo X, Ma Y, Qiao Q, Guo J (2019) PlWRKY13: A Transcription Factor Involved in Abiotic and Biotic Stress Responses in *Paeonia lactiflora*. Int J Mol Sci 20:5953

Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot 100:681–697

Wei KA, Chen JU, Wang Y, Chen Y, Chen S, Lin Y, Pan S, Zhong X, Xie D (2012) Genome-wide analysis of bZIP-encoding genes in maize. DNA Res 19:463–476

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Huang HD, Jin H (2013a) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science 342:118–123

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Huang HD, Jin H (2013b) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science 4:118–123

Willmann R, Lajunen HM, Erbs G, Newman MA, Kolb D, Tsuda K, Katagiri F, Fliegmann J, Bono JJ, Cullimore JV, Jehle AK (2011) Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. Proc Natl Acad Sci 108:19824–19829

Xia Y, Yu K, Gao QM, Wilson E, Navarre D, Kachroo P, Kachroo A (2012) Acyl CoA binding proteins are required for cuticle formation and plant responses to microbes. Front Plant Sci 3:224

Xiong C, Xie Q, Yang Q, Sun P, Gao S, Li H, Zhang J, Wang T, Ye Z, Yang C (2020) WOOLLY, interacting with MYB transcription factor MYB31, regulates cuticular wax biosynthesis by modulating expression in tomato. Plant J 103:323–337

Xu L, Yao X, Zhang N, Gong BQ, Li JF (2019) Dynamic G protein alpha signaling in Arabidopsis innate immunity. Biochem Biophys Res Commun 516:1039–1045

Yi SY, Kim JH, Joung YH, Lee S, Kim WT, Yu SH (2004) The pepper transcription factor CaPF1 confers pathogen and freezing tolerance in *Arabidopsis*. Plant Physiol 136:2862–2874

Yu Y, Chakravorty D, Assmann SM (2018) The G protein β-subunit, AGB1, interacts with feronia in RALF1-regulated stomatal movement. Plant Physiol 176:2426–2440

Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, Wang J, Wang M, Li Q, Yang D, He Z (2007) Functional analysis of rice NPR1-like genes reveals that OsNPR1/NH1 is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. Plant Biotechnol J 5:313–324

Zeng J, Gupta VK, Jiang Y, Yang B, Gong L, Zhu H (2019) Cross-kingdom small RNAs among animals, plants and microbes. Cell 8:1–15

Zhai J, Jeong DH, De Paoli E, Park S, Rosen BD, Li Y, González AJ, Yan Z, Kitto SL, Grusak MA, Jackson SA (2011) MicroRNAs as master regulators of the plant NB-LRR defense gene family via the production of phased, trans-acting siRNAs. Genes Dev 25:2540–53

Zhan Y, Sun X, Rong G, Hou C, Huang Y, Jiang D, Weng X (2017) Identification of two transcription factors activating the expression of OsXIP in rice defence response. BMC Biotechnol 1:1–11

Zhang H, Gao Z, Zheng X, Zhang Z (2012) The role of G-proteins in plant immunity. Plant Signal and Behav 7:1284–1288

Zhang T, Zhao YL, Zhao JH, Wang S, Jin Y, Chen ZQ, Fang YY, Hua CL, Ding SW, Guo HS (2016) Cotton plants export micro RNAs to inhibit virulence gene expression in a fungal pathogen. Nat Plants 2:16153

Zhao JH, Guo HS. (2019) Trans-kingdom RNA interactions drive the evolutionary arms race between hosts and pathogens. Curr Opin Genet Dev 58:62–9

Zhou M, Memelink J (2016) Jasmonate-responsive transcription factors regulating plant secondary metabolism. Biotechnol Adv 34:441–449

Zhou Y, Memelink J, Linthorst HJ (2018) AP/ERF and WRKY transcription factors involved in the coordinated regulation of the salicylic acid signaling pathway in *Arabidopsis thaliana*. Exploring novel regulators and enzymes in salicylic acid-mediated plant defense 2018:35

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. Cell 125:749–760

Understanding Rhizosphere Through Metatranscriptomic Approaches

11

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Abstract

The rhizosphere is a closely proximate area where plant roots and PGPR live together and produce various organic molecules, which boost plant growth and immunity. Metatranscriptomics is the best technique to study unexplored or unidentified microbes that help plants in terms of growth and also elicit induced systemic resistance against various plant pathogens. Metatranscriptomics provides direct access to the microbial community, which is not culturable on growth media. We have provided a holistic view in this chapter about rhizosphere, types of microbes which live in the rhizosphere, and how they affect plant health. We have discussed techniques that are used to study metatranscriptomic and bioinformatic tools to interpret the most valuable knowledge from sequencing data.

Keywords

Rhizosphere · PGPR · PGPF · ISR · Metagenomics · Metatranscriptomics

11.1 Introduction

In 1904, Lorenz Hiltner coined the term "rhizosphere" which means the word "root" (Hiltner 1904; Hartmann et al. 2008), and related it to plant–root interaction. The microbial population found in the rhizosphere is different from that found in the bulk

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soil. Root exudates provide a source of nutrients for microbial growth. Weller and Thowshow (1994) proved that the rhizosphere area is rich in nutrients for bacteria, fungi, and other microorganisms as compared to normal soil. Plant roots contain 10–100 times more microorganisms than the normal soil. The area around plant roots is divided into three parts depending upon microorganisms' community or niche, that is, rhizosphere, phyllosphere, and endosphere. The rhizosphere is an area of soil derivative, influenced by sedimentation of plant mucilage and root exudates (Kent and Triplett 2002). Phyllosphere is a space or area which is relatively nutritionally poor and faces a lot of extreme temperatures, radiation, and moisture. Microorganisms found in the rhizosphere and phyllosphere are termed as epiphytes while microbes within plant tissues are called endophytes. Microorganisms in these niches can establish a beneficial, neutral, or detrimental relation with their host plants. One of the best examples of this kind of association is rhizobium–legume symbiosis, which paved the way for crop rotation system, that led to increased agriculture output (Oldroyd et al. 2011). In this chapter, we mainly focus on the metatranscriptomic approach which is used to study the rhizosphere microbial diversity, plant–microbial interaction, scope, and limitation of this technology.

11.2 Plant–Microbial Community Interactions in Rhizosphere

Plant roots produce a diverse range of chemical compounds in the rhizosphere (including enzymes, plant growth hormones, and secondary metabolites), which are associated to cater microorganisms and form mutualistic association in the rhizosphere. Plant roots and fungus form a relationship that results in exchanges of exudates and nutrients. A large number of plant genes are identified which play a vital role in facilitating arbuscular mycorrhizal fungi (AM fungi) interaction. Due to lack of literature about the signaling process between symbionts, we do not have correct information about pathways involved in symbiotic interactions resulting in the exchange of nutrients between plant roots and fungi (Hirsch and Mauchline 2012). Symbiotic relation lacks host specificity, and the symbiotic relationship between AM fungi and plants is one of the most common and the earliest plant symbioses. Glomeromycotina, Ascomycotina, and Basidiomycotina include 6000 species in their family; however, there could be an expansion in this number with advances in molecular biology techniques.

The mechanism of the signaling process between AM fungi and plants and the pathways involved in nutrient exchange between the two partners are not very much understood. However, it has been speculated that plant-derived signals or related compounds, which are conserved throughout the plant kingdom, play a major role in such interactions (Whipps et al. 2008). The most studied among these signals include flavonoids and strigolactones produced by *Lotus japonicus*. Strigolactones have been reported to encourage growth and branching in AM fungal hyphae, which are needed for effective root colonization by AM fungi.

11.2.1 Plant-Derived Compounds Affecting Rhizosphere Microbes

The production of plant-released exudates is dependent on numerous factors such as plant types, climatic conditions, biological, physical, and chemical characteristics of the neighboring soil. Plant root-released compounds that deposit in the surrounding soil are termed as rhizodeposits (Rovira 1969). Rhizodeposits are composed of parts of dead root caps, mucilage, and root exudates. The mucilage secreted by root caps and epidermal cells contains polysaccharide-rich material, which protects roots from desiccation and assist in nutrient gain and thus forming soil aggregates. It also attracts useful microbes including AM fungi (Klironomos et al. 1993).

Scientific community lays emphasis on the effects of chemical compounds released by plant roots on microbial diversity and on the nutrient acquisition of Fe and P (i.e., allelopathy) or biochemical signs to attract beneficial microbes such as AM fungi and rhizobia (chemotaxis) and encourage establishment of beneficial (biocontrol) microbes including bacteria (*Bacillus subtilis*, *Pseudomonas flores-cence*) and fungi (*Trichoderma* sp.) on the root surface. Chemicals in root exudates abet plants for accessing nutrients by acidifying or altering redox conditions within the rhizosphere. Malic acid and citric acid released by plant roots help in reducing the pH of the soil within the rhizosphere and solubilizing phosphorus. Fe binds strongly with phytosiderophores which is then absorbed by roots through diffusion. Flavonoids, which are root exudates, modulate the interaction between plants and plant growth-promoting rhizobacteria (PGPR), but clear-cut signaling molecules that recruit specific bacterial species are hardly understood. PGPR require a definite signal to colonize the host and initiate symbiotic interaction with their partner.

11.2.2 Rhizosphere Microbe-Derived Compounds Affecting Plant Growth and Health

The rhizosphere is a place and contagion court for soil-borne disease-causing agents and similarly in the field, here both flora and fauna relate to plant pathogens and affect the result of diseases causing agent infection though numerous useful microbes that exist in the rhizosphere region can constrain the development and action of soil-inhabiting plant pathogens (Widder et al. 2016). The effect of interaction of helpful rhizosphere fungi such as *Trichoderma* and *Gliocladium* and bacteria such as *Pseudomonas* and *Burkholderia* on plant growth, development, and health are well recognized. All PGPR have incidental beneficial ramifications on plant well-being by hindering plant pathogens through rivalry and antibiosis. PGPR also produce straight optimistic results on plant well-being by stimulating induced systemic resistance (ISR), thereby protecting plants from devastating pathogen outbursts or by revealing the plants to PGPR-synthesized chemicals such as 2,3-butanediol, pyoverdine, and lipopeptide disinfectants. Although some experiments were conducted to find out the pathways underlying the interaction between PGPR and plants, more studies are required to fully understand these

interactions and to improve their use in agriculture with special reference to management of soil-borne plant diseases (Zimmerman and Vitousek 2012).

11.2.2.1 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal (AM) fungi are soil-stomached microorganisms that form a mutualistic symbiotic association with maximum land plants. As obligate biotrophs, these fungi are incapable of finishing life cycle in the absence of a host plant. The Arbuscular mycorrhizal fungi association is characteristically beneficial. Being obligate symbionts, AMF are primarily dependent on plants for carbon fixation and the plant get benefited from such interactions in the form of improved growth (Davies et al. 1993), abiotic and biotic resistance, improved nutrient uptake particularly of immobile nutrients such as P and Zn over non-mycorrhizal controls, and alteration of root morphology. The improved nutrient uptake results from the uptake of nutrients by the extra-radical hyphae of the AMF that spread up to 8 cm outside the root which mocks and functions as the extension of the root structure in obtaining nutrients from the soil.

The AMF hyphae produce a glycoprotein termed glomalin (Wright et al. 1996; Wright and Upadhyaya 1996), which helps plants by sustaining a steady water level in the soil. Furthermore, AMF improves nutrient cycling and carbon flow, which significantly controls soil microbial diversity (Linderman 1991). For instance, *Glomus mosseae* have been reported to expand the diversity of rhizosphere bacteria. Thus, AMF are a crucial part of agricultural ecosystems that could be good substitutes for chemical fertilizers and could work as biocides for the management of both abiotic and biotic stresses.

11.2.2.2 Plant Growth-Promoting Rhizobacteria and Fungi

Plant growth-promoting rhizobacteria (PGPR) are bacteria present in agricultural ecosystems that act positively in plant development though alterative reproducibility as well as the possible effects of inoculation upon plant root-related microbial groups can be sources of concern. PGPR reside in the rhizosphere of diverse plant species and produces useful effects, such as better plant growth and low vulnerability to diseases produced by plant-pathogenic fungi, bacteria, viruses, and nematodes. Usually, PGPR endorse plant growth and development by enabling nutrient acquisition (N_2, P, and other vital minerals), modifying levels of plant growth hormones or through biological control of plant diseases. Some PGPR are also sources of physical or chemical alterations related to plant defense, a process stated as "induced systemic resistance" (ISR) (Suryadi et al. 2019). ISR induced by PGPR has suppressed plant diseases prompted by a range of pathogens through "induced systemic tolerance" (IST) for PGPR-encouraged physical and chemical changes in plants that result in enhanced tolerance to abiotic stress.

The characteristic features of PGPR include (1) inhabiting root surface; (2) living, proliferating, and striving with microbiota present in the rhizosphere, and helping plant growth promotion/defense actions; and (3) boosting plant growth (Kloepper 1994). PGPR could be extracellular PGPR (e-PGPR), which inhabit within the rhizosphere, rhizoplane, or in the root cortex, and intracellular (i-PGPR), which

Table 11.1 List of PGPR and PGPF capable of plant growth promotion

Genera	Species/strain	Growth promotion in	Reference
Alternaria	*Alternaria* sp.	*Nicotiana tabacum*	Zhou et al. (2014)
Aspergillus	*Aspergillus* spp. *PPA1*	*Cucumis sativus*	Islam et al. (2014)
	As. Fumigatus	*Oryza sativa*	Hamayun et al. (2009)
	Aspergillus niger	*Brassica chinensis Linn.*	Chuang et al. (2007)
Penicillium	*Penicillium citrinum*	*Helianthus annuus*	Waqas et al. (2015)
Aspergillus	*Aspergillus terreus*	*Helianthus annuus*	Waqas et al. (2015)
Trichoderma	*Trichoderma virens*	*Pinus sylvestris* var. *mongolica*	Yedidia et al. (2001)
	Trichoderma virens	*Arabidopsis thaliana*	Contreras-Cornejo et al. (2011)
Achromobacter	*Achromobacter xylosoxidans* strain *Ax10*	*Brassica juncea*	Ma et al. (2009)
Azospirillium	*Azospirillium lipoferum*	Hordeum vulgare	Belimov et al. (2004)
	Azospirillum brasilence	*Zea mays, glycine max*	Orlandini et al. (2014)
Azotobacter	*Azotobacter chroococcum*	*Brassica juncia*	Narozna et al. (2014)
Bradyrhizobium	*Bradyrhizobium japonicum*	*Zea mays, glycine max*	Orlandini et al. (2014)

live in root cells, predominantly in nodules (Figueiredo et al. 2011). Apart from PGPR, many actinomycetes such as *Micromonospora* sp., *Streptomyces* spp., *Streptosporangium* sp., and *Thermobifida* sp., etc. are also known to interfere with rhizosphere microbial groups and support plant growth, development, and other useful traits such as abiotic and biotic stress tolerance. Like PGPR and plant growth-promoting fungi (PGPF), actinomycetes have also offered huge biocontrol potential for the management of diverse soil-borne plant pathogens (Tables 11.1 and 11.2).

PGPF belong to the group of nonpathogenic fungi that help plants by improving plant growth, development, and stress tolerance. Several findings suggested that PGPF have a wide range of hosts and are different to each for their systematics, the environments they live in, functioning, and their interaction with plants and other microbes in the rhizosphere. PGPF species are universal saprophytes and occupy the soil near the plant roots. Despite the name PGPF, they may not always improve plant growth and development, as their effect may not be the same for all the plant species. For instance, one PGPF that encourages the growth and development of one plant species may have a reverse effect on another plant species, or the effect may vary between two different environments. Likewise, all the fungi encouraging plant growth are not grouped under PGPF (Berg and Smalla 2009). For instance, AM fungi, though enhance plant growth as discussed earlier, are not included under PGPF.

Microorganisms recognized as PGPF have varied taxonomy. Majority of them belong to the phylum Ascomycota and others to Zygomycota and Basidiomycota.

Table 11.2 List of PGPR and PGPF with biocontrol activity

Genera	Species/strain	Effective against		Host	Reference
		Pathogen	Disease		
Bacillus	*Bacillus sp*	*Fusarium oxysporum* f. sp. *ciceri*, *Fo. phaseoli* and *Fo. Melonis*	Chickpea wilt	Chickpea	Landa et al. (1997)
	B. subtilis	*F. solani*	Chickpea wilt	Chickpea	Singh et al. (2014)
	B. subtilis	*Macrophomina phaseolina*	Dry root rot of chickpea	Chickpea	Singh et al. (2014)
	B. subtilis	*Blumeria graminis* f. sp	Powdery mildew	Barley	Prathap and Ranjith (2015)
	B. subtilis	*Meloidogyne incognita, M. arenaria*	Root knot	*Cotton*	Prathap and Ranjith (2015)
Pseudomonas	*P. fluorescens*	*Fo. ciceris*	Chickpea wilt	Chickpea	Landa et al. (1997)
	P. cepacian	*Phytophthora megasperma* f. sp. *medicaginis*	Root rot of chickpea	Chickpea	Myatt et al. (1993)
	P. fluorescens	*Pm. medicaginis*	Root rot of chickpea	Chickpea	Myatt et al. (1993)
	Pseudomonas sp. isolate NBRI9926	*Fo. ciceris*	Chickpea wilt	Chickpea	Nautiyal (1997)
	Pseudomonas sp. isolate NBRI9926	*R. bataticola*	Dry root rot in chickpea	Chickpea	Nautiyal (1997)
Pseudomonas	*Pseudomonas* sp. isolate NBRI9926	*Pythium* sp.	Seed rot in chickpea	Chickpea	Nautiyal (1997)
	P. putida, P. aeuroginosa	*Fo. ciceris*	Chickpea wilt	Chickpea	Karimi et al. (2012)
	P. putida, P. alcaligenes, Pseudomonas isolate (Ps28)	*M. phaseolina*	Dry root rot of chickpea	Chickpea	Akhtar and Siddiqui (2009)
	Pseudomonas sp.	*Fo. ciceris*	Chickpea wilt	Chickpea	Landa et al. (2001)

Stenotrophomonas	*S. maltophilia*	*Verticillium dahliae*	Wilt of oilseed rape	Chickpea	Landa et al. (1997)
Rhizobium	*Rhizobium* sp. isolate NBRI9513	*Fo. ciceris*	Chickpea wilt	Chickpea	Nautiyal (1997)
	Rhizobium sp. isolate NBRI9513	*R. bataticola*	Dry root rot in chickpea	Chickpea	Nautiyal (1997)
	Rhizobium sp. isolate NBRI9513	*Pythium* sp.	Seed rot in chickpea	Chickpea	Nautiyal (1997)
Paenibacillus	*Paenibacillus* sp	*Fo. ciceris*	Chickpea wilt	Chickpea	Landa et al. (2001)
Stenotrophomonas	*Stenotrophomonas* sp.	*Fo. ciceris*	Chickpea wilt	Chickpea	Landa et al. (2001)
Penicillium	*Penicillium citrinum* LWL4 and *Aspergillus terreus* LWL5	*Sclerotium rolfsii*	Collar rot	Sunflower	Waqas et al. (2015)
Aureobasidium and Rhodotorula	*Aureobasidium pullulans* YA05, *Rhodotorula mucilaginosa* YR07	*Phytophtora infestans and F. graminearum*	Chestnut blight	Dark chestnut soil	Ignatova et al. (2015)
Rhizoctonia	*Rhizoctonia solani*	*Fo, F. crown*	Root rot	Tomato	Muslim et al. (2003)
Phytophthora	*Phytophthora cryptogea*	*Fo* f. sp. *lycopersici*	Wilt disease	Tomato	Attitalla et al. (2001)
Trichoderma	*Trichoderma viride*	*Fo* f. sp. lycopersici	Wilt disease	Tomato	Moosa et al. (2017)

Some of the members of PGPF, for example, *Colletotrichum* sp., *Pythium oligandrum*, *Phytophthora cryptogea*, *Fusarium oxysporum*, *Alternaria* sp. and binucleate *Rhizoctonia*, are phylogenetically close to their counterpart plant pathogens, but they lack virulence factors required to cause disease in plants. This suggests that huge portions of rhizosphere microorganisms belong to PGPF.

11.3 Understanding Plant Microbiome

The plant microbiome bacteria are very important for plant health and fitness, and thus contribute to the range and improvement of microbial taxa covering essential functional genes for the benefit of the plant holobiont (Hugenholtz and Tyson 2008). Molecular evidence suggests that plant–microbial interactions involving AM fungi, PGPR, and PGPF were important for improvement in plant species for the past 700 million years. A noteworthy amount (nearly 5–20%) of the photosynthate is produced mostly through plant roots. Furthermore, plants produce a significant amount of methanol and isoprene, two of the important sources of carbon and energy for microbes, into the atmosphere. Moreover, the management of plant microbiome has got the interesting prospect of biological control, surge of agricultural production, reduced use of chemicals, and cut down on greenhouse gas secretion for more sustainable agricultural practices. Some of the promising approaches for understanding plant microbiome are discussed in the coming sections.

11.3.1 DNA Fingerprinting

Microorganism ecology has observed a large variation in the last two decades with regard to techniques utilized for the study of ecological communities. Paramount focus changed from inoculation to interpretation of conserved biomolecules including DNA-based techniques that are founded on cloning and sequencing of DNA segment or largely based on previous amplification of precise sequences by use of PCR. The mixture of PCR product yield could be cloned and sequenced or can be exposed to a growing variety of genetic characterizing methods, through amplified ribosomal DNA restriction analysis. DNA fingerprinting method was first reported by Avaniss-Aghajani et al. (1994) and utilized for Mycobacterial identification. Ding et al. (2013) applied terminal-restriction fragment-length polymorphism (T-RFLP) to understand (1) the genetic changes that are present in the endophyte microbe population surviving in diverse plant species and (2) the effect of bacterial and fungal populations on the plethora and diversity of endophyte microbes. They evaluated a collection of ten different maize varieties for the occurrence of different endophytes by growing the endophytes in vitro, cloning them, and DNA fingerprinting using 16S rDNA T-RFLP.

11.3.2 Phylogenetics of rRNA and Other Genes

Molecular marker technology is one of the most preferred choices for characterizing a variety of microbes including bacteria. Molecular markers have been utilized for different kinds of studies including microbial taxonomy and evolution biology of different organisms. Moreover, with the availability of whole-genome sequence information of numerous fungi, bacteria, actinomycetes, etc., the identification of orthologous families of genes in different organisms and understanding of the gene function have progressively become easier and more cost-effective (Eisen 1998).

RNAs belonging to different groups of microorganisms have been recognized through the phylogenomic approach that may be expected as new genes or governing elements. Likewise, microbial molecular investigation offers substantial evolutionary understandings when mapped onto *rRNA* phylogeny; meanwhile, microbial *rRNA* phylogenetic studies have been revealed to be curiously reliable (Siefert and Fox 1998). A phylogenomic investigation method that comprises information about the purpose of genes, exaptation, and biochemistry in upcoming time could be worthy in inter- and intra-genomic studies of microorganisms.

Studies confirming the arrangements of 16 *rRNA* genes are one more significant milestone in the study of evolution and categorization of microorganisms. Earlier, microorganisms were grouped based on the resemblances and changes in their phenotypic traits, into prokaryotes and eukaryotes, which were further categorized into different taxonomic ranks. However, taxonomic classification based on these approaches could be tedious because differences in phenotypic features of different organisms may not be too prominent. To overcome such limitations, nucleic acid-based approaches were identified and developed. Phylogenetic analysis of 16S *rRNA* gene sequence characterization from several bacterial species was first conducted by Woese and George (1977). This approach has been exploited efficiently for phylogenic and evolutionary studies in gold nanoparticle-producing bacteria (Nangia et al. 2009). Rainey et al. (1997) described phylogenetic diversity among five species of genus *Deinococcus* (*D. radiopugnuns*, *D. proteolyticus, D. rudioduruns, D. radiophilus,* and *D. erythromym*) and *Deinobacter grandis* using 16S rDNA sequence comparison. Bond et al. (2000) reported a phylogenetic study of *Leptospirillum* sp. and *Ferromicrobium acidophilus* using 16S analysis. Sourath and Subramanian (2014) investigated the molecular identification of *Pseudomonas aeruginosa* by 16S *rRNA* sequence-based method as per standard protocol. Dehnad et al. (2015) described *Arthrobacter nitroguajacolicus* using 16S *rRNA*. Similarly, Alam et al. (2016) reported a phylogenetic relation of *Pseudomonas putida* strain MDH1 with other species of *Pseudomonas* using 16S *rRNA* gene.

11.3.3 Metagenomics

The genomics of diverse microbes by direct DNA extraction and cloning from the direct soil sample is termed as metagenomics. Rhizosphere soil or marine samples hold significantly high genetic information as these samples possess a highly diverse

population of microorganisms. The literature on metagenomic studies characteristically include cloning of microbial DNA fragments, their sequencing, and functional characterization.

Recent molecular biology techniques, which are being utilized for investigating unknown ecological DNA samples, have unlocked a paradigm shift of thrilling research discoveries. During the last few decades, numerous vital research investigations devoted to metagenomics have occurred. Now, with the advancement of sequencing technology, larger chromosome fragments have been cloned and sequenced from the same genome to find out the structure and function of entire unidentified or uncultured genomes from environmental samples. These kinds of studies lead to the development of novel DNA isolation methods along with better cloning systems. Metagenomic methods depict communities based on the comparative abundance of genes, and the purpose of these studies is to deliver a complete, all-inclusive opinion of these communities, though the emphasis is frequently on only a fraction of the physiological functions represented.

11.3.4 Metatranscriptomics

Metatranscriptomics offers a valued understanding of the complete transcriptomic analysis of all the microbes representing a specific ecosystem. It delivers the insight into all the functional genes present in that ecosystem, their role in the ecosystem, and how their expression change in response to prevailing ecological changes. Metatranscriptomic findings are very useful in studying diverse ecosystems including soil and aquatic ecosystems. It offers appropriate techniques to study and elucidate the eukaryotic gene pool for the betterment of these ecosystems. Metatranscriptomics is a vibrant tool in streamlining the new bioinformatic approaches to analyze the data generated from metatranscriptomic analysis.

11.4 Metatranscriptomics: Background, Methodologies, Scope, and Limitations

Metatranscriptomics provide information related to culturable and non-culturable microbial species. It provides transcriptomic information through high-throughput sequencing of transcripts from different bacterial or fungal communities in exact environmental samples. Metatranscriptomic sequencing provides the opportunity to randomly sequence mRNAs as a unit for insight into the complex pathways and metabolites in microbial communities. The study of the metatranscriptome by the NSG technique allows us to find gene expression profiles from whole microbial inhabitants, providing new understandings of poorly known biological systems, and reduce technical limitations connected to individual bacteria isolation.

There are very few metatranscriptomic studies that were done to investigate rhizosphere ecosystem. Metatranscriptomics can produce comprehensive functional outlines of rhizosphere microbial communities. It could be utilized to understand the

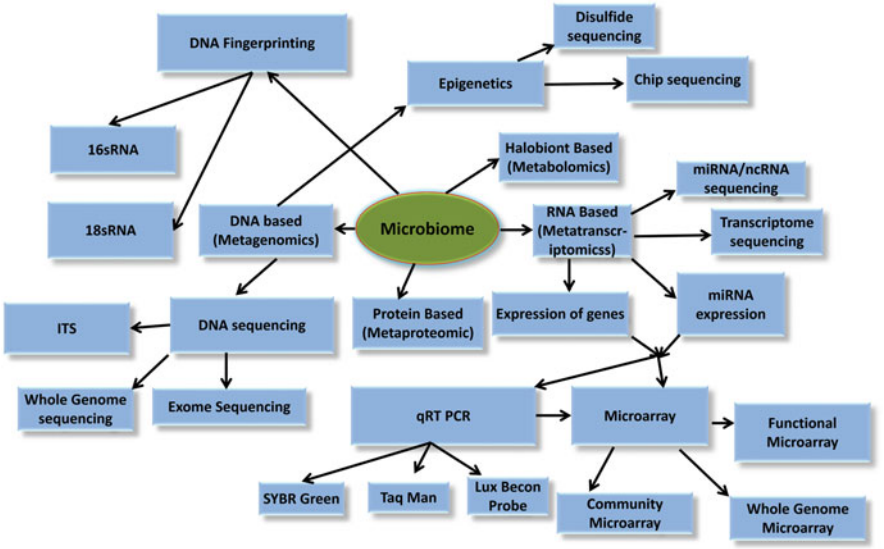

Fig. 11.1 Diagrammatic representation of different approaches for studying metatranscriptomics

mechanism of ISR in plants by a specific group of microbes including PGPR, PGPF, actinomycetes, etc. (De Vleesschauwer and Hofte 2009). Metatranscriptomics of bacterial RNA from the rhizospheres of test plants and plants applied with signaling molecules such as salicylic acid, jasmonic acid, ethylene, etc. have been elucidated to understand the mechanism of inducing defense responses (Anderson et al. 2004).

On the contrary, we can compare and correlate the microbial metatranscriptome of the rhizospheres of wild-type plants with the defense pathways in mutant plants such as npr1, etr1, eds5, coi1, jar1, jin1, pad4, cpr5, and ein2 (Jones and Dangl 2006). The availability of various advanced tools and techniques has helped the field of metatranscriptomics including rhizosphere metatranscriptomics to reach great heights (Fig. 11.1). Some of the techniques are discussed here.

11.4.1 Microarray

Microarray, a tool used to identify the expression of a large number of genes at the same time, is an influential genomic tool, which has been used extensively to investigate gene expression profiles under diverse cell growth conditions, sense exact mutations in microbial DNA, and describe the microbial population in ecological samples. Metatranscriptomic analysis-based microarrays are of three types.

11.4.2 Functional Gene Arrays (FGAs)

The functional gene array (FGA) is a very important tool for the characterization and functional analysis of microbial diversity in different ecosystems. FAGs could be constructed from oligos and DNA fragments after analysis of functional genes similar to the microarray which are to examine gene expression (Wu et al. 2001). Microarray hybridization consequences specified that genes owning 80–85% sequence uniqueness could be distinguished under high-stringency hybridization conditions (65.8 °C). The recognition perimeter for *nirS* (nitrite reductase genes) was around 1 ng of pure genomic DNA and 25 ng of soil community DNA via the enhanced protocol. Such a level of accuracy is considered to be adequate in microbial ecology studies (Pinkel et al. 1998).

11.4.3 Community Genome Arrays (CGAs)

Very little is known about the genome sequence characteristics of many microbes isolated from their native habitats. The big collection of pure cultures of diverse microorganisms is a valuable resource for investigating microbial population dynamics and structure in their natural habitat. CGAs are suitable for the identification of diverse microbes up to the species/strain/race level. CGA is theoretically similar to membrane-based reverse sample genome probing (RSGP) except that arranging substrate and signal detection plans in RSGP is different. Moreover, a nonporous surface is used for fabrication and fluorescence-based recognition in CGA (Voordouw 1998). A strong association has been reported between sequence similarity values and CGA hybridization ratios resulting from small subunit rRNA and gyrB genes, DNA–DNA reassociation, or repetitive element-based and BOX-PCR fingerprints (r2 ¼ 0.80–0.95) (Wu et al. 2001). Due to high capacity, constructing CGAs containing bacterial and other related strains is much easier. With CGAs, we could rapidly recognize unidentified bacterial strains, as long as an appropriately related probe is on the array, through the hybridization of genomic DNA from unidentified strains. A glass-based microarray, comprising 132 small subunits of rRNA-targeted oligonucleotide probes and representing all documented sulfate-reducing prokaryotes, was developed (Loy et al. 2002). This system could perfectly differentiate among perfectly matched and mismatched probes under similar hybridization conditions when 41 reference strains were used (Loy et al. 2002).

11.4.4 Whole-Genome Open Reading Frame (ORF) Microarray

The whole-genome open reading frame (ORF) microarray-based hybridization method is in use for explaining variability in genomes and differences between nearly identical organisms. This method was applied to assess the genomic variability and similarity among several related metal-plummeting bacteria within

the *Shewanella* genus (Murray et al. 2001). They identified both conserved and less conserved genes among nine species of *Shewanella* studied and found that related *Shewanella* sp. had more than 93% and 80% sequence similarities in SSU rRNA and gyrB, respectively.

11.4.4.1 Quantitative Real-Time PCR (qRTPCR)

Quantitative real-time PCR (qRTPCR), chiefly used to quantify the amount of a definite RNA, is attained by studying the extension reaction using fluorescence, a method called real-time PCR or quantitative PCR (qPCR) (Lacava et al. 2006). Besides SYBR green and Taqman chemistries, there are additional variants such as Lux and Beacon probes available for qPCR. SYBR green and Taqman chemistries were used to quantify bacterial species associated with plants (Zhang and Fang 2006). These approaches were also applied to know the bacterial pathogen *Xylella fastidiosa* in the citrus specimen (Oliveira et al. 2002), and the endophytic bacterium *Methylobacterium mesophilicum* in the model plant *Catharanthus roseus* (Ruppel et al. 2006). Similarly, the population dynamics and distribution of PGPR *Enterobacter radicincitans* outside and within the plant system were monitored using qPCR in *Brassica oleracea* (Ruppel et al. 2006). Likewise, qPCR can be used in studying the gene expression profiles of microorganisms.

11.4.4.2 Pyro Sequencing

Pyrosequencing has come up as a fascinating technology in the field of microbial diversity that has limited the restriction of sequencing. Pyrosequencing can deliver megabases of sequences in a few hours and permits a deep survey of microbial species in any ecosystem (Edwards et al. 2006). Pyrosequencing is a bit different from the Sanger methodology as the latter measures combination and additional recognition of dideoxynucleotide triphosphate (ddNTP) that is fluorescently labeled (Margulies et al. 2005).

During pyrosequencing, an enormously low quantity of reaction is required for the up-scaling of the procedure. With the availability of advanced instruments, pyrosequencing can yield about 3 lakh reads of around 200–400-bp size in 5 h. Pyrosequencing has been exploited to study the diverse microbial populations in various environments including the deep ocean biosphere and soil bacterial population (Roesch et al. 2007; Sogin et al. 2006). These studies reported that the microbial population was highly variable in deep ocean biosphere and rhizosphere. Although they could obtain 30,000 sequences, the complete description of microbial species/strain and their DNA sequences in both the ecosystems was not accomplished (Roesch et al. 2007; Sogin et al. 2006). The microbial diversity linked with plants appears mostly identical, mainly for endophytes. Therefore, the application of pyrosequencing in revealing the population structure and dynamics in different ecosystems including rhizosphere is promising.

11.4.4.3 Internal Transcribed Spacer (ITS) Sequencing

The internal transcribed spacer 1 (ITS1), the cistron region of the rRNA, is one of the most commonly used molecular markers for the identification and characterization

of diverse microbes including fungi and bacteria from ecologically diverse samples such as water, plant, soil, etc. without culturing the microbes (Schoch et al. 2012). ITS as a molecular marker has been used to identify several fungal endophytes including *Gibberella moniliformis, C. oxysporum, C. boninense, C. gloeosporioides, Curvularia lunata, F. fujikuroi, C. lunata, Epicoccum sorghinum, Penicillium* sp., *Nemania* sp., *Rigidoporus vinctus, Scopulariopsis gracilis, and Sarocladium zeae* (Renuka and Ramanujam 2016). ITS was utilized to identify fungal species from organic and nonorganic crop fields by Xia et al. (2019), who identified and characterized 190 and 550 fungal species, respectively, from these fields. They further classified these fungi into eight orders and 22 species, of which *Trichoderma* sp. and *Pichia guilliermondi* were highly populated in both the samples (Xia et al. 2019).

11.4.4.4 Bioinformatics Tool

Storage and analysis of a huge amount of data is a challenging task in the post-genomic era. There is a need to develop software, which can help us in drawing some meaningful conclusions from simple DNA or RNA sequences and could support in comparing the data generated by researchers across the globe (Yilmaz et al. 2011). Numerous online software and tools have been developed to ease the analysis of such data. Some of the software and tools being used to analyze metatranscriptome data sequences include SAMSA, MetaModules, rRNAFilter, MetaTrans, IDBA-MT, COMAN, etc. (Table 11.3).

The data analyzed using these tools are generated in different forms; some of them can be downloaded, which allow user-defined data analysis as per the requirement of the experimental objectives. The data analysis using different tools involving different parameters and procedure generates different explanations for a similar set of data within a given environment. Metagenome Analyzer (MEGAN) is a convincing bioinformatic software for the study of metagenomic data for samples collected from different habitats. MEGAN results in a small amount of false-positive results, though with a limited number of underprediction. The advanced version of MEGAN permits assessments among different metagenomic data groups, which will be mostly beneficial for probing diverse rhizospheres and their comparisons. This approach might be utilized to investigate metatranscriptomic information similarly, by assigning mRNA reads to already known taxa. Thus, the field of metatranscriptomics is expected to witness a fast growth with improvements in the

Table 11.3 List of online software for metatranscriptomic analysis

Tools	Link
SAMSA	http://creativecommons.org/licenses/by/4.0
MetaModules	https://github.com/njsmith/metamodule
rRNAFilter	http://hulab.ucf.edu/research/projects/rRNAFilter/rRNAFilter.html
MetaTrans	http://www.metatrans.org
IDBA-MT	http://i.cs.hku.hk/~alse/hkubrg/projects/idba_mt/index.html
COMAN	http://sbb.hku.hk/COMAN/

methodological challenges of rRNA extraction from a different array of samples, and sequencing depth and price drops for these analyses. Even then the task of handling huge data groups and biological queries relevant to metatranscriptome will keep on increasing gradually with improvements in the understanding of other research questions.

11.4.4.5 Scope and Limitations

Numerous scopes related by metatranscriptome investigation are worth stating. The extraction of superior RNA specimens from few biological samples could be a problem if not an intimidating job. Moreover, the possibility of host RNA contamination in the sample can prove to be problematic. Additional subject to include is mRNA has a petite half-life and therefore it can be difficult to notice rapid/short-lived signals to ecological stimuli. Furthermore, the presence of mRNA is not continuously identical with the occurrence of protein. By itself, pipelines assimilating metagenomic, metatranscriptomic, metabolomic, and metaproteomic datasets might firmly allow to improve the all-inclusive sight of microbiome arrangement and function at numerous layers. Lastly, now, numerous metagenomic study approaches may at times yield mutable results, even if matching databases are applied in the investigation. Thus, calibration of RNA isolation, refining, sequencing, and an investigation are necessary to allow additional distribution of metatranscriptomic approaches and their incorporation into microbiome research.

Usually, large-scale expression investigations using approaches, for example, microarrays and serial analysis of gene expression, have been escorted by authentication of results by a sovereign technique, that is, qPCR, being measured as the gold standard. Today, as the large-scale expression method has moved toward using NGS approaches, explicitly RNA-seq, authentication of findings using this technology with the use of qPCR must not be ignored which can deliver added value to the experiential expression patterns.

11.5 Conclusion and Future Perspective

Metatranscriptomics is a promising approach to explore community-level metabolic interactions among different individuals in a community and improve our understanding of the role of rhizosphere in diverse ecosystems. However, performing metatranscriptomic analysis is a highly challenging task in the sense that extraction of pure mRNA from different samples, in-depth sequencing, and cost of such analysis are not that easier and convenient for every researcher. Besides, methodological challenges of finding representative mRNA and creating metatranscriptome datasets are comparatively modest related to their investigation and understanding. Our capability to understand solitary transcriptomes and metatranscriptomic information is presently inadequate due to lack of accessibility to high-quality, exactly interpreted, and phylogenetically various genomes. Good quality, precisely interpreted standard genomes are vital for all metatranscriptome investigations so that specific transcripts and specific genomes could be recognized. Such databases

are being generated. Due importance is required to be given in the cell biology of wider taxonomic groups of fungi to ease definite task of gene functions and describe mechanisms of key metabolic pathways, particularly for the "hypothetical" genes. Supplementing metatranscriptome investigations with improvements in mass spectrometry-based metaproteome studies will help improve precision of functional stint of transcribed genes and decrease our dependence on sequence homology-based approaches.

References

Akhtar MS, Siddiqui ZA (2009) Use of plant growth-promoting rhizobacteria for the biocontrol of root-rot disease complex of chickpea. Australas Plant Pathol 38(1):44–50

Alam N, Sarkar M, Chowdhury T, Ghosh D, Chattopadhyay B (2016) Characterization of a novel MDH1 bacterium from a virgin hot spring applicable for gold nanoparticle (GNPs) synthesis. Adv Microbiol 6:724–732

Anderson JP, Badruzsaufari E, Schenk PM, Manners JM, Desmond OJ, Ehlert C et al (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. Plant Cell 16:3460–3479

Attitalla IH, Johnson P, Brishammar S, Quintanilla P (2001) Systemic resistance to Fusarium wilt in tomato induced by *Phytophthora cryptogea*. J Phytopathol 149:373–380

Avaniss-Aghajani E, Jones K, Chapman D, Brunk C (1994) A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences. BioTechniques 17:144–149

Belimov AA, Kunakova AM, Safronova VI et al (2004) Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. Microbiology 7:99–106

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Bond PL, Druschel GK, Banfield JF (2000) Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. Appl Environ Microbiol 66:4962–4971

Chuang CC, Kuo YL, Chao CC, Chao WL (2007) Solubilization of inorganic phosphates and plant growth promotion by Aspergillus Niger. Biol Fertil Soils 43:575–584

Contreras-Cornejo HA, Macias-Rodriguez L, Beltran-Pena E, Herrera-Estrella A, Lopez-Bucio J (2011) Trichoderma-induced plant immunity likely involves both hormonal- and camal exindependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungi *Botrytis cinerea*. Plant Signal Behav 6(10):1554–1563

Davies FT, Porter JR, Linderman RG (1993) Drought resistance of mycorrhizal pepper plants—independent of leaf phosphorus concentration, response in gas exchange, and water relations. Physiol Plant 87:45–53

De Vleesschauwer D, Hofte M (2009) Rhizobacteria-induced systemic resistance. Adv Bot Res 51:223–281

Dehnad A, Hamedi J, Derakhshan-Khadivi F, Abusov R (2015) Green synthesis of gold nanoparticles by a metal resistant *Arthrobacter nitroguajacolicus* isolated from gold mine. IEEE Trans Nanobiosci 14:393–394

Ding T, Palmer MW, Melcher U (2013) Community terminal restriction fragment length polymorphisms reveal insights into the diversity and dynamics of leaf endophytic bacteria. BMC Microbiol 13(1):1

Edwards RA, Rodriguez-Brito B, Wegley L, Haynes M, Breitbart M, Peterson DM, Saar MO, Alexander S, Alexander EC Jr, Rohwer F (2006) Using pyrosequencing to shed light on deep mine microbial ecology. BMC Genomics 7:57

Eisen JA (1998) A phylogenomic study of the MutS family of proteins. Nucleic Acids Res 26:4291–4300

Figueiredo MVB, Seldin L, Araujo FF, Mariano RLR (2011) Plant growth promoting rhizobacteria: fundamentals and applications. Plant Signal Behav 6(10):1554–1563

Hamayun M, Khan SA, Ahmad N et al (2009) *Cladosporium sphaerospermum* as a new plant growth-promoting endophyte from the roots of Glycine max (L.) Merr. World J Microbiol Biotechnol 25:627–632

Hartmann A, Rothballer M, Schmid M (2008) Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant Soil 312:7–14

Hiltner L (1904) Uber neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderden berucksichtigung und Brache. Arb Dtsch Landwirtsch Gesellschaft 98:59–78

Hirsch PR, Mauchline TH (2012) Who's who in the plant root microbiome? Nat Biotechnol 30:961–962

Hugenholtz P, Tyson GW (2008) Microbiology - metagenomics. Nature 455:481–483

Ignatova LV, Brazhnikova YV, Berzhanova RZ, Mukasheva TD (2015) Plant growth-promoting and antifungal activity of yeasts from dark chestnut soil. Microbiol Res 175:78–83

Islam S, Akanda AM, Sultana F, Hossain MM (2014) Chilli rhizosphere fungus Aspergillus spp. PPA1 promotes vegetative growth of cucumber (Cucumis sativus) plants upon root colonisation. Arch Phytopathol Plant Protect 47:1231–1238

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329

Karimi K, Amini J, Harighi B, Bahramnejad B (2012) Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against fusarium wilt of chickpea. Aust J Crop Sci 6(4):695

Kent AD, Triplett EW (2002) Microbial communities and their interactions in soil and rhizosphere ecosystems. Annu Rev Microbiol 56:211–236

Klironomos JN, Moutogolis P, Kendrick B, Widden PS (1993) A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils. Can J Bot 71:1472–1480

Kloepper JW (1994) Plant growth-promoting rhizobacteria (other systems). In: Okon Y (ed) Azospirillum/plant associations. CRC Press, Boca Raton, pp 111–118

Lacava PT, Li WB, Araujo WL, Azevedo JL, Hartung JS (2006) Rapid, specific and quantitative assays for the detection of the endophytic bacterium Methylobacterium mesophilicum in plants. J Microbiol Methods 65(3):535–541

Landa BB, Hervás A, Bettiol W, Jiménez-Díaz RM (1997) Antagonistic activity of bacteria from the chickpea rhizosphere against *Fusarium Oxysporum* f. sp. *ciceris*. Phytoparasitica 25 (4):305–318

Landa BB, Navas-Cortés JA, Hervás A, Jiménez-Díaz RM (2001) Influence of temperature and inoculum density of *Fusarium oxysporum* f. sp. *ciceris* on suppression of Fusarium wilt of chickpea by rhizosphere bacteria. Phytopathology 91:807–816

Linderman RG (1991) Mycorrhizal interactions in the rhizosphere. In: Keister DL, Cregan PB (eds) The Rhizosphere and plant growth. Kluwer Academic Publishers, Dordrecht, pp 343–348

Loy A, Lahner A, Lee N, Adamczyk J, Meier H, Ernst J, Schleifer KH, Wagner M (2002) Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. Appl Environ Microbiol 68:5064–5081

Ma Y, Rajkumar M, Freitas H (2009) Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J Environ Manag 90:831–837

Margulies M, Egholm M, Altman WE et al (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380

Moosa A, Shahbaz TS, Haq IU, Farzand A, Khan SA, Javaid K (2017) Antagonistic potential of *Trichoderma* isolates and manures against Fusarium wilt of tomato. Int J Veg Sci 23(3):207–218

Murray AE, Lies D, Li G, Nealson K, Zhou J, Tiedje JM (2001) DNA–DNA hybridization to microarrays reveals gene-specific differences between closely related microbial genomes. Proc Natl Acad Sci U S A 98:9853–9858

Muslim A, Horinouchi H, Hyakumachi M (2003) Control of Fusarium crown and root rot of tomato with hypovirulent binucleate Rhizoctonia in soil and rock wool systems. Plant Dis 87:739–747

Myatt PM, Dart PJ, Hayward AC (1993) Potential for biological control of Phytophthora root rot of chickpea by antagonistic root-associated bacteria. Aust J Agric Res 44(4):773–784

Nangia Y, Wangoo N, Goyal N, Shekhawat G, Suri CR (2009) A novel bacterial isolate *Stenotrophomonas maltophilia* as living factory for synthesis of gold nanoparticles. Microb Cell Factories 8:39–45

Narozna D, Pudełko Kroliczak KJ, Golinska B, Sugawara M, Cezary J et al (2014) Survival and competitiveness of *Bradyrhizobium japonicum* strains 20 years after introduction into field locations in Poland. Appl Environ Microbiol 81:5551–5559

Nautiyal CS (1997) Rhizosphere competence of pseudomonas sp. NBRI9926 and rhizobium sp. NBRI9513 involved in the suppression of chickpea (*Cicer arietinum* L.) pathogenic fungi. FEMS Microbiol Ecol 23(2):145–158

Oldroyd GE, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144

Oliveira AC, Vallim MA, Semighini CP, Araujo WL, Goldman GH, Machado MA (2002) Quantification of Xylella fastidiosa from citrus trees by real-time polymerase chain reaction assay. Phytopathology 92(10):1048–1054

Orlandini V, Emiliani G, Fondi M, Maida E, Perrin E, Fani R (2014) Network analysis of Plasmidomes: the Azospirillum Brasilense Sp245 case. Hindawi Publishing Corporation, London, pp 1–14

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y et al (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207–211

Prathap M, Ranjith KBD (2015) A critical review on plant growth promoting rhizobacteria. J Plant Pathol Microbiol 6(4):1–4

Rainey AF, Nobre MF, Schumann P, Stackebrandt E (1997) Phylogenetic diversity of the *Deinococci* as determined by 16S ribosomal DNA sequence comparison. Int J Syst Bacteriol 47:510–514

Renuka S, Ramanujam B (2016) Fungal endophytes from maize (Zea mays L.): isolation, identification and screening against maize stem borer, Chilo partellus (Swinhoe). J Pure Appl Microbiol 10(1):523–529

Roesch LFW, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, Daroub SH, Camargo FAO, Farmerie WG, Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 1:283–290

Rovira AD (1969) Plant root exudates. Bot Rev 35:35–37. https://doi.org/10.1007/bf02859887

Ruppel S, Ruhlmann J, Merbach W (2006) Quantification and localization of bacteria in plant tissues using quantitative real-time PCR and online emission fingerprinting. Plant Soil 286 (1–2):21–35

Schoch CL, Seifert KA, Robert V et al (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci 109(16):6241–6246

Siefert JL, Fox GE (1998) Phylogenetic mapping of bacterial morphology. Microbiology 144:2803–2808

Singh RK, Kumar DP, Singh P et al (2014) Multifarious plant growth promoting characteristics of chickpea rhizosphere associated Bacilli help to suppress soil-borne pathogens. Plant Growth Regul 73(1):91–101

Sogin ML, Morrison HG, Huber JA et al (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci U S A 103(32):12115–12120

Sourath E, Subramanian S (2014) Synthesis and characterization of silver nanoparticles by a soil isolate of *Pseudomonas aeruginosa* and their potential antimicrobial property. Green Chem 1:182–186

Suryadi Y, Susilowati DN, Fauziah F (2019) Management of plant diseases by PGPR-mediated induced resistance with special reference to tea and rice crops. J Exp Bot 64:3829–3842

Voordouw G (1998) Reverse sample genome probing of microbial community dynamics. ASM News 64:627–633

Waqas M, Khan AL, Hamayun M et al (2015) Endophytic fungi promote plant growth and mitigate the adverse effects of stem rot: an example of *Penicillium citrinum* and *Aspergillus terreus*. J Plant Interact 10:280–287

Weller DM, Thomashow LS (1994) Current challenges in introducing beneficial microorganisms into the rhizosphere. In: O'Gara F, Dowling DN, Boesten B (eds) Molecular ecology of rhizosphere microorganisms biotechnology and the release of GMOs. VCH Verlagsgesellschaft, Weinheim, pp 1–18

Whipps J, Hand P, Pink D, Bending GD (2008) Phyllosphere microbiology with special reference to diversity and plant genotype. J Appl Microbiol 105(6):1744–1755

Widder S, Allen RJ, Pfeiffer T, Curtis TP, Wiuf C, Sloan WT, Shou W (2016) Challenges in microbial ecology: building predictive understanding of community function and dynamics. ISME J 10(11):2557

Woese CR, George EF (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci U S A 74:5088–5090

Wright SF, Upadhyaya A (1996) Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci 161:575–586

Wright SF, Franke-Snyder M, Morton JB, Upadhyaya A (1996) Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil 181:193–203

Wu L, Thompson D, Li G, Hurt RA, Tiedje JM, Zhou J (2001) Development and evaluation of functional gene arrays for detection of selected genes in the environment. Appl Environ Microbiol 67:5780–5790

Xia Y, Sahib MR, Amna A, Opiyo SO, Zhao Z, Gao YG (2019) Culturable endophytic fungal communities associated with plants in organic and conventional farming systems and their effects on plant growth. Sci Rep 9(1):1669

Yedidia I, Srivastva AK, Kapulnik Y, Chet I (2001) Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil 235:235–242

Yilmaz P, Gilbert JA, Knight R, Amaral-Zettler L, Karsch-Mizrachi I, Cochrane G et al (2011) The genomic standards consortium: bringing standards to life for microbial ecology. ISME J 5:1565–1567

Zhang T, Fang HH (2006) Applications of real-time polymerase chain reaction for quantification of microorganisms in environmental samples. Appl Microbiol Biotechnol 70(3):281–289

Zhou Z, Zhang C, Zhou W et al (2014) Diversity and plant growth-promoting ability of endophytic fungi from the five flower plant species collected from Yunnan, Southwest China. J Plant Interact 9:585–591

Zimmerman NB, Vitousek PM (2012) Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proc Natl Acad Sci 109(32):13022–13027

Rhizospheric Microbial Communities: Occurrence, Distribution, and Functions

12

Vikram Poria, Surender Singh, Lata Nain, Balkar Singh, and Jitendra Kumar Saini

Abstract

Plant–microbe interactions are crucial for many ecological processes. These interactions majorly take place in the rhizosphere and are mediated by the secretion of organic compounds by plant roots. These compounds act as signalling molecules and also as carbon sources for microbes. Microbial community in the rhizosphere is very diverse and consists of bacteria, archaebacteria, viruses, fungi, actinomycetes, protozoans, arthropods, algae, and nematodes. The rhizospheric microbes promote plant growth by different mechanisms such as biocontrol activity, phytohormone secretion, siderophore production, mineral solubilization, nitrogen fixation, and enzyme production. Since, a large proportion of microbial diversity is still not cultured, the detection and phylogenetic characterization of such un-/non-cultured microorganisms require advanced molecular techniques viz. metagenomics, metabolomics, metatranscriptomics, and metaproteomics. Several factors affect the rhizosphere microbial population, including root exudates, type and age of the plant, status of plant health, and application of fertilizers, pesticides, and amendments. Plant growth-promoting microbes of the rhizosphere can be used as biofertilizers and biocontrol agents and rhizosphere competence is an important factor that determines their success. This chapter discusses all these aspects of rhizospheric microbial communities, especially their occurrence, distribution, and functions.

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Rhizosphere · Rhizosphere engineering · Metagenomics · Microbial communities · PGPR · Rhizosphere competence

12.1 Introduction

The interplay between plants and microorganisms play a crucial role in ecosystem processes, such as nutrient cycling. These interactions benefit both plants and microbes. The beneficial impact of these interactions on plant includes increased nutrient availability, protection against diseases, and increased tolerance to biotic and abiotic stresses. The plant secretes different root exudates which act as signalling molecules and also as substrates for many microbes. The rhizosphere is the zone where all these interactions take place. About 10–60% of the carbon which is fixed by the process of photosynthesis is released as root exudates from the plant as soluble sugars, amino acids, or products of plant secondary metabolism for the benefit of microbes (Prashar et al. 2014).

The rhizospheric microorganisms interact with the roots of the plants, soil, and other microbes in many ways and are crucial for the growth and development of plants. Plants can shape the microflora according to their needs by changing the composition of the exudates secreted by their roots (Meena et al. 2017). The rhizospheric microbial community is very diverse and the various groups present in the rhizosphere include fungi, virus, bacteria, nematodes, etc. Various microbes from the rhizosphere show beneficial activities in terms of the promotion of growth and development of plants. Therefore, the use of these beneficial rhizospheric microbes as biofertilizers can be a very important environment-friendly alternative for sustainable crop production (Dubey et al. 2016).

Rhizospheric microbial communities are very complex and affect plant health, and hence it is very important to study their interactions and exact role in the rhizosphere. Many advanced molecular techniques including metagenomics, metabolomics, metatranscriptomics, and metaproteomics are now employed routinely to gain information about the rhizospheric microbes interacting with the plants.

12.2 The Rhizosphere: History and Scope

The term "rhizosphere" (meaning "root and environment of influence") was first defined by Hiltner (1904), as "the compartment of soil which is influenced by plant roots" and is the site of interaction of roots, soil microbiota, and soil.

There are three separate zones in the rhizosphere: the endo- and ecto-rhizosphere and the rhizoplane (Fig. 12.1). The ecto-rhizosphere consists of soil in close contact with the roots of the plant, whereas the endorhizospheric zone majorly includes the inner tissue as well as the endodermis and cortical layers of the root. The

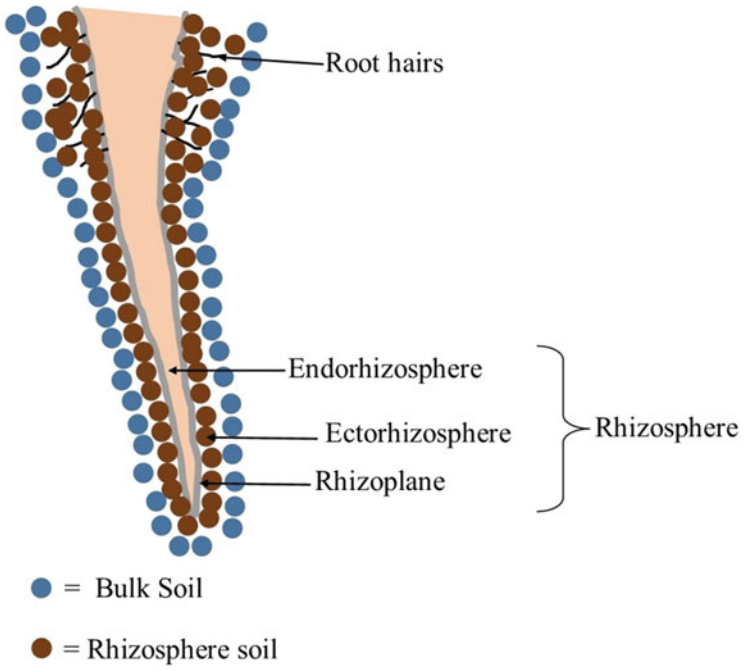

Fig. 12.1 The diagrammatic representation of the three separate zones in the rhizosphere

intermediate zone, i.e. the rhizoplane, includes the root surface and harbors most of the microorganisms and includes the mucilage and epidermal layers as well as the cortex. The physico-chemical soil characteristics are directly or indirectly affected by the interaction between root, soil, and rhizospheric microbes which ultimately change the rhizospheric microbial population (Huang et al. 2014; Shaikh et al. 2018).

The quantity of the microorganisms present in the rhizosphere is far greater than that in the non-rhizospheric soil since different carbon sources as well as other nutrients are present in higher quantities in the rhizosphere and the inhabiting microbes utilize these as energy sources. These organic compounds are deposited in the rhizosphere by plant roots. The rhizosphere is also the location of the interaction of soil-borne pathogens and plant roots. These pathogens compete with rhizospheric microbes for space and nutrients to cause infection the plants. In disease suppressive soil, the growth of the pathogens is suppressed by the rhizosphere microbes. Thus, the interaction between pathogens, rhizosphere microbes, and plant roots are key elements in shaping plant protective microbiome (Chapelle et al. 2016).

12.3 Structure and Abundance of Microbial Groups in the Rhizosphere

Rhizosphere harbors all types of microorganisms—bacteria, archaebacteria, viruses, fungi, actinomycetes, protozoans, arthropods, algae, and nematodes. All types of microbe–microbe and plant–microbe interactions occur in the rhizosphere. Rhizospheric soil contains up to 10^{11} microbial cells/gram of root (White et al. 2017). The approximate number of bacteria in the rhizosphere is 1.2×10^8 per gram of dry soil which is very high as compared to other groups of microbes. The number (Ahmad and Baker 1988) of fungi, algae, and actinomycetes is 12×10^5, 5×10^5, and 4.6×10^7, respectively (Shaikh et al. 2018). A summary of the most abundant members of different groups of microorganisms is provided in Table 12.1.

12.3.1 Bacteria

Different bacterial species are found in the rhizosphere depending on the root zone, plant health, and growth phase of the plant. *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Acidobacteria*, and *Bacteroidetes* dominate in the rhizospheric zones of different field crops, horticultural crops, and conifer plantations. *Proteobacteria* constitute the most abundant group followed by *Acidobacteria* (Lagos et al. 2015).

The high nutrient availability in the rhizosphere generally promotes bacterial species, like *Pseudomonas* sp., which are a fast-growing and opportunistic species. However, few studies have also shown that the *Actinobacteria* and *Coryneform* and not the Pseudomonads are the dominating bacteria in the rhizosphere of different plants belonging to family Gramineae (Miller et al. 1989, 1990).

Lee et al. (2015) determined the distribution of bacteria in general as well as some specific bacteria, such as archaebacteria, methanotrophic-, and methanogenic bacteria using pyrosequencing of 16S rRNA, in both the rhizospheric as well as bulk soil

Table 12.1 List of most abundant members of different groups of microorganisms in the rhizosphere

S. No.	Groups	Most abundant Genus/class/phylum
1.	Bacteria	*Pseudomonas* sp., *Bacillus* sp., *Arthrobacter* sp., *Rhizobia* sp., *Agrobacterium* sp., *Alcaligenes* sp., *Azotobacter* sp., *Mycobacterium* sp., *Flavobacter* sp., *Cellulomonas* sp. and *Micrococcus* sp.
2.	Actinobacteria	*Streptomyces* sp., *Micromonospora* sp., and *Nocardia* sp.
3.	Fungi	Ascomycota and Basidiomycota
4.	Arbuscular mycorrhizal fungi	*Glomus* sp., *Acaulospora* sp., *Gigaspora* sp.
5.	Virus	Myoviridae, Podoviridae, and Siphoviridae
6.	Archaebacteria	Crenarchaeota, Euryarchaeota, Thaumarchaeota

of paddy fields flooded with water. In the case of bacteria, major phyla at all depths in both the soils included, *Proteobacteria*, *Cyanobacteria*, *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, and *Firmicutes*. With the depth gradient of both the soils, the relative abundance of *Chloroflexi*, *Acidobacteria*, and *Actinobacteria* increased and that of *Cyanobacteria* and *Bacteroides* decreased. Archaebacteria belonging to phylum *Euryarchaeota* were found predominantly in both the soils at all depths.

Bacteria belonging to groups like *Actinobacteria*, *Firmicutes*, *Acidobacteria*, *Verrumicrobiaceae*, and *Chloroflexi* were isolated from *Jatropha curcas* rhizosphere (Dubey et al. 2016). The analysis of the bacterial population of the rhizosphere of the wild and domesticated barley using 16S rRNA pyrosequencing proved *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* to be the dominating ones (Bulgarelli et al. 2015). The rhizosphere and the non-rhizospheric soils of *Morus alba* commonly had the species of *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Ensifer*, *Flavobacterium*, and *Brevibacillus* (Zhang et al. 2016). However, some bacteria were specific to the rhizosphere soil of *Morus alba*. These bacteria include the species of *Stenotrophomonas*, *Burkholderia*, *Acinetobacter*, *Sphingobium*, and *Variovorax*. Bacteria such as *Cupriavidus*, *Microbacterium*, *Sinomonas*, and *Agrobacterium* were only found in bulk soils.

Gaya Karunasinghe et al. (2020) isolated salt-tolerant *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Alcaligenes faecalis* from the rhizosphere soil of tomato and checked their antagonism against *Pythium aphanidermatum.* Among these isolates, *Serratia marcescens* showed the highest activity and suppressed the disease by 68%.

Besides supporting the growth and development of associated plants, the rhizospheric microorganisms also enhance the rate of phytoremediation of heavy metals in the rhizospheric region by both direct and indirect pathways. Phytoremediation through direct processes involves phytostabilization or phytoextraction and through indirect promotion involves plant metal tolerance conferred by microbes. Wang et al. (2020) have found that rhizosphere bacteria in mining areas assist indigenous weeds as these can accumulate or exclude the heavy metals. Among all the weeds (*Cyperus difformis*, *C. iria*, *Digitaria sanguinalis*, *Echinochloa crusgalli*, *Fimbristylis miliacea,* and *Ludwigia prostrata*), the highest accumulation of copper, lead, and zinc occurred in the leaves and stems of *L. prostrata*. However, cadmium accumulation in the roots of *Ludwigia prostrata* was found lower than that in *D. sanguinalis* and *F. miliacea*. Highest Cd accumulation was recorded in *D. sanguinalis*. The bacterial diversity in the rhizosphere followed the order *C. difformis* (highest diversity) > *E. crusgalli* > *D. sanguinalis* > *L. prostrata* > *C. iria* > *F. miliacea*.

Cordero et al. (2019) isolated microbes from the rhizoplane and the rhizosphere of lentil, wheat, field pea, and canola and reported that the rhizosphere bacterial communities varied depending on the crops and sampling site location whereas root interior bacterial communities varied with plant species only. *Acidobacteria*, *Firmicutes*, and *Gemmatimonadetes* dominated the rhizosphere soil. The root interior of all crops was dominated by *Acinetobacter* sp., *Arthrobacter* sp., *Rhizobium*

sp., *Streptomyces* sp., *Variovorax* sp., and *Xanthomonas* sp. *Pseudomonas* sp., and *Stenotrophomonas* sp. were present in both the rhizosphere and interior of the root. In another study, 15 rhizospheric bacteria from tomato were found to possess at least one of the tested plant growth-promoting (PGP) activities, such as antibiotic resistance, P-solubilization, amylase activity, IAA production, etc. (Sunera et al. 2020). Two selected bacteria, *Klebsiella variicola* and *Bacillus cereus* increased the mineral (K, Fe, Cu, Zn, etc.,) uptake after their application on tomato and mung bean plants. Imriz et al. (2020) investigated the rhizosphere bacteria of wheat and barley for their biocontrol activity against fungal pathogen *Fusarium culmorum* using dual culture technique. From 463 isolates, only 31 showed the biocontrol activity against *F. culmorum*. In another study, Rana et al. (2011) isolated 100 bacteria from the rhizosphere of wheat and based on screening for different PGP attributes found that the species of *Bacillus*, *Providencia*, and *Brevundimonas* were most efficient in improving wheat growth under controlled conditions. Further, Rana et al. (2012) also demonstrated that the use of *Providencia* sp. and *Anabaena* sp. could help save half of the N-fertilizer and at the same time improving the protein content of wheat by 18.6%.

12.3.2 Fungi

Diverse kinds of fungi, both beneficial and harmful, are present in the rhizosphere. The diversity of the rhizospheric fungi depends on the type of plant, its health status, the characteristics of root exudates, as well as the presence of antagonists. Mahamuni et al. (2012) isolated *Aspergillus niger*, *Aspergillus awamori*, *Aspergillus fumigatus*, *Alternaria alternata*, *Curvularia pallescens*, *Penicillium oxalicum*, *Penicillium rubrum,* and *Trichoderma viride* from the rhizosphere of sugarcane and sugar beet, which were capable of solubilizing phosphate from soil. Different fungi belonging to Ascomycota and Basidiomycota were isolated from the rhizosphere of soybean in a continuous cropping system (Bai et al. 2015). Sugiyama et al. (2014) analyzed rhizospheric soil of soybean using pyrosequencing and reported that the rhizospheric microflora changes with the growth stage of the plant. Ascomycota group of fungi were dominant in all types of soil (Moshiri et al. 2019) followed by Basidiomycota.

Gqozo et al. (2020) investigated the fungal diversity in the bulk and rhizosphere soil of wheat using next-generation sequencing and found Ascomycota to be the dominating phylum in the rhizosphere soil followed by Basidiomycota. Among Ascomycota, members of classes Sordariomycetes, Dothideomycetes, Eurotiomycetes, and Orbiliomycetes were found to be the dominating. The prevalent genera were *Fusarium*, *Aureobasidium*, and *Colletotrichum*. Agaricomycetes and Tremellomycetes belonging to class Basidiomycota.

Manici and Caputo (2020) investigated the effect of binucleate *Rhizoctonia* sp., which is the anamorphic stage of *Ceratobasidium* sp., on the growth of apple plants. Binucleate *Rhizoctonia* after colonizing the rhizosphere increased the fresh and dry shoot biomass and also helped the plant to mitigate water stress. Temperature or the

season also influenced the fungal population. Ascomycota was found to dominate during summer and Basidiomycota during winter in the rhizosphere soil of *Coptis chinensis* fields which were not cropped for more than 3 years (Alami et al. 2020).

Gao et al. (2019) investigated how the continuous cropping of sweet potato affected its rhizosphere fungal community and found a significant increase in the diversity and richness of the fungi. Ascomycota dominated the rhizosphere soil which decreased after continuous cropping. The number of pathogenic fungi belonging to *Verticillium*, *Colletotrichum*, *Fusarium,* etc. increased whereas that of beneficial fungi such as *Chaetomium* sp. decreased. The increased number of pathogenic fungi led to decreased yield and quality of sweet potato.

Salinity also affects rhizospheric fungal communities. The effect of salinity was investigated on the rhizospheric fungal population of the halophytic black mangrove, *Avicennia germinans,* from a semi-arid mangrove. Samples were taken from three different sites having different salinity (23.2%, 14.61%, and 2.8%). *Aspergillus* sp., *Saitozyma* sp., *Trichoderma* sp., *Podosphaera* sp., and *Cystofilobasidium* sp. were dominant in samples from high salt containing location (23.2%) while *Penicillium*, *Trichoderma*, *Cystobasidium*, and *Aspergillus* dominated the samples taken from lowest salinity conditions (2.8%). Genus *Amorosia*, *Phaeoacremonium*, *Aspergillus*, *Talaromyces*, and *Trichoderma* were prevalent in the samples having 14.61% salinity. *Aspergillus* sp. was found to be present in all three levels of salinity (Vanegas et al. 2019).

In another study, Kazerooni et al. (2017) compared the diversity of tomato rhizosphere fungi under the conventional as well as desert farming systems. They used two different techniques, pyrosequencing and culture-based technique for this purpose. Culture-based techniques revealed that in both conventional and desert farming systems, Ascomycota was found to be the most abundant phylum. Zygomycota and Oomycota were found only in desert farming and conventional farming, respectively. *Aspergillus* was the most abundant genera in both farming systems. Pyrosequencing methods indicated that Microsporidia was the most abundant taxa in the conventional farming system followed by Ascomycota, Chytridiomycota, Basidiomycota, and Zygomycota. In the desert farming system, Ascomycota was found to be the most abundant taxa. Zygomycota and Chytridiomycota were absent in the desert farming system.

12.3.3 Arbuscular Mycorrhizal Fungi (AMF)

AMFs are present as symbionts of most of the higher plant roots, except the members of Cruciferae, Chenopodiaceae, and Caryophyllaceae families, and cover nearly about 80% of plant's root. Plant roots provide photosynthetically fixed carbon to AMF and obtain mineral nutrients in return. AMFs are divided into two major groups: (a) ectomycorrhiza and (b) endomycorrhiza. Ectomycorrhiza have dense mycelial sheath invading the root cortex and are limited to most temperate forest trees; while endomycorrhiza form external hyphal networks in the soil and grow extensively within the cells of the root cortex of most of the field crops. In addition to

improving plant nutrition, AMF provides resistance to plant against soil-borne pathogenic microorganisms and insects feeding on areal parts, drought, salinity, and heavy metals as well as in improving soil aggregate stability (Tripaldi et al. 2017).

Various species of AMF have been reported from different plants with *Glomus*, *Acaulospora*, *Gigaspora* being the most common one. Jefwa et al. (2012) isolated 22 AMFs from the rhizospheric zone of banana and plantain. These fungi belonged to *Acaulospora* sp., *Archaeospora* sp., *Glomus* sp., *Scutellospora* sp., and *Gigaspora* sp., and the highest abundance in the banana rhizosphere was of *Acaulospora scrobiculata*. Whereas, the most abundant species of AMF in the rhizosphere of soybean were *Glomus fasciculatum* and *Glomus mosseae* (Danesh et al. 2006). In a study, Kumalawati et al. (2014) isolated different AMF from the rhizospheric region of sugarcane belonging to the genus, *Glomus*, *Acaulospora*, *Gigaspora,* and *Sclerocystis*. They also found that two genera, *Glomus* and *Gigaspora,* have similar abundance and spore characteristics indicating their widespread capability to associate with sugarcane.

AMF increases the plant efficiency for the absorption of phosphate from the soil solution and also increase the growth of plants under salt stress. Phosphate solubilizing rhizobacteria solubilize phosphate which is absorbed and transferred by mycelium of external arbuscular mycorrhiza. In a study, seeds of *Kosteletzkya virginica* were inoculated with *Glomus mosseae* and *Glomus aggregatum*, both from saline soil and *Mortierella* sp. which was first grown on solid Martin culture media. Co-inoculation of AMF and *Mortierella* sp. enhanced the root colonizing efficiency under saline conditions (Zhang et al. 2011).

Hyphae and spores of AMF secrete glomalin protein in the soil from which it can be estimated quantitatively as "glomalin-related soil protein (Wu et al. 2015a)." It plays an important role in carbon storage, soil aggregation, carbon storage, and stress tolerance. AMF is also a source of different soil enzymes (Wu et al. 2015a). Root exudates and mycorrhiza act as sources of energy to the rhizosphere microbiota, which secrete extracellular enzymes for degrading soil organic matter (Shahzad et al. 2015). Synergism of AMF and *Bradyrhizobium* with beneficial rhizospheric microbes has also been found to increase nitrogen and phosphorous acquisition in soybean and improve the rhizospheric environment (Meena et al. 2018).

The diversity of AMF is correlated with plant diversity rather than bacterial diversity in soil. Bi et al. (2020) isolated AMF from the rhizosphere of *Leymus chinensis*, *Calamagrostis rigidula*, *Lespedeza hedysaroides*, *Vicia amoena*, *Carex* sp., and *Artemisia* sp. to check the specificity and diversity of AMF in the Sonnen grassland of China. A total of 24 species belonging to six different genera (*Acaulospora* sp., *Glomus* sp., *Gigaspora* sp., *Pacispora* sp., *Racocetra* sp., and *Rhizophagus* sp.) were isolated, among which *Glomus* sp. and *Acaulospora* sp. dominated. *Glomus* sp. also dominated in the rhizosphere of all plants except *Artemisia lavandulaefolia,* in which *Acaulospora laevis* was the most dominating species.

In an interesting experiment of tomato-potato onion intercropping, Gao et al. (2020) found that the AMF abundance in the rhizosphere of tomato was increased by

intercropping, whereas that in case of potato onion declined (*Allium cepa* var. aggregatum). Phosphorus fertilization moderated these effects and was found to be the key factor in driving the AMF communities. When phosphorous fertilizers are applied in a higher amount, the AMF root colonization was negatively affected but the moderate application of phosphorous stimulated the AMF root colonization. AMF species namely *Diversispora*, *Archaeospora*, and *Paraglomus* were found in soil where no phosphorous fertilization was done, while phosphorous fertilized soil was dominated by *Glomus*.

AMF is also crucial for the nitrogen cycle as AMF competes with ammonia oxidizers for ammonium ion (NH_4^+). Wattenburger et al. (2020) examined the abundance of ammonia-oxidizing bacteria and archaebacteria in the rhizosphere and bulk soil of corn under conventional cultivation and diversified cultivation systems and found it to be significantly affected by the cropping system and rhizosphere, but not by AMF in nitrogen-enriched soil.

Jamiołkowska et al. (2019) concluded that when AMF *Claroideoglomus etunicatum* was inoculated on tomato plants, it directly affected the rhizosphere population of fungi and increased the number of saprotrophs. A total of 3086 fungal colonies were isolated from the tomato rhizosphere during a 3-year mycological analysis using Warcup's method. These fungi belonged to 42 different genera mainly dominated by *Fusarium*, *Mucor*, *Penicillium*, and *Trichoderma.*

Plant hormones strigolactones are released from roots which induce branching in AMF. In a study, Carvalhais et al. (2019) reported that the fungal rhizosphere community was affected by the release of strigolactones from the roots of *Arabidopsis thaliana*. However, no effect on the bacterial rhizosphere community was observed. Fungi attracted to the roots due to the release of strigolactones comprise mainly, *Epicoccum* sp., *Penicillium* sp., *Fibulochlamys* sp., *Herpotrichiellaceae* sp., *Mycosphaerella* sp., and *Mycosphaerellaceae* sp.

12.3.4 Viruses

Viruses present in soil are of great ecological importance. Viruses can transfer genes from host to host and potentially cause microbial mortality, which affects microbial evolution in the rhizosphere. Rhizosphere has a high population of various kinds of microorganisms is high compared to bulk soil which can be linked to high viral diversity and abundance in the rhizosphere. The viral abundance of different soils can be enumerated using plaque assay, epifluorescence microscopy (EFM), and transmission electron microscopy (TEM) and it is reported that around 10^8 virus particles are present per gram dry weight of soil (Williamson et al. 2003). The viral particles are most abundant in forests and wetlands followed by cold deserts, fields, and agricultural soils. The lowest abundance of viral particles is found in hot deserts. In the soil systems, tailed phages belonging to Myoviridae, Podoviridae, and Siphoviridae (Pratama et al. 2020) are more abundant. Swanson et al. (2009) took different samples from the rhizosphere of *Triticum aestivum* and analyzed the presence of different virus particles in the samples. The majority of tailed phages

isolated belonged to family *Podoviridae,* whereas members of family *Myoviridae* and *Siphoviridae* were almost equal in numbers. Different spherical viruses, filamentous particles, rod-shaped type viruses, and bacilliform particles were also found in the rhizospheric samples.

Similarly, Cubo Sánchez et al. (2020) studied the virulent phage diversity of the *Medicago marina* rhizosphere, using seven different strains of bacteria *Sinorhizobium meliloti* as a host for viruses. Eight new sinorhizobiophage lytic phages were isolated from the rhizosphere. These viruses belonged to family Myoviridae, Syphoviridae, Podoviridae, and Inoviridae. Berrios and Ely (2019) isolated the Kronos virus from the rhizosphere of the dichotomous plant by using *Caulobacter* wild type strain. Hence, the phage numbers and diversity depend on the type of bacterial/fungal species present in the rhizosphere.

12.3.5 Archaebacteria

The diversity of archaebacteria in the rhizosphere is less known when compared to bacteria due to a limited number of such studies. Archaebacteria can be crucial for plant survival by transforming soil mercury by reduction, methylation, and demethylation (Ma et al. 2019). In the recent past, many studies have explored the archaeal diversity of rhizosphere of many crops, such as *Jatropha curcas* (Dubey et al. 2016), rice fields (Srivastva et al. 2018; Ma et al. 2019), rhizosphere of *Suaeda nudiflora* and Banni grass (Yadav et al. 2019), and tomato rhizosphere (Taffner et al. 2020), etc.

The rhizosphere bacterial and archaebacterial diversity associated with *J. curcas* was explored by employing terminal restriction fragment length (T-RFLP) and targeted the 16S rDNA region. Bacterial indicative terminal restriction fragments were *Actinobacteria*, *Firmicutes*, *Acidobacteria*, *Verrumicrobiaceae*, and *Chloroflexi* while the archaeal terminal restriction fragments were majorly crenarchaeota and euryarchaeota (Dubey et al. 2016). Ma et al. (2019) isolated archaebacteria from the rhizosphere of the rice fields containing mercury gradient and reported that Thaumarchaeota was prevalent in the rhizosphere as well as non-rhizospheric soil of rice fields, followed by Crenarchaeota and Euryarchaeota. *Methanolobus tindarius*, *Methanomethylovorans hollandica*, *Methanospirillum hungatei*, *Methanomassiliicoccus luminyensis*, *Methanocorpusculum bavaricum*, *Methanofollis liminatans*, *Methanosphaerula palustris*, and *Methanocella paludicola* are the well-known archaebacteria which methylate mercury. These are all known to possess the *hgcAB* gene cluster which is linked to mercury methylation. In another study, archaebacterial diversity and abundance of nitrogen fertilized rice fields were studied and it was reported that the *Methanocellales*, *Methanomicrobiaceae*, *Methanobacteriaceae*, *Methanisarcinaceae*, and *Methanosaetaceae* were found in all types of soils but their abundance varied with the type of soil (Srivastva et al. 2018). Yadav et al. (2019) isolated archaebacteria from different plant rhizospheres in Rann of Kutch and reported that the culturable archaebacterial diversity associated with the rhizospheres of *Suaeda nudiflora* and

Banni grass were maximum and minimum, respectively, with a seasonal fluctuation in their number and genera. The amplification and sequencing of the16s rDNA region showed that 16 different genera, majorly of halophilic archaea were found during all the seasons. Taffner et al. (2020) isolated archaea from the tomato rhizosphere and found Thaumarchaeota and Euryarchaeota as dominated rhizospheric archaebacterial groups.

12.4 Plant Growth-Promoting (PGP) Rhizobacteria and the Rhizosphere

Microbes that are present in the rhizosphere soil can secrete phytohormones (indole acetic acid, gibberellins, siderophores), enzymes, and antibiotics, and solubilize minerals, thereby, improving the growth and developments of plants. Different plant beneficial rhizobacteria like *Pseudomonas* sp., *Azospirillum* sp., *Azotobacter* sp., *Bacillus* sp., *Burkholderia* sp., *Enterobacter* sp., *Rhizobium* sp., *Erwinia* sp., *Mycobacterium* sp., *Mesorhizobium* sp., *Flavobacterium* sp., etc., have been reported from the rhizosphere of different crop and wild plants (Table 12.2).

12.4.1 Antagonistic Plant Growth-Promoting (PGP) Rhizobacteria

Different mechanisms are employed by rhizospheric microbes to control the phytopathogens including the production of antibiotic, bacteriocin, siderophore, volatile and low molecular weight organic compounds, hydrolytic enzymes (e.g., chitinases and glucanases, etc.), phytoalexins (Sindhu et al. 2016) as well as induction of systemic response. Therefore, many microbes in the rhizosphere act as antagonists of the pathogenic macro- and microorganisms and enhance crop productivity.

Research by Zhao et al. (2018) showed that five bacterial strains, namely *Bacillus cereus*, *B. subtilis*, *Pseudomonas putida*, *P. fluorescens*, and *Serratia proteamaculans,* isolated from fields of cucumber, tomatoes, and other crops, showed antagonistic activity against root-knot disease-causing nematode. *Serratia proteamaculans* was the superior bacteria, causing more than 99% and 61% mortality in *Meloidogyne incognita* juveniles and eggs, respectively, resulting in the highest root and shoot growth during pot experiment.

Biocontrol activity of bacteria *Bacillus amyloliquefaciens* Ba13 against yellow leaf curl virus disease in tomato was reported by Guo et al. (2019). It was mediated by the induction of systemic response against virus and also influenced the rhizospheric microbial community, by decreasing the number of pathogenic *Fusarium solani,* and *F. oxysporium*. Actinomycete *Streptomyces pactum* was also reported for its biocontrol activity against the leaf curl virus by Li et al. (2019). Figueroa-López et al. (2016) isolated bacteria viz. *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Lysinibacillus* from maize rhizosphere which exhibited biocontrol activity against *Fusarium verticillioides,* the causative agent of rot in maize. Etesami

Table 12.2 Different PGPR isolated from different crops and their PGP attributes

S. No.	Crop	PGPR	PGPR attribute	Reference
1.	Tomato	*Bacillus* sp., *Pseudomonas* sp.,	Biocontrol	Zhao et al. (2018)
2.	Wheat	*Lysinibacillus* sp.	Salt tolerance, IAA production	Damodaran et al. (2019)
3.	*Salicornia* sp.	*Staphylococcus* sp.	Salt tolerance, IAA, and ACC-deaminase production, phosphate solubilization	Razzaghi Komaresofla et al. (2019)
4.	Wheat	*Pseudomonas* sp.	IAA production, P-solubilization	Emami et al. (2019)
5.	Rice	*Brevibacterium sediminis*	Biocontrol, IAA and HCN production, P-solubilization, ammonia generation, chitinase synthesis	Chopra et al. (2020)
6.	Maize	*Enterobacter cloacae*	IAA, and ACC deaminase, and Siderophore production, P-solubilization	Abedinzadeh et al. (2019)
7.	Wheat	*Pseudomonas* sp.	P-solubilization, auxin production, HCN production, siderophore production	Karimzadeh et al. (2020)
8.	*Salicornia bigelovii*	*Streptomyces* sp.	IAA synthesis, generation of ACC deaminase	Mathew et al. (2020)
9.	Wheat	*B. pumilus*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*	P-solubilization, HCN production, Ammonia production, Siderophore production	Kumar et al. (2019)
10.	Maize	*Bacillus* sp.	Salt stress, ethylene metabolism, IAA production	Misra and Chauhan (2020)
11.	Tomato	*Bacillus* sp.	Biocontrol, ammonia production, IAA production, P-solubilization	Pathania et al. (2020)
12.	Soybean	*Streptomyces* sp.	IAA production, P-solubilization	Wahyudi et al. (2019)
13.	Rice	*B. subtilis* *P. fluorescens*	Biocontrol siderophore production, Chitinase production	Karnwal and Mannan (2018)
14.	Chilli	*Bacillus amyloliquefaciens*	Biocontrol, IAA synthesis, Siderophore and ammonia generation, Cellulose production, P-solubilization	Passari et al. (2018)

and Alikhani (2018) tested the antagonism of rhizospheric and endorhizospheric bacteria (majorly *Bacillus* sp.) of rice, clover, and rapeseed, grown under rotation, against five different fungal pathogens belonging to *Magnaporthe* sp., and *Fusarium* sp. The biocontrol activity of microbial agents, thus, depends upon the rhizospheric microbial community also. In a similar study, Gómez-Lama Cabanás et al. (2018)

have reported the biocontrol efficacy of olive rhizosphere inhabiting *Pseudomonas* sp. against *Verticillium* wilt causing pathogen.

12.4.2 Mineral Solubilizers

Rhizospheric microbial population supports the growth and development of plants by solubilizing different minerals in the rhizosphere. Different mineral-solubilizing rhizospheric microbes help in increasing the solubility of macro- and micro-nutrients, such as phosphate, potassium, zinc, silicon, aluminum, iron, etc. (Zhang et al. 2016; Dhaked et al. 2017). Zhang et al. (2016) reported the presence of highly efficient bacteria, capable of solubilizing zinc, silicon, aluminum, and iron, in the rhizosphere soils of *Morus alba*. These bacteria were dominated by *Arthrobacter* sp., *Bacillus* sp., and *Stenotrophomonas* sp., in contrast to *Arthrobacter* sp. and *Pseudomonas* sp. dominating in the bulk soil. Furthermore, *Bacillus licheniformis* isolated from the rhizosphere of different crops showed the highest efficiency for solubilizing phosphate and potassium from tri-calcium phosphate and waste muscovite, respectively (Bahadur et al. 2017). Different fungi namely, *Aspergillus* sp., *Trichoderma* sp., and *Penicillium* sp. have also been reported for their phosphate solubilizing activity (Mahamuni et al. 2012).

Verma and Kaur (2015) isolated bacteria from the rhizosphere of apple, which was found to solubilize the mineral phosphate. *Pseudomonas* sp. was the most potential solubilizer with other plant growth supporting activities, such as the production of HCN, IAA, siderophore, and ammonia apart from acting as a biological control agent against pathogenic fungi, *Dematophora nectarix,* and *Phytophthora cactorum*.

The mechanism of phosphate solubilization by these bacteria includes the production of organic acids which convert the complex insoluble forms of phosphate into soluble forms by chelating the cations bound to phosphorous (Zheng et al. 2018). Many different organic acids are produced by bacteria which include oxalic, fumaric, malic, 2-ketoglutaric acid, malate, gluconate, citric, tartaric acid, etc. (Babana et al. 2013; Illmer and Schinner 1992).

12.4.3 Nitrogen Fixation and Phytohormone Production

Many bacteria in the crop rhizosphere can fix atmospheric nitrogen (N). Some of the predominating nitrogen-fixing bacteria are *Azotobacter* sp., *Azospirillum* sp., *Herbaspirillum* sp., *Burkholderia* sp., and *Pseudomonas* sp.

Li et al. (2017) isolated *Pseudomonas* from the sugarcane rhizosphere which showed the ability to fix nitrogen as well as the production of IAA, ACC deaminase, and antibiotics. Tam and Diep (2015) reported the presence of N-fixing *Bacillus*, *Proteobacteria*, *Acidobacteria*, and *Bacteroides,* in the sugarcane rhizosphere. All these have shown the ability to solubilize phosphate and IAA production. Similar research by Majeed et al. (2015) reported seven out of nine isolates bacterial isolates

for PGP activities, such as N-fixation, as well as abilities to produce IAA and solubilize inorganic phosphate.

Phytohormones (IAA, auxin, etc.,) are secreted by the rhizospheric microbial population which directly affects the growth and development of plants. For example, Bahadur et al. (2017) isolated mineral solubilizers from the rhizosphere of different crops grown in the Gangetic planes, exhibiting PGP activities through the synthesis of IAA, ammonia, HCN, etc. The highest amount of IAA production was shown by *Brevibacillus formosus* followed by *Bacillus subtilis*.

12.5 Advanced Techniques for Studying Rhizospheric Microbial Communities

The major proportion of microbes, nearly 99% present in the rhizosphere are un-culturable. These microbes cannot be isolated using simple techniques; therefore, advanced biochemical and molecular techniques are used for their isolation and studies. Various techniques, traditional as well as modern molecular techniques, used to study the rhizospheric microbes have been discussed in detail in various reviews (Nannipieri et al. 2017; Vitorino and Bessa 2018; Salmonová and Bunešová 2017; van Elsas and Boersma 2011). These different techniques with their advantages and disadvantages are summarized in Table 12.3 and an overview of some important advanced techniques is presented in the following discussion.

12.5.1 Fingerprinting Techniques

For the study of rhizosphere, microbiome fingerprinting techniques, T-RFLP, denaturing gradient gel electrophoresis (DGGE), and single-strand conformation polymorphism (SSCP) are generally used. Yang et al. (2001) employed a PCR-DGGE 16S rDNA fingerprinting technique to study the difference in the healthy and *Phytophthora* infected avocado root rhizosphere population. They found different microbial communities dominated the healthy and infected plant rhizosphere. However, the rhizospheric population of trees treated with antagonist *Pseudomonas fluorescens* and nontreated healthy trees indicated the role of Pseudomonas in restoring the rhizospheric microbes in diseased plants. Kawasaki et al. (2016) analyzed the rhizosphere microbiome of *Brachypodium distachyon* using the T-RFLP technique (by targeting the 16S rDNA and ITS region of bacteria and fungi, respectively, and root exudates using HPLC analysis. They reported the similarity between the rhizosphere microbial communities and root exudates of *Brachypodium distachyon* and wheat, in contrast to differences between those from *B. distachyon* and *Arabidopsis* rhizosphere.

Zachow et al. (2014) carried out SSCP analysis to check the difference between the rhizospheric microbiome of modern sugar beet and *Beta vulgaris* ssp. *maritima*. They reported that *Beta vulgaris* spp. *Maritima*, which is an ancestor of all beet crops

Table 12.3 Techniques commonly used for the study of rhizospheric microorganisms

S.No.	Technique	Description	Advantages	Disadvantages
A. Culture-based techniques				
1.	Direct plating	Microbes are cultures on solid growth medium	Simple and sensitive, Isolates can be utilized for various purposes	Only 0.5–1.0% of total microbes are culturable due to different growth requirements of microbes
2.	Community-level physiological profiles (CLPP)	Heterotrophic microbial communities are classified and characterized based on carbon source utilization patterns	Simple and high throughput, requires less time	Sample preparation and inoculation needs accuracy, data analysis and interpretation is difficult
3.	Most probable number	Used for enumeration of low abundance specific groups of microorganisms	Useful for enumeration of low abundance microorganisms	Labor intensive, only specific groups can be enumerated
B. Culture-independent techniques				
1.	Phospholipid fatty acid (PLFA) analysis/fatty acid methyl ester analysis (FAME)	Fatty acid acids are extracted and unique fatty acids for each group of microorganisms is quantified to estimate the microbial activity	Culturing of microorganisms is not required, information on all organisms is acquired simultaneously	The sample has to be processed immediately after sampling, biased towards the more abundant group of microorganisms, not a high throughput method
2.	Fluorescent in situ hybridization (FISH)	16S or 23S rDNA region is hybridized with fluorescent dye-tagged taxon-specific oligonucleotide probes after fixing the whole cell and then visualized under scanning confocal laser microscopy	Microbial detection across various phylogenetic levels, more sensitive than immunofluorescence due to non-specific bonding with the particles of the soil	Not as sensitive as nucleic acid hybridization using environmental samples, absence of low copy number sequences are difficult to detect
3.	DNA microarray	A very small array of complementary DNA probes which are between 5×10^2 to 5×10^3 bases, or oligonucleotides having between 15–17 base pairs, linked directly to a solid matrix, Allows concomitant hybridization of many probe sets complementary to target DNA/RNA	Analysis of genes of the magnitude of 10^3, highly specific detection	Species having high abundance are detected, only culturable, low microbial diversity can be analyzed, non-specific hybridization may produce misleading signals, lack of specificity, sensitivity, and quantification

(continued)

Table 12.3 (continued)

S.No.	Technique	Description	Advantages	Disadvantages
4.	Single-strand conformation polymorphism (SSCP)	Distinct ssDNA is resolved through gel electrophoresis on the basis of differences in the nucleotide sequences leading to hetero duplexes formation and changes in movement of DNA through gel	Simultaneous and multiple-sample analysis with high speed, reliability and reproducibility	Sometimes PCR-induced sequence artifacts or bias occurs
5.	Restriction fragment length polymorphism (RFLP)	Based upon polymorphisms of the DNA, run on electrophoresis followed by restriction digestion, blotting on suitable membranes, and hybridization with suitable probes (made by cloning)	Detection of the structure related differences in the population of microorganisms	Sometimes PCR-induced sequence artifacts or bias occurs
6.	Terminal restriction fragment length polymorphism (T-RFLP)	An extension of the RFLP/ ARDRA analysis, Different from RFLP in that one primer is fluorescently tagged with either 4, 7, 2′, 7′-tetrachloro-6-carboxyfluorescein or 6-phosphoramidite fluorochrome-5-carboxyfluorescein	Pattern of bands is not complicated as in RFLP, automation is possible, numerous sample analysis is possible with better reproducibility when comparing different populations of microorganisms	Sometimes PCR-induced sequence artifacts or bias occurs, extraction and lysis need to be efficient, Taq polymerase dependent variation can occur, restriction enzyme needs to be well chosen for analysis
7.	Ribosomal intergenic spacer analysis Swanson et al. (2009)/automated ribosomal intergenic spacer analysis (ARISA)/ amplified ribosomal DNA restriction analysis (ARDRA)	IGS region of 16S and 23S rDNA is subjected to amplification, denaturation, and separation on denaturing-PAGE	Highly reproducible community profiles of different bacterial strains or the closely related species	Requires large quantities of DNA Swanson et al. (2009) PCR biases
8.	Denaturing and temperature gradient gel electrophoresis (DGGE and TGGE)	Separation of DNA segments having with only single nucleotide differences is possible, due to distinct T_m and resolution	Simultaneous and multiple-sample analysis with high speed, reliability and reproducibility	Sometimes PCR-induced sequence artifacts or bias occurs, extraction, lysis, storage need to be efficient, multiple-species can be migrated in one band, detection of dominating species

harbored a distinctive set of rhizospheric microflora than modern domesticated sugar beet.

12.5.2 Quantitative PCR and Gene Expression

The quantitative PCR technique detects and quantifies specific genes and their expression. Dudenhöffer et al. (2016) quantified the total and specific disease suppressive bacteria using quantitative real-time PCR. Growth of disease suppressive bacteria especially fluorescent pseudomonads was selectively enhanced by the barley plant for biocontrol of pathogenic fungi, *Fusarium graminearum*.

Shrestha et al. (2010) used PCR for amplification of the *pmoA* gene and quantitative RT-PCR to determine the copy number of *pmoA* gene to check the efficacy of nitrogen fertilizers on the metabolism, microbial diversity, and gene expression in methanotrophic bacteria present in the rhizosphere of rice. It was found that type-I methanotrophic bacteria dominated during the whole season whether nitrogen fertilizer was applied or not, while the population of type-II methanotrophic bacteria increased only under the more conducive conditions, like ammonium sulfate fertilizer application. Studies have also employed quantitative PCR analysis (Marques et al. 2014) to determine the effect of resistance breeding on the microbial communities of the common bean rhizosphere, by analyzing the bacterial gene expression in rhizospheric and non-rhizospheric soil (Mendes et al. 2018). It was found that the pseudomonads, bacilli, and members of solibacteraceae and cytophagaceae dominated in the rhizospheric soil of the *Fusarium oxysporium* (Mendes et al. 2018) resistant varieties than in susceptible ones. Microarrays and real-time quantitative PCR are used for rhizospheric studies of microbial communities. Despite being powerful techniques, these are not without limitations. Using quantitative PCR only a few genes can be analyzed at once and for microarrays analysis, previous knowledge about targeted sequences is generally required (Carvalhais et al. 2013).

12.5.3 Meta-Omics Techniques

Apart from the techniques for studying the diversity and characteristics of the culturable microorganisms, many methods have also been developed for studying the microbial diversity and community structure of rhizospheric and non-rhizoshperic soils, namely, metagenomics, metatranscriptomics, metaproteomics, and metabolomics. These recent techniques based on the principles and tools of molecular biology have shown that the abundance of microbes in the rhizosphere and bulk soil is far more than predicted earlier (Lagos et al. 2015) using culturable methods. Basic principles and applications of these techniques concerning rhizosphere microbial diversity are discussed below.

The relative abundance and microbial types can be easily predicted using metagenomics, as it focuses on the DNA (Carvalhais et al. 2013). Mukhtar et al.

(2016) estimated the microbial diversity of the rhizosphere and rhizoplane, as well as that of histoplane of para grass cultivated in the saline environment. Culturable microbes were estimated and characterized by amplification and sequencing of 16S rDNA region, and biochemical analysis. While non-culturable microbes were characterized using 16S rRNA gene amplification from the metagenome. The most probable numbers of microbes from the studied regions of para grass were 150×10^7, 47×10^7, and 130×10^7, respectively. Using 16S rRNA gene sequencing analysis, a total of 26 operational taxonomic units (OTUs) were obtained from the rhizosphere. While for non-culturable microbes, a total of 48 16S rRNA clones, grouped into 25 OTUs, were obtained from the rhizosphere.

In contrast to metagenomics, metatranscriptomics focuses on RNA and involves the characterization of mRNA produced in the cells. This technique provides information about genes that are transcribed by the microbes and thus, helps in understanding the metabolism of the microbial population (Carvalhais et al. 2013; Lagos et al. 2015; Verma et al. 2018). Bacterial metabolism and gene expression in the rhizosphere before and after treatment with glyphosate has been analyzed using a metatranscriptomic approach (Newman et al. 2016b). The rhizospheric bacteria were reported to invest most of their energy in carbohydrate metabolism and transcription. After treatment with glyphosate, expression of genes encoding ATP-synthase and cytochrome c-553 increased significantly, denoting increased respiration after glyphosate treatment.

Metaproteomics provides information about the role of soil microbiota in different biogeochemical processes, degradation, or bioremediation processes by measuring the proteins present in the rhizospheric samples (Verma et al. 2018; Abiraami et al. 2020). Bona et al. (2018) characterized the rhizosphere of the *Vitis vinifera* using metaproteomics. They isolated protein from the soil, digested it with trypsin, and analyzed using mass spectroscopy. More than 570 proteins from over a hundred of bacterial genera were identified from bulk and rhizospheric soil, out of which 20 proteins were under constitutive expression due to their involvement in nutrient transport. It was analyzed that 56 proteins were expressed by bulk soil specific bacteria, 54 proteins were expressed by rhizosphere specific bacterial genera. Furthermore, a total of 59 bacterial genera were common in both the soil types.

For root exudate analysis, different metabolomics techniques can be used. Metabolomics can be used to analyze multiple compounds in one go. Metabolomic techniques may be used to identify compounds present in root exudate depends on the class of the compound. For example, in the case of volatile root exudates, GC-MS can be used, while for analysis of phenolics, flavonoids, or other water-soluble root exudates, liquid chromatography or nuclear magnetic resonance techniques can be used. A combination of different techniques can be used when there is insufficient knowledge about the types of molecules present in root exudates that are needed to be analyzed. Metabolomics also provides information about signalling networks present in the rhizosphere (van Dam and Bouwmeester 2016).

12.6 Factors Affecting Rhizospheric Microbial Population

Various factors influence the rhizospheric microbial population qualitatively as well as quantitatively (Fig. 12.2). The higher number of microbes in rhizospheric soil than bulk soil is due to the rhizospheric effect. The rhizospheric effect is measured by calculating R:S ratio (root:soil ratio) which is the proportion of the quantity of microbes present in the rhizosphere and bulk soil (Hiltner 1904). Both biotic and abiotic factors, directly as well indirectly affect the colonization of the microbial population in the rhizosphere. Such factors include pH of the soil, temperature, root exudates, competition, and inorganic nutrients, etc. and are summarized in Table 12.4 and discussed below.

12.6.1 Root Exudates

Microbes present in soil compete for available nutrients and other resources, which affect the growth of these microbes. Roots of plants influence the activity of these microbes by secreting root exudates, which are the compounds that are released from roots into the soil. The plant secretes these root exudates that promote specific microbes for colonization in the rhizosphere (Doornbos et al. 2012). These compounds include primary as well as secondary metabolites.

Various plant-related and external factors determine the quantitative as well as qualitative characteristics of the root exudates. In soil, the roots are exposed to different microbes which can be beneficial or pathogenic. Plants attract only specific microbes and can alter the diversity and composition of the rhizospheric microbial

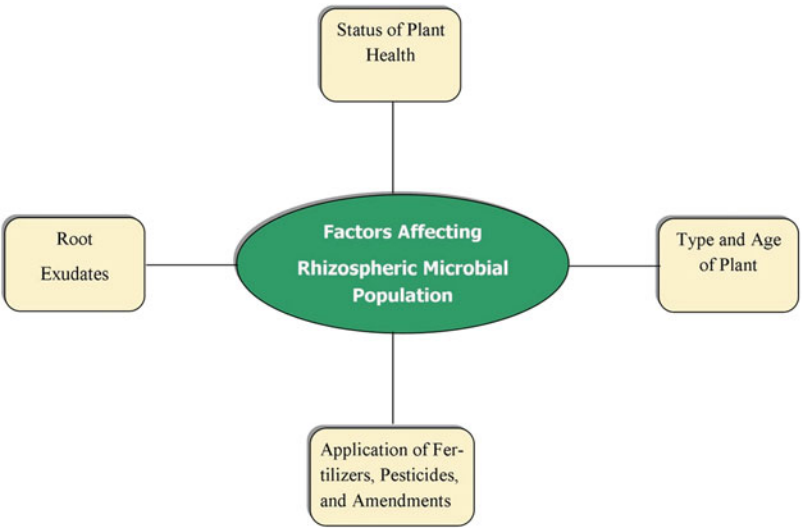

Fig. 12.2 Various factors influencing the rhizospheric microbial population

Table 12.4 Factors affecting the rhizospheric population

S. No.	Factor	Impact on microbial community	Reference
1.	Root exudates	Specific microbes are attracted to colonization and affect the rhizosphere microbial population both qualitatively and quantitatively	Doornbos et al. (2012) and van Loon et al. (1998)
2.	Type of plant	Rhizosphere microbial population varies with plant genotype and cultivar	Pérez-Jaramillo et al. (2016) and Weinert et al. (2011)
3.	Age of plant	Rhizosphere microbial community composition depends upon plant growth stage	Marques et al. (2014) and Sinegani and Sharifi (2007)
4.	Plant health status	Rhizospheric microbial population dynamics of healthy plant differs with that of diseased plant. Both rhizospheric bacteria and fungi are abundant in healthy plant than diseased plant	Wu et al. (2015b)
5.	Application of fertilizers	After N-fertilizers application number of *Azospirillum lipoferum* and *Gluconacetobacter diazotrophicus* in rhizosphere is negatively affected, Bacillales, Nitrosomonadales, and Rhodocyclales become more dominating, while Chloroflexales, Gemmatimonadetes, and Phycisphaerae become less abundant	Zhu et al. (2016)
6.	Application of pesticides	Glyphosate application increased the number of pathogenic fungi (*Fusarium*) in the rhizosphere and negatively affected the fluorescent *Pseudomonads.* The number of Acidobacteria decreased after treatment with glyphosate and that of proteobacteria increased	Newman et al. (2016a) and Zobiole et al. (2011)
7.	Application of biofertilizers and compost	The number of beneficial microbial populations increased and the number of fungi decreased in the rhizosphere	Fu et al. (2017) and Mickan et al. (2018)
8.	Application of vermicompost	The activity of pathogens, *Pythium aphanidermatum*, *Pythium ultimum*, and *Rhizoctonia solani* was suppressed, the relative abundance of Ascomycota and Chytridiomycota increased and that of Glomeromycota and Zygomycota decreased	You et al. (2019)

population. Plant roots release different compounds in the form of organic-, fatty- or amino acids, simple carbohydrates, sterols, growth factors, etc. The process of release of these compounds is also known as rhizodeposition. These secreted compounds from roots are grouped into two classes: (1) low molecular weight

compounds, like phenolics, sugars, amino acids, organic acids, etc. and (2) higher molecular weight compounds viz., polysaccharides and proteins (Huang et al. 2014; Prashar et al. 2014). Root exudates are also classified as active and passive, depending upon the role and mode of secretion from roots. Active exudates have a specific function and are released via open pores of the cell membrane while passive exudates have an unknown function and constitute approximately 3–5% of total photosynthetically fixed carbon. Passive root exudates are released from the roots via diffusion. Further, exudates can be classified, based on their biological activity, as signal molecules, phytoalexins, phytohormones, enzymes, or allelochemicals (Prashar et al. 2014).

Limited plant nutrients also affect the root exudates and rhizospheric microbiome. In a study, it was shown that limiting the amount of nitrogen negatively influenced amino acid secretion in maize rhizosphere, which further suppressed the transcription of genes affecting translation in the bacterium *B. amyloliquefaciens* (Carvalhais et al. 2011).

12.6.2 Type and Age of the Plant

Rhizospheric microbial population is influenced by the plant characteristics (Pérez-Jaramillo et al. 2016; Sinegani and Sharifi 2007). Each plant recruits a particular set of the rhizospheric microbiome and rhizospheric microbial composition of plant species also varies with the phylogenetic distance (Pérez-Jaramillo et al. 2016). Rhizospheric microbial composition also varies with the genotypes of the same species. In a study, the relation between rhizospheric microbial composition and the growth of three cultivars of potato was analyzed using PhyloChips, which detected 2432 operational taxonomic units. Further, the rhizospheric microbial composition varied with cultivar, and varying microbial populations belonged mainly to the *Pseudomonadales*, *Actinomycetales,* and *Enterobacteriales* (Weinert et al. 2011). Marques et al. (2014) reported that age as well as the genotype significantly affected the rhizospheric population of sweet potato.

In a study, Sinegani and Sharifi (2007) investigated the number of AMF spores in 14 rhizospheric soils of different crops. They found the abundance of AMF varied based on the type of crop and vegetative stage of the crop. The rhizospheric AMF spore counts were the highest for *Zea mays* and the lowest for *Raphanus sativa,* during the mid-vegetative growth. After the vegetative growth culminated, the AMF spore counts were the highest in the *Allium cepa* rhizosphere and lowest in *Raphanus sativa*. During termination of the vegetative phase of development, the rhizosphere of *Triticum aestivum*, *Zea mays*, *Trifolium repens*, *Solanum tuberosum*, *Satureja hortensis*, and *Allium cepa* had the elevated counts of AMF spores.

12.6.3 Status of Plant Health

The rhizospheric microbial communities associated with the diseased plants differ significantly from the healthy plants of the same species. Wu et al. (2015b) demonstrated this in their experiment on rhizosphere soils from roots of diseased (root-rot disease) and healthy plants of *Panax notoginseng*. Microbial community of rhizosphere soil of both diseased and healthy plants was analyzed using throughput sequencing of the amplified bacterial 16S or fungal 18S rDNA region and higher abundance of both bacteria and fungi in the rhizosphere of the healthy plants were found. Many bacteria were found dominant in the rhizosphere of both healthy and diseased plants, while some were specific to the rhizosphere of either healthy or diseased plants. Comparative analysis using Paired-T tests showed that Proteobacteria were more abundant while Acidobacteria, Cyanobacteria, Firmicutes, Verrucomicrobia were low in abundance in the rhizosphere of diseased plants. The rhizospheric fungi belonging to Ascomycota were more abundant while those belonging to Glomeromycota were less abundant in diseased plants. Basidiomycota and Zygomycota were the major phyla that were equally abundant in the rhizosphere of both the plants.

12.6.4 Application of Fertilizers, Pesticides, and Amendments

Rhizospheric microbial communities are very important for plant growth. The application of different amendments alters the rhizospheric microbial populations. In different studies, the effect of pesticide application on the quantity and quality of rhizospheric microbes has been established.

For example, glyphosate amendment the quantity of *Fusarium* in the rhizosphere, simultaneously affecting the population of fluorescent *Pseudomonads* as well as indole acetic acid-producing bacteria, and Mn-reducing bacteria (Zobiole et al. 2011). In another similar study, the initial rhizosphere population of soybean and corn was dominated by *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* but after treatment with glyphosate, the number of *Acidobacteria* decreased in the rhizosphere of both soybean and corn. These bacteria were primarily involved in the cellulose biodegradation. The relative abundance of *Proteobacteria* increased after treatment with glyphosate (Newman et al. 2016a).

Application of N-fertilizers in high amounts also negatively affects the number of many bacteria, such as *Azospirillum lipoferum*, *Gluconacetobacter diazotrophicus,* etc. in the rhizosphere. Zhu et al. (2016) concluded after GC-MS analysis of the rhizospheric region that when N-fertilizers were amended, the amount of root exudates also increased. They also analyzed the effect of increasing N-rates on rhizospheric microbial population and found that Bacillales, Nitrosomonadales, and Rhodocyclales capable of ammonia oxidation, were significantly abundant relative to other groups of bacteria. Conversely, Chloroflexales, Gemmatimonadetes, and Phycisphaerae got significantly reduced.

In a study on banana rhizosphere, *Bacillus amyloliquefaciens* NJN-6, and compost-based biofertilizer were found to enhance beneficial microbes and simultaneously decreased wilt causing *Fusarium* sp. in the rhizosphere (Fu et al. 2017). Nevita et al. (2018) inoculated chopped rice straw residue with indigenously isolated *B. cereus*, *Stenotrophomonas maltophilia*, and *K. pneumonia* (10^9 CFU/kg) individually for its application as bacterial probiotic compost to enhance the growth of rice. The microbial communities and their numbers were significantly altered after the application of probiotic compost. In the rhizosphere treated with *B. cereus* probiotic bacterial compost, the relative abundance of Proteobacteria decreased while that of Acidobacteria, Actinobacteria, and Firmicutes increased as compared to control rhizosphere. In the case of *S. maltophilia* probiotic bacterial compost application, the relative abundance of both Proteobacteria and Actinobacteria decreased and that of Acidobacteria, Firmicutes, and Bacteroidetes increased in contrast to untreated control. When treated with *K. pneumonia* probiotic bacterial compost Bacteroidetes and Firmicutes increased, while Proteobacteria decreased. Mickan et al. (2018) determined the correlation between the application of clay and compost and the rhizosphere population of *Trifolium subterraneum* under water stress conditions. Compost application decreased AMF colonization by 29.8%.

Underwater stress conditions, AMF colonization in unamended soil decreased in contrast that in clay supplemented soil. Different treatments (clay, compost, and clay +compost) had a large positive impact on microbial populations. Gram-negative phyla Bacteroidetes, Gemmatimonadetes, and Proteobacteria dominated in clay amended soil, Chloroflexi and Proteobacteria dominated in compost amended soil, and Chloroflexi, Bacteroidetes, and Proteobacteria showed dominance in clay+compost amended soil (Mickan et al. 2018). However, Acidobacteria, Planctomycetes, Firmicutes, Actinobacteria, and Verrucomicrobia had decreased abundance when the soil was supplemented with clay. In compost amended soil, Verrucomicrobia, Acidobacteria, Planctomycetes, and Firmicutes had decreased abundance, while in clay+compost amended soil, Acidobacteria, Verrucomicrobia, Planctomycetes, Firmicutes, and Actinobacteria had a low abundance. Underwater stress conditions, the relative abundance of Actinobacteria decreased in compost amended soil and increased in all other treatments (clay, clay+compost, and unamended). The relative abundance of Proteobacteria decreased in clay+compost amended soil when there was water scarcity.

You et al. (2019) reported that while applying vermicompost-bamboo powder suppression of damping-off disease in cucumber occurred. The activity of pathogens, *Pythium aphanidermatum*, *Pythium ultimum*, and *Rhizoctonia solani* was suppressed by the use of vermicompost-bamboo powder. Zhao et al. (2017) reported that Ascomycota and Chytridiomycota had elevated, while Glomeromycota and Zygomycota had decreased abundance in cucumber rhizosphere after the treatment of soil with vermicompost and inorganic fertilizer mixture as compared to unamended soil. When treated with the mixture of both fertilizers (Zheng et al. 2018), Glomeromycota and Zygomycota decreased. However, an increase in abundance of Chytridiomycota and a decrease in that of Glomeromycota and Zygomycota occurred when the treatment of soil with inorganic fertilizer was

done. In all the three treatments (vermicompost and inorganic fertilizer mixture, inorganic and organic fertilizer mixture, and inorganic fertilizer), the relative abundance of Basidiomycota decreased.

12.7 Rhizosphere Competence and PGPR Development

The microbes applied as PGPR first need to multiply and colonize in the rhizosphere in the presence of other microbial populations. Rhizosphere competence defines the growth and functional capacity of the microbes in the plant rhizosphere by competing with other resident microbes present there for nutrition and space on the root surface of plants (Monfil and Casas-Flores 2014). The multiplication and colonization of PGPR inoculants are affected by different factors including soil type, presence of grazers, moisture content of the soil, edaphic factors like soil pH, competition from native microbes, availability of nutrients, and suitable host plant root. Several rhizosphere competence traits help in colonization and multiplication of applied inoculants. These traits include the formation of biofilm, the production of siderophores, motility, antagonistic activity, protease activity, and the ability to utilize root exudates (Kaur et al. 2017).

Microbes present in the rhizosphere interact with plant roots, soil, and other microbes in several distinct ways. These interactions in the rhizosphere can be beneficial, harmful, or neutral. Beneficial interactions that promote plant growth and improve soil quality include biocontrol, bioremediation, phytostimulation, and bio fertilization (de Weert and Bloemberg 2006).

Bach et al. (2016) studied the rhizospheric competence and biological control activity of three biocontrol bacteria *Bacillus mycoides*, *Burkholderia cepacia*, and *Paenibacillus riograndensis* and reported that these bacteria enhance their growth and survive under high competition in the rhizosphere. These bacteria have shown the proteolytic activity, production of hydrolytic enzymes, and catalase activity. Antagonistic activity of these bacteria was checked against filamentous fungi and all of them inhibited the growth of filamentous fungi.

The efficacy of PGPR generally decreases when used at the field scale. For a successful biocontrol activity, the biocontrol agent must have high rhizosphere competence so that it can easily compete with the rhizospheric population for nutrition and space, and can perform its function (Schreiter et al. 2018). Similarly, *Pseudomonas* sp. has been found to grow in potato and lettuce rhizosphere grown under three types of soils (Schreiter et al. 2018) was investigated to observe the biocontrol ability against fungal pathogen *Rhizoctonia solani* and rhizospheric competence. The population of *Pseudomonas* remained unaffected in both the rhizospheres under each soil type and the presence of *Rhizoctonia solani* (Schreiter et al. 2018).

12.8 Rhizosphere Engineering for Better Plant and Soil Health

Plant health and productivity can be improved by manipulating the rhizosphere by various methods. To alleviate the different environmental stresses, plants use different strategies to modify the rhizosphere. Rhizosphere engineering can enhance plant stress tolerance ability under several harsh environments. Rhizosphere engineering can be done for improving the overall plant health and growth. It is generally carried out by amending the soil, plant engineering, engineering the microbial partners, and engineering the plant–microbe interactions (Dessaux et al. 2016). It can also be done in several other ways, including transcriptome engineering which can be used to overexpress genes encoding enzymes related to the accumulation of osmolytes and proteins. These osmolytes and proteins improve abiotic stress tolerance ability by ion transporting ions and scavenging the reactive oxygen species. Another strategy is the isolation and identification of stress-tolerant microbes from the rhizosphere of different plants and inoculating them in the rhizosphere of different plants to reduce abiotic stress (Ahkami et al. 2017).

12.9 Conclusions

The rhizosphere of the plants constitutes an interesting environment, where several types of interactions interplay between plant, soil, and microorganisms. Many plant beneficial microorganisms are found in this region, which is in the vicinity of the plant roots. These microorganisms include bacteria, archaebacteria, viruses, fungi, actinomycetes, protozoans, arthropods, algae, and nematodes. The bacteria generally outnumber other microbes in the rhizosphere. All types of microbe–microbe and plant–microbe interactions occur in the rhizosphere, which may be positive or negative, and beneficial or detrimental to plant growth and crop productivity. Many different methods and techniques are applied to study the rhizospheric microbial communities. Both culturable and non-culturable microorganisms and their influence can be studied and predicted in the plant rhizosphere. The modern techniques include fingerprinting techniques such as terminal restriction fragment length polymorphism, denaturing gradient gel electrophoresis, and single-strand conformation polymorphism quantitative PCR based gene expression analysis and meta-omics-based techniques (namely, metagenomics, metatranscriptomics, metaproteomics, and metabolomics). These recent techniques based on the principles and tools of molecular biology have shown great capabilities in studying the details of the rhizospheric microbial populations in lesser time.

Many different plant-associated, as well as external environment associated factors, affect the population of rhizospheric microorganisms. Further, the dominant rhizospheric microbes are found to possess rhizospheric competence, i.e. their ability to survive the close competition with other microorganisms for nutrition and space. The rhizospheric microbial communities are not constant and can dynamically change, depending upon the different amendments, such as organic or inorganic fertilizers, biofertilizers, pesticides, compost, biocontrol agents, etc. All the

principles of rhizosphere–microbe interactions can be made use of, in enhancing the overall crop productivity and disease management by engineering the rhizosphere. It is generally carried out by amending the soil, plant engineering, engineering the microbial partners, and engineering the plant–microbe interactions. It can also be done in several other ways, including transcriptome engineering or bioprospecting followed by the application of stress-tolerant microbes. In conclusion, rhizosphere engineering is one of the practical solutions to achieve the goals of enhanced productivity and lead to sustainable agriculture.

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References

Abedinzadeh M, Etesami H, Alikhani HA (2019) Characterization of rhizosphere and endophytic bacteria from roots of maize (*Zea mays* L.) plant irrigated with wastewater with biotechnological potential in agriculture. Biotechnol Rep 21:e00305. https://doi.org/10.1016/j.btre.2019.e00305

Abiraami TV, Singh S, Nain L (2020) Soil metaproteomics as a tool for monitoring functional microbial communities: promises and challenges. Rev Environ Sci Biotechnol 19(1):73–102. https://doi.org/10.1007/s11157-019-09519-8

Ahkami AH, Allen White R, Handakumbura PP, Jansson C (2017) Rhizosphere engineering: enhancing sustainable plant ecosystem productivity. Rhizosphere 3:233–243. https://doi.org/10.1016/j.rhisph.2017.04.012

Ahmad J, Baker R (1988) Rhizosphere competence of *Trichoderma harzianum*. Can J Microbiol 34:229–234. https://doi.org/10.1139/m88-043

Alami MM, Xue J, Ma Y, Zhu D, Abbas A, Gong Z, Wang X (2020) Structure, function, diversity, and composition of fungal communities in Rhizospheric soil of *Coptis chinensis* Franch under a successive cropping system. Plan Theory 9(2):244

Babana AH, Dicko A, Maïga K, Traoré D (2013) Characterization of rock phosphate-solubilizing microorganisms isolated from wheat (*Triticum aestivum* L.) rhizosphere in Mali. J Mıcrobıol Mıcrobıal Res 1(1):1–6

Bach E, Seger GDS, Fernandes GC, Lisboa BB, Passaglia LMP (2016) Evaluation of biological control and rhizosphere competence of plant growth promoting bacteria. Appl Soil Ecol 99:141–149. https://doi.org/10.1016/j.apsoil.2015.11.002

Bahadur I, Maurya BR, Meena VS, Saha M, Kumar A, Aeron A (2017) Mineral release dynamics of Tricalcium phosphate and waste muscovite by mineral-solubilizing Rhizobacteria isolated from indo-Gangetic plain of India. Geomicrobiol J 34(5):454–466. https://doi.org/10.1080/01490451.2016.1219431

Bai L, Cui J, Jie W, Cai B (2015) Analysis of the community compositions of rhizosphere fungi in soybeans continuous cropping fields. Microbiol Res 180:49–56. https://doi.org/10.1016/j.micres.2015.07.007

Berrios L, Ely B (2019) The isolation and characterization of Kronos, a novel *Caulobacter* Rhizosphere phage that is similar to Lambdoid Phages. Curr Microbiol 76(5):558–565. https://doi.org/10.1007/s00284-019-01656-1

Bi Y, Ma W, Xing F, Gao Y, Li Z, Chen C, Mu X, Li X, Zhu X (2020) Diversity and specificity of arbuscular mycorrhizal fungi in the rhizosphere of six plants in the Songnen grassland, China. Écoscience 27(1):11–21. https://doi.org/10.1080/11956860.2019.1662969

Bona E, Massa N, Novello G, Boatti L, Cesaro P, Todeschini V, Magnelli V, Manfredi M, Marengo E, Mignone F, Berta G, Lingua G, Gamalero E (2018) Metaproteomic characterization of the *Vitis vinifera* rhizosphere. FEMS Microbiol Ecol 95(1):fiy204. https://doi.org/10.1093/femsec/fiy204

Bulgarelli D, Garrido-Oter R, Münch Philipp C, Weiman A, Dröge J, Pan Y, McHardy Alice C, Schulze-Lefert P (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe 17(3):392–403. https://doi.org/10.1016/j.chom.2015.01.011

Carvalhais LC, Dennis PG, Fedoseyenko D, Hajirezaei M-R, Borriss R, von Wirén N (2011) Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. J Plant Nutr Soil Sci 174(1):3–11. https://doi.org/10.1002/jpln.201000085

Carvalhais LC, Dennis PG, Tyson GW, Schenk PM (2013) Rhizosphere metatranscriptomics: challenges and opportunities. Mol Microb Ecol Rhizosphere 2:1137–1144

Carvalhais LC, Rincon-Florez VA, Brewer PB, Beveridge CA, Dennis PG, Schenk PM (2019) The ability of plants to produce strigolactones affects rhizosphere community composition of fungi but not bacteria. Rhizosphere 9:18–26. https://doi.org/10.1016/j.rhisph.2018.10.002

Chapelle E, Mendes R, Bakker PAHM, Raaijmakers JM (2016) Fungal invasion of the rhizosphere microbiome. ISME J 10(1):265–268. https://doi.org/10.1038/ismej.2015.82

Chopra A, Kumar Vandana U, Rahi P, Satpute S, Mazumder PB (2020) Plant growth promoting potential of *Brevibacterium sediminis* A6 isolated from the tea rhizosphere of Assam, India. Biocatal Agric Biotechnol:101610. https://doi.org/10.1016/j.bcab.2020.101610

Cordero J, de Freitas JR, Germida JJ (2019) Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. Can J Microbiol 66(1):71–85. https://doi.org/10.1139/cjm-2019-0330

Cubo Sánchez MT, Alías Villegas C, Balsanelli E, Mesa D, Espuny Gómez MR, de Souza E (2020) Diversity of *Sinorhizobium* (*Ensifer*) *meliloti* bacteriophages in the Rhizosphere of *Medicago marina*: Myoviruses, filamentous and N4-like Podovirus. Front Microbiol 11(art 22):1–14

Damodaran T, Mishra VK, Jha SK, Pankaj U, Gupta G, Gopal R (2019) Identification of Rhizosphere bacterial diversity with promising salt tolerance, PGP traits and their exploitation for seed germination enhancement in Sodic soil. Agric Res 8(1):36–43. https://doi.org/10.1007/s40003-018-0343-5

Danesh YR, Goltapeh EM, Alizadeh A, Varma A, Mukerjii K (2006) Distribution and abundance of arbuscular mycorrhizal fungi from soybean rhizosphere in Iran. J Agric Technol 2:251–257

de Weert S, Bloemberg GV (2006) Rhizosphere competence and the role of root colonization in biocontrol. In: Gnanamanickam SS (ed) Plant-associated bacteria. Springer, Dordrecht, pp 317–333. https://doi.org/10.1007/978-1-4020-4538-7_9

Dessaux Y, Grandclément C, Faure D (2016) Engineering the Rhizosphere. Trends Plant Sci 21(3):266–278. https://doi.org/10.1016/j.tplants.2016.01.002

Dhaked BS, Triveni S, Reddy RS, Padmaja G (2017) Isolation and screening of potassium and zinc solubilizing bacteria from different rhizosphere soil. Int J Curr Microbiol App Sci 6(8):1271–1281

Doornbos RF, van Loon LC, Bakker PAHM (2012) Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agron Sustain Dev 32(1):227–243. https://doi.org/10.1007/s13593-011-0028-y

Dubey G, Kollah B, Gour VK, Shukla AK, Mohanty SR (2016) Diversity of bacteria and archaea in the rhizosphere of bioenergy crop *Jatropha curcas*. 3 Biotech 6(2):257. https://doi.org/10.1007/s13205-016-0546-z

Dudenhöffer J-H, Scheu S, Jousset A (2016) Systemic enrichment of antifungal traits in the rhizosphere microbiome after pathogen attack. J Ecol 104(6):1566–1575. https://doi.org/10.1111/1365-2745.12626

Emami S, Alikhani HA, Pourbabaei AA, Etesami H, Sarmadian F, Motessharezadeh B (2019) Effect of rhizospheric and endophytic bacteria with multiple plant growth promoting traits on wheat growth. Environ Sci Pollut Res 26(19):19804–19813. https://doi.org/10.1007/s11356-019-05284-x

Etesami H, Alikhani HA (2018) *Bacillus* species as the most promising bacterial biocontrol agents in rhizosphere and endorhiza of plants grown in rotation with each other. Eur J Plant Pathol 150 (2):497–506. https://doi.org/10.1007/s10658-017-1276-8

Figueroa-López AM, Cordero-Ramírez JD, Martínez-Álvarez JC, López-Meyer M, Lizárraga-Sánchez GJ, Félix-Gastélum R, Castro-Martínez C, Maldonado-Mendoza IE (2016) Rhizospheric bacteria of maize with potential for biocontrol of *Fusarium verticillioides*. Springerplus 5(1):330. https://doi.org/10.1186/s40064-016-1780-x

Fu L, Penton CR, Ruan Y, Shen Z, Xue C, Li R, Shen Q (2017) Inducing the rhizosphere microbiome by biofertilizer application to suppress banana *Fusarium* wilt disease. Soil Biol Biochem 104:39–48. https://doi.org/10.1016/j.soilbio.2016.10.008

Gao Z, Han M, Hu Y, Li Z, Liu C, Wang X, Tian Q, Jiao W, Hu J, Liu L, Guan Z, Ma Z (2019) Effects of continuous cropping of sweet potato on the fungal community structure in Rhizospheric soil. Front Microbiol 10:2269–2269. https://doi.org/10.3389/fmicb.2019.02269

Gao D, Pan X, Zhou X, Wei Z, Li N, Wu F (2020) Phosphorus fertilization and intercropping interactively affect tomato and potato onion growth and rhizosphere arbuscular mycorrhizal fungal community. Arch Agron Soil Sci. https://doi.org/10.1080/03650340.2020.1768530. 10.1080/03650340.03652020.01768530

Gaya Karunasinghe T, Hashil Al-Mahmooli I, Al-Sadi AM, Velazhahan R (2020) The effect of salt-tolerant antagonistic bacteria from tomato rhizosphere on plant growth promotion and damping-off disease suppression under salt-stress conditions. Acta Agric Scand Sect B Soil Plant Sci 70 (1):69–75. https://doi.org/10.1080/09064710.2019.1668956

Gómez-Lama Cabanás C, Legarda G, Ruano-Rosa D, Pizarro-Tobías P, Valverde-Corredor A, Niqui JL, Triviño JC, Roca A, Mercado-Blanco J (2018) Indigenous *Pseudomonas* spp. strains from the olive (*Olea europaea* L.) Rhizosphere as effective biocontrol agents against *Verticillium dahliae*: from the host roots to the bacterial genomes. Front Microbiol 9:277. https://doi.org/10.3389/fmicb.2018.00277

Gqozo MP, Bill M, Siyoum N, Labuschagne N, Korsten L (2020) Fungal diversity and community composition of wheat rhizosphere and non-rhizosphere soils from three different agricultural production regions of South Africa. Appl Soil Ecol 151:103543. https://doi.org/10.1016/j.apsoil.2020.103543

Guo Q, Li Y, Lou Y, Shi M, Jiang Y, Zhou J, Sun Y, Xue Q, Lai H (2019) *Bacillus amyloliquefaciens* Ba13 induces plant systemic resistance and improves rhizosphere microecology against tomato yellow leaf curl virus disease. Appl Soil Ecol 137:154–166. https://doi.org/10.1016/j.apsoil.2019.01.015

Hiltner L (1904) Uber nevere Erfahrungen und Probleme auf dem Gebiet der Boden Bakteriologie und unter besonderer Beurchsichtigung der Grundungung und Broche. Arbeit Deut Landw Ges Berlin 98:59–78

Huang X-F, Chaparro JM, Reardon KF, Zhang R, Shen Q, Vivanco JM (2014) Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92(4):267–275. https://doi.org/10.1139/cjb-2013-0225

Illmer P, Schinner F (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soils. Soil Biol Biochem 24(4):389–395. https://doi.org/10.1016/0038-0717(92)90199-8

Imriz G, Özdemir F, Karaca MS, Taş MN, Topal I, Ercan B (2020) Biological control potential of rhizosphere bacteria with ACC-deaminase activity against *Fusarium culmorum* in wheat. Zemdirbyste-Agriculture 107(2):105–112

Jamiołkowska A, Thanoon AH, Patkowska E, Grządziel J (2019) Impact of AMF *Claroideoglomus etunicatum* on the structure of fungal communities in the tomato rhizosphere. Acta Mycol 54:1

Jefwa JM, Kahangi E, Losenge T, Mung'atu J, Ngului W, Ichami SM, Sanginga N, Vanluawe B (2012) Arbuscular mycorrhizal fungi in the rhizosphere of banana and plantain and the growth of tissue culture cultivars. Agric Ecosyst Environ 157:24–31. https://doi.org/10.1016/j.agee.2012.03.014

Karimzadeh J, Alikhani HA, Etesami H, Pourbabaei AA (2020) Improved phosphorus uptake by wheat plant (*Triticum aestivum* L.) with Rhizosphere fluorescent pseudomonads strains under water-deficit stress. J Plant Growth Regul:1–17

Karnwal A, Mannan M (2018) Application of *Zea mays* L. Rhizospheric bacteria as promising biocontrol solution for Rice sheath blight. Pertanika J Trop Agric Sci 41:4

Kaur C, Selvakumar G, Ganeshamurthy AN (2017) Rhizocompetence of applied bioinoculants. In: Singh DP, Singh HB, Prabha R (eds) Plant-microbe interactions in agro-ecological perspectives: volume 1: fundamental mechanisms, methods and functions. Springer, Singapore, pp 501–511. https://doi.org/10.1007/978-981-10-5813-4_25

Kawasaki A, Donn S, Ryan PR, Mathesius U, Devilla R, Jones A, Watt M (2016) Microbiome and exudates of the root and Rhizosphere of *Brachypodium distachyon*, a model for wheat. PLoS One 11(10):e0164533–e0164533. https://doi.org/10.1371/journal.pone.0164533

Kazerooni EA, Maharachchikumbura SSN, Rethinasamy V, Al-Mahrouqi H, Al-Sadi AM (2017) Fungal diversity in tomato Rhizosphere soil under conventional and desert farming systems. Front Microbiol 8:1462. https://doi.org/10.3389/fmicb.2017.01462

Kumalawati Z, Musa Y, Amin N, Asrul L, Ridwan I (2014) Exploration of arbuscular mycorrhizal fungi from sugarcane rhizosphere in South Sulawesi. Int J Sci Technol Res 3(1):201–203

Kumar V, Sharma N, Kansal S (2019) Characterization of potential PGPR's from Rhizosphere of wheat from trans-Himalayas and their efficacy on seed germination and growth promotion of wheat under net house conditions. J Plant Dev Sci 11(3):121–131

Lagos L, Maruyama F, Nannipieri P, Mora M, Ogram A, Jorquera M (2015) Current overview on the study of bacteria in the rhizosphere by modern molecular techniques: a mini–review. J Soil Sci Plant Nutr 15(2):504–523

Lee HJ, Jeong SE, Kim PJ, Madsen E, Jeon CO (2015) High resolution depth distribution of bacteria, Archaea, methanotrophs, and methanogens in the bulk and rhizosphere soils of a flooded rice paddy. Front Microbiol 6:639. https://doi.org/10.3389/fmicb.2015.00639

Li H-B, Singh RK, Singh P, Song Q-Q, Xing Y-X, Yang L-T, Li Y-R (2017) Genetic diversity of nitrogen-fixing and plant growth promoting *Pseudomonas* species isolated from sugarcane Rhizosphere. Front Microbiol 8:1268. https://doi.org/10.3389/fmicb.2017.01268

Li Y, Guo Q, Li Y, Sun Y, Xue Q, Lai H (2019) *Streptomyces pactum* Act12 controls tomato yellow leaf curl virus disease and alters rhizosphere microbial communities. Biol Fertil Soils 55 (2):149–169. https://doi.org/10.1007/s00374-019-01339-w

Ma M, Du H, Sun T, An S, Yang G, Wang D (2019) Characteristics of archaea and bacteria in rice rhizosphere along a mercury gradient. Sci Total Environ 650:1640–1651. https://doi.org/10.1016/j.scitotenv.2018.07.175

Mahamuni S, Wani P, Patil A (2012) Isolation of phosphate solubilizing fungi from rhizosphere of sugarcane & sugar beet using TCP & RP solubilization. Asian J Biochem Pharm Res 2 (1):237–244

Majeed A, Abbasi MK, Hameed S, Imran A, Rahim N (2015) Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Front Microbiol 6:198. https://doi.org/10.3389/fmicb.2015.00198

Manici LM, Caputo F (2020) Growth promotion of apple plants is the net effect of binucleate *Rhizoctonia* sp. as rhizosphere-colonizing fungus. Rhizosphere 13:100185. https://doi.org/10.1016/j.rhisph.2020.100185

Marques JM, da Silva TF, Vollu RE, Blank AF, Ding G-C, Seldin L, Smalla K (2014) Plant age and genotype affect the bacterial community composition in the tuber rhizosphere of field-grown sweet potato plants. FEMS Microbiol Ecol 88(2):424–435. https://doi.org/10.1111/1574-6941.12313

Mathew BT, Torky Y, Amin A, Mourad A-HI, Ayyash MM, El-Keblawy A, Hilal-Alnaqbi A, AbuQamar SF, El-Tarabily KA (2020) Halotolerant marine Rhizosphere-competent Actinobacteria promote *Salicornia bigelovii* growth and seed production using seawater irrigation. Front Microbiol 11:552. https://doi.org/10.3389/fmicb.2020.00552

Meena M, Dubey MK, Swapnil P, Zehra A, Singh S, Kumari P, Upadhyay R (2017) 15 the Rhizosphere microbial community and methods of its analysis. Adv PGPR Res:275

Meena RS, Vijayakumar V, Yadav GS, Mitran T (2018) Response and interaction of *Bradyrhizobium japonicum* and arbuscular mycorrhizal fungi in the soybean rhizosphere. Plant Growth Regul 84(2):207–223. https://doi.org/10.1007/s10725-017-0334-8

Mendes LW, Raaijmakers JM, de Hollander M, Mendes R, Tsai SM (2018) Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. ISME J 12 (1):212–224. https://doi.org/10.1038/ismej.2017.158

Mickan BS, Abbott LK, Fan J, Hart MM, Siddique KHM, Solaiman ZM, Jenkins SN (2018) Application of compost and clay under water-stressed conditions influences functional diversity of rhizosphere bacteria. Biol Fertil Soils 54(1):55–70. https://doi.org/10.1007/s00374-017-1238-5

Miller H, Henken G, Veen JV (1989) Variation and composition of bacterial populations in the rhizospheres of maize, wheat, and grass cultivars. Can J Microbiol 35(6):656–660

Miller H, Liljeroth E, Henken G, Veen JV (1990) Fluctuations in the fluorescent pseudomonad and actinomycete populations of rhizosphere and rhizoplane during the growth of spring wheat. Can J Microbiol 36(4):254–258

Misra S, Chauhan PS (2020) ACC deaminase-producing rhizosphere competent *Bacillus* spp. mitigate salt stress and promote *Zea mays* growth by modulating ethylene metabolism. 3 Biotech 10(3):119. https://doi.org/10.1007/s13205-020-2104-y

Monfil VO, Casas-Flores S (2014) Chapter 32 - molecular mechanisms of biocontrol in *Trichoderma* spp. and their applications in agriculture. In: Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG (eds) Biotechnology and biology of Trichoderma. Elsevier, Amsterdam, pp 429–453. https://doi.org/10.1016/B978-0-444-59576-8.00032-1

Moshiri F, Ebrahimi H, Ardakani MR, Rejali F, Mousavi SM (2019) Biogeochemical distribution of Pb and Zn forms in two calcareous soils affected by mycorrhizal symbiosis and alfalfa rhizosphere. Ecotoxicol Environ Saf 179:241–248. https://doi.org/10.1016/j.ecoenv.2019.04.055

Mukhtar S, Mirza MS, Awan HA, Maqbool A, Mehnaz S, Malik KA (2016) Microbial diversity and metagenomic analysis of the rhizosphere of Para grass (*Urochloa mutica*) growing under saline conditions. Pak J Bot 48(2):779–791

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2017) Microbial diversity and soil functions. Eur J Soil Sci 68(1):12–26. https://doi.org/10.1111/ejss.4_12398

Nevita T, Sharma GD, Pandey P (2018) Differences in rice rhizosphere bacterial community structure by application of lignocellulolytic plant-probiotic bacteria with rapid composting traits. Ecol Eng 120:209–221. https://doi.org/10.1016/j.ecoleng.2018.06.007

Newman MM, Hoilett N, Lorenz N, Dick RP, Liles MR, Ramsier C, Kloepper JW (2016a) Glyphosate effects on soil rhizosphere-associated bacterial communities. Sci Total Environ 543:155–160. https://doi.org/10.1016/j.scitotenv.2015.11.008

Newman MM, Lorenz N, Hoilett N, Lee NR, Dick RP, Liles MR, Ramsier C, Kloepper JW (2016b) Changes in rhizosphere bacterial gene expression following glyphosate treatment. Sci Total Environ 553:32–41. https://doi.org/10.1016/j.scitotenv.2016.02.078

Passari AK, Lalsiamthari PC, Zothanpuia LVV, Mishra VK, Yadav MK, Gupta VK, Singh BP (2018) Biocontrol of Fusarium wilt of *Capsicum annuum* by rhizospheric bacteria isolated from turmeric endowed with plant growth promotion and disease suppression potential. Eur J Plant Pathol 150(4):831–846. https://doi.org/10.1007/s10658-017-1325-3

Pathania P, Bhatia R, Khatri M (2020) Cross-competence and affectivity of maize rhizosphere bacteria *Bacillus* sp. MT7 in tomato rhizosphere. Sci Hortic 272:109480. https://doi.org/10.1016/j.scienta.2020.109480

Pérez-Jaramillo JE, Mendes R, Raaijmakers JM (2016) Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol 90(6):635–644. https://doi.org/10.1007/s11103-015-0337-7

Prashar P, Kapoor N, Sachdeva S (2014) Rhizosphere: its structure, bacterial diversity and significance. Rev Environ Sci Biotechnol 13(1):63–77. https://doi.org/10.1007/s11157-013-9317-z

Pratama AA, Terpstra J, de Oliveria ALM, Salles JF (2020) The role of Rhizosphere bacteriophages in plant health. Trends Microbiol. https://doi.org/10.1016/j.tim.2020.04.005

Rana A, Saharan B, Joshi M, Prasanna R, Kumar K, Nain L (2011) Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann Microbiol 61 (4):893–900. https://doi.org/10.1007/s13213-011-0211-z

Rana A, Joshi M, Prasanna R, Shivay YS, Nain L (2012) Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. Eur J Soil Biol 50:118–126. https://doi.org/10.1016/j.ejsobi.2012.01.005

Razzaghi Komaresofla B, Alikhani HA, Etesami H, Khoshkholgh-Sima NA (2019) Improved growth and salinity tolerance of the halophyte *Salicornia* sp. by co–inoculation with endophytic and rhizosphere bacteria. Appl Soil Ecol 138:160–170. https://doi.org/10.1016/j.apsoil.2019.02.022

Salmonová H, Bunešová V (2017) Methods of studying diversity of bacterial Comunities: a review. Sci Agric Bohem 48(3):154–165. https://doi.org/10.1515/sab-2017-0022

Schreiter S, Babin D, Smalla K, Grosch R (2018) Rhizosphere competence and biocontrol effect of *Pseudomonas* sp. RU47 independent from plant species and soil type at the field scale. Front Microbiol 9:97. https://doi.org/10.3389/fmicb.2018.00097

Shahzad T, Chenu C, Genet P, Barot S, Perveen N, Mougin C, Fontaine S (2015) Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biol Biochem 80:146–155. https://doi.org/10.1016/j.soilbio.2014.09.023

Shaikh S, Wani S, Sayyed R (2018) Impact of interactions between rhizosphere and rhizobacteria: a review. J Bacteriol Mycol 5:1058

Shrestha M, Shrestha PM, Frenzel P, Conrad R (2010) Effect of nitrogen fertilization on methane oxidation, abundance, community structure, and gene expression of methanotrophs in the rice rhizosphere. ISME J 4(12):1545–1556. https://doi.org/10.1038/ismej.2010.89

Sindhu SS, Sehrawat A, Sharma R, Dahiya A (2016) Biopesticides: use of rhizosphere bacteria for biological control of plant pathogens. Strain 90:166

Sinegani AAS, Sharifi Z (2007) The abundance of Arbuscular Mycorrhizal fungi spores in Rhizospheres of different crops. Turk J Biol 31(3):181–185

Srivastva N, Singh A, Dubey SK (2018) Methanogenic Archaea in Paddy agricultural fields. In: Choudhary DK, Kumar M, Prasad R, Kumar V (eds) In Silico approach for sustainable agriculture. Springer, Singapore, pp 51–68. https://doi.org/10.1007/978-981-13-0347-0_3

Sugiyama A, Ueda Y, Takase H, Yazaki K (2014) Pyrosequencing assessment of rhizosphere fungal communities from a soybean field. Can J Microbiol 60(10):687–690. https://doi.org/10.1139/cjm-2014-0443

Sunera A, Saqib S, Uddin S, Zaman W, Ullah F, Ayaz A, Asghar M, Rehman S, Munis MFH, Chaudhary HJ (2020) Characterization and phytostimulatory activity of bacteria isolated from tomato (*Lycopersicon esculentum* Mill.) rhizosphere. Microb Pathog 140:103966. https://doi.org/10.1016/j.micpath.2020.103966

Swanson MM, Fraser G, Daniell TJ, Torrance L, Gregory PJ, Taliansky M (2009) Viruses in soils: morphological diversity and abundance in the rhizosphere. Ann Appl Biol 155(1):51–60. https://doi.org/10.1111/j.1744-7348.2009.00319.x

Taffner J, Bergna A, Cernava T, Berg G (2020) Tomato-associated Archaea show a cultivar-specific Rhizosphere effect but an unspecific transmission by seeds. Phytobiomes J 4(2):133–141. https://doi.org/10.1094/PBIOMES-01-20-0017-R

Tam HM, Diep CN (2015) Isolation and identification of Rhizospheric bacteria in sugarcane (*Saccharum* spp. L.) cultivated on Acrisols and Ferrasols of Dong Nai Province, the southeast of Vietnam. Am J Life Sci 3:109–118

Tripaldi C, Novero M, Di Giovanni S, Chiarabaglio P, Lorenzoni P, Meo Zilio D, Palocci G, Balconi C, Aleandri R (2017) Impact of Mycorrhizal fungi and rhizosphere microorganisms on maize grain yield and chemical composition. Pak J Agric Sci 54(4):857–865

van Dam NM, Bouwmeester HJ (2016) Metabolomics in the Rhizosphere: tapping into below-ground chemical communication. Trends Plant Sci 21(3):256–265. https://doi.org/10.1016/j.tplants.2016.01.008

van Elsas JD, Boersma FGH (2011) A review of molecular methods to study the microbiota of soil and the mycosphere. Eur J Soil Biol 47(2):77–87. https://doi.org/10.1016/j.ejsobi.2010.11.010

van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by Rhizosphere bacteria. Annu Rev Phytopathol 36(1):453–483. https://doi.org/10.1146/annurev.phyto.36.1.453

Vanegas J, Muñoz-García A, Pérez-Parra KA, Figueroa-Galvis I, Mestanza O, Polanía J (2019) Effect of salinity on fungal diversity in the rhizosphere of the halophyte *Avicennia germinans* from a semi-arid mangrove. Fungal Ecol 42:100855. https://doi.org/10.1016/j.funeco.2019.07.009

Verma PP, Kaur M (2015) Characterization of mineral phosphate solubilizing activity of fluorescent *Pseudomonas* sp. isolated from rhizosphere of apple. Trends Biosci 8(23):6600–6604

Verma A, Kumar S, Kumar G, Saini JK, Agrawal R, Satlewal A, Ansari MW (2018) Rhizosphere metabolite profiling: An opportunity to understand plant-microbe interactions for crop improvement. In: Ram P, Singh GS, Tuteja N (eds) Crop improvement through microbial biotechnology. Elsevier, Amsterdam, pp 343–361

Vitorino LC, Bessa LA (2018) Microbial diversity: the gap between the estimated and the known. Diversity 10(2):46

Wahyudi AT, Priyanto JA, Afrista R, Kurniati D, Astuti RI, Akhdiya A (2019) Plant growth promoting activity of Actinomycetes isolated from soybean Rhizosphere. Online J Biol Sci 19:1–8

Wang J, Xiong Y, Zhang J, Lu X, Wei G (2020) Naturally selected dominant weeds as heavy metal accumulators and excluders assisted by rhizosphere bacteria in a mining area. Chemosphere 243:125365. https://doi.org/10.1016/j.chemosphere.2019.125365

Wattenburger CJ, Gutknecht J, Zhang Q, Brutnell T, Hofmockel K, Halverson L (2020) The rhizosphere and cropping system, but not arbuscular mycorrhizae, affect ammonia oxidizing archaea and bacteria abundances in two agricultural soils. Appl Soil Ecol 151:103540. https://doi.org/10.1016/j.apsoil.2020.103540

Weinert N, Piceno Y, Ding G-C, Meincke R, Heuer H, Berg G, Schloter M, Andersen G, Smalla K (2011) PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. FEMS Microbiol Ecol 75(3):497–506

White RA, Rivas-Ubach A, Borkum MI, Köberl M, Bilbao A, Colby SM, Hoyt DW, Bingol K, Kim Y-M, Wendler JP, Hixson KK, Jansson C (2017) The state of rhizospheric science in the era of multi-omics: a practical guide to omics technologies. Rhizosphere 3:212–221. https://doi.org/10.1016/j.rhisph.2017.05.003

Williamson KE, Wommack KE, Radosevich M (2003) Sampling natural viral communities from soil for culture-independent analyses. Appl Environ Microbiol 69(11):6628. https://doi.org/10.1128/AEM.69.11.6628-6633.2003

Wu Q-S, Li Y, Zou Y-N, He X-H (2015a) Arbuscular mycorrhiza mediates glomalin-related soil protein production and soil enzyme activities in the rhizosphere of trifoliate orange grown under different P levels. Mycorrhiza 25(2):121–130. https://doi.org/10.1007/s00572-014-0594-3

Wu Z, Hao Z, Zeng Y, Guo L, Huang L, Chen B (2015b) Molecular characterization of microbial communities in the rhizosphere soils and roots of diseased and healthy *Panax notoginseng*. Antonie Van Leeuwenhoek 108(5):1059–1074. https://doi.org/10.1007/s10482-015-0560-x

Yadav AN, Gulati S, Sharma D, Singh RN, Rajawat MVS, Kumar R, Dey R, Pal KK, Kaushik R, Saxena AK (2019) Seasonal variations in culturable archaea and their plant growth promoting attributes to predict their role in establishment of vegetation in Rann of Kutch. Biologia 74 (8):1031–1043. https://doi.org/10.2478/s11756-019-00259-2

Yang CH, Crowley DE, Menge JA (2001) 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. FEMS Microbiol Ecol 35(2):129–136. https://doi.org/10.1111/j.1574-6941.2001.tb00796.x

You X, Kimura N, Okura T, Murakami S, Okano R, Shimogami Y, Matsumura A, Tokumoto H, Ogata Y, Tojo M (2019) Suppressive effects of vermicomposted-bamboo powder on cucumber damping-off. Japan Agric Res Q 53(1):13–19

Zachow C, Müller H, Tilcher R, Berg G (2014) Differences between the rhizosphere microbiome of *Beta vulgaris* ssp. *maritima*—ancestor of all beet crops—and modern sugar beets. Front Microbiol 5:415. https://doi.org/10.3389/fmicb.2014.00415

Zhang H, Wu X, Li G, Qin P (2011) Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella* sp.) and their effects on *Kostelelzkya virginica* growth and enzyme activities of rhizosphere and bulk soils at different salinities. Biol Fertil Soils 47(5):543. https://doi.org/10.1007/s00374-011-0563-3

Zhang Z, Huang J, He L, Sheng X (2016) Distinct weathering ability and populations of Culturable mineral-weathering bacteria in the Rhizosphere and bulk soils of *Morus alba*. Geomicrobiol J 33 (1):39–45. https://doi.org/10.1080/01490451.2015.1004142

Zhao H-T, Li T-P, Zhang Y, Hu J, Bai Y-C, Shan Y-H, Ke F (2017) Effects of vermicompost amendment as a basal fertilizer on soil properties and cucumber yield and quality under continuous cropping conditions in a greenhouse. J Soils Sediments 17(12):2718–2730. https://doi.org/10.1007/s11368-017-1744-y

Zhao D, Zhao H, Zhao D, Zhu X, Wang Y, Duan Y, Xuan Y, Chen L (2018) Isolation and identification of bacteria from rhizosphere soil and their effect on plant growth promotion and root-knot nematode disease. Biol Control 119:12–19. https://doi.org/10.1016/j.biocontrol.2018.01.004

Zheng B-X, Ibrahim M, Zhang D-P, Bi Q-F, Li H-Z, Zhou G-W, Ding K, Peñuelas J, Zhu Y-G, Yang X-R (2018) Identification and characterization of inorganic-phosphate-solubilizing bacteria from agricultural fields with a rapid isolation method. AMB Express 8(1):47. https://doi.org/10.1186/s13568-018-0575-6

Zhu S, Vivanco JM, Manter DK (2016) Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Appl Soil Ecol 107:324–333. https://doi.org/10.1016/j.apsoil.2016.07.009

Zobiole LHS, Kremer RJ, Oliveira RS Jr, Constantin J (2011) Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. J Appl Microbiol 110(1):118–127. https://doi.org/10.1111/j.1365-2672.2010.04864.x

Psychrotrophic Microbes: Biodiversity, Adaptation, and Implications

13

Anita Kumari, Jyoti Upadhyay, and Rohit Joshi

Abstract

Extreme cold environments harbor novel psychrotrophic microbes bestowed with the characteristic to grow in diverse cold habitats worldwide ranging from permanently ice-covered lakes, glaciers, snow, ice cap cores of deep oceans, cloud droplets, and Antarctica. To study the survival mechanism under low temperature, diverse psychrotrophic microbes act as model organisms. These microbes have potentially important and multiple commercial utilities as enzymes, peptides, biodetergents, antibiotics, and bioactive compounds in different areas of industries, agriculture, and pharmaceutics along with multifunctional plant growth-promoting traits. In addition, it also provides an environment-friendly and economically captivating means for improving nutrition acquisition, plant hormone production, and release of siderophores to trigger crop growth under cold stress. Such psychrotrophic microbes are of immense potential for high-altitude and psychrotrophic agroecosystems due to their unique climatic adaptations. Hence, it is of utmost importance to isolate, characterize, and conserve these economically important microbes to reveal their functional

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characteristics under cold temperature. The present chapter provides insights into the biodiversity of psychrotrophic microbes, their adaptation strategies, and their potential applications in agriculture, medicine, industry, food, and allied sectors.

Keywords

Adaptation · Biodiversity · Cryoconite · Diversity · Implications · Plant growth promotion · Psychrophiles · Psychrotolerant · Psychrotrophic

13.1 Introduction

Temperature plays an essential role in the ecology of microbial communities that are known globally as the most efficient colonizers (Mishra et al. 2020). The microbiome of cold habitat is of particular importance as about two third of the global terrestrial and aquatic ecosystem is affected by sub-zero temperatures, while three fourth area remains below 5°C either permanently or seasonally (Awasthi et al. 2019; Margesin and Collins 2019). Psychrotolerant microbes grow near sub-zero temperatures with optimum growth above 30°C and thus are termed as cold-tolerant mesophiles (Mishra et al. 2020). Similarly, the optimum temperature for psychrophiles is below 15°C, while psychrotrophic microbes show optimum growth above 15°C (Srivastava et al. 2013; Kushwaha et al. 2020). Several researchers have isolated, identified, characterized, and sequenced a wide diversity of psychrotrophic microbiomes from different cold habitats worldwide such as snow, Antarctic lakes, Arctic glaciers, permafrost soils, cloud droplets, ice cap cores, cold desert of mountains, subalpine regions, deep-sea permafrost, subglacial lakes, and plants growing at low-temperature conditions (Du et al. 2015; Shen et al. 2015; Verma et al. 2015; Yadav et al. 2015a, b, 2019a, b; Dhar et al. 2016; Margesin et al. 2016; Saxena et al. 2016; Singh et al. 2016; Cai et al. 2017; Zachariah et al. 2017; Jiang et al. 2018; Zhou et al. 2018; Zhang et al. 2018; Kumar et al. 2020; Thakur et al. 2020). In addition, a whole genome analysis of various potential and novel psychrotrophic microbes has been performed (Singh et al. 2016). This diversity of psychrotrophic microbes will serve as a database for selection and utilization of plant growth-promoting (PGP) bio-inoculants for crop improvement at high altitudes (Yadav et al. 2017a, b, 2018).

Psychrophilic/psychrotolerant microorganisms have recently gained our focus because of their vast biotechnological applicability in various industrial, agricultural, and allied sectors (Verma et al. 2015; Yadav et al. 2017a, 2019a). These microbes produce innumerable enzymes, peptides, antifreezing compounds, and antibiotics, besides their multiple plant growth-promoting attributes (Yadav et al. 2017a). In addition, it was also reported that psychrotrophic microbes play a prominent role in enhancing plant stress tolerance against factors such as cold stress (Kushwaha et al. 2020), besides understanding other biotechnological prospects and primitive analogues during primitive earth environments (Yadav et al. 2017a, b). Psychrophilic microbes are even utilized for biodegradation of agro-waste and production of

antifreezing compounds used in cryosurgery and cryopreservation and antifreezing proteins to improve food industry (Yadav et al. 2018). Despite this fact, few reports are available on these psychrotrophic microbes due to their slow growth rate and complicated in vitro culturing (Mishra et al. 2020). This chapter presents the biodiversity, adaptations, and implications of psychrotolerant microbes in different perspectives for future endeavors.

13.2 Biodiversity

Cold environment dominates a major area of earth's biosphere ranging from high altitudes to deep seas at below 15°C temperature (Singh et al. 2020). The average temperature of most of the ocean's portion ranges between −1 and +5°C, while 25% of the global terrestrial area is either alpine or polar region (Yadav et al. 2018). These harsh natural ecosystems are the prosperous hot spots of psychrophilic and psychrotrophic microbiomes with their ability to grow well with reduced metabolic activity below 0°C temperature (Kumar et al. 2019). Extensive studies have demonstrated that commonly found soil microbes, even in the Antarctic region, are either psychrotrophic or psychrotolerant strains of mesophilic species and true psychrophiles are relatively uncommon (Cowan et al. 2011). Among the 14 different phyla of psychrotrophic microbes present in the dry valley of Antarctica are dominated by aerobic groups such as *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes*, but few anaerobic species also occur prominently such as *Arthrobacter*, *Brevibacterium*, and *Corynebacterium* along with few archaeal phylotypes and *Proteobacteria* (Aislabie et al. 2008; Khan 2008; Niederberger et al. 2008; Cary et al. 2010).

Globally several psychropiezophilic microbes have been reported from the sea primarily from three domains eukarya, bacteria, and archaea which include *Vibrio*, *Polaribacter*, *Psychroflexus*, *Psychrobacter*, *Polaromonas*, *Pseudomonas*, *Pseudoalteromonas*, *Moritella*, *Moraxella*, *Micrococcus*, *Flavobacterium*, *Bacillus*, and *Arthrobacter* and belonging to 17 different phyla such as *Verrucomicrobia*, *Thaumarchaeota*, *Spirochaetes*, *Proteobacteria*, *Planctomycetes*, *Nitrospirae*, *Mucoromycota*, *Gemmatimonadetes*, *Firmicutes*, *Euryarchaeota*, *Cyanobacteria*, *Chloroflexi*, *Chlamydiae*, *Basidiomycota*, *Bacteroidetes*, *Ascomycota*, and *Actinobacteria*. The members belonging to *Proteobacteria* are the most dominant followed by *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Basidiomycota* (Yadav et al. 2019a; Kushwaha et al. 2020), while a least number of microbes were isolated from *Mucoromycota* followed by *Gemmatimonadetes*, *Nitrospirae*, and *Thaumarchaeota*. After examining different cold habitats, it was concluded that out of 120 genera of psychrophilic microbes, only *Sphingobacterium*, *Psychrobacter*, *Pseudomonas*, *Exiguobacterium*, and *Bacillus* are found ubiquitously (Yadav et al. 2018).

Over the past several years, researchers have reported various psychrotolerant microbes from diverse environments of Alaska and Tennessee such as *Trichoderma* (Johnson et al. 1987); Canadian soils such as *Mesorhizobium* sp. and *Rhizobium*

leguminosarum (Prevost et al. 1999); cave soil of Russia such as *Pseudochrobactrum kiredjianiae* (Qin et al. 2017); Trans-Himalayan region such as *Pseudomonas* strains with plant growth-promoting activity (Negi et al. 2005; Vyas et al. 2010); subalpine regions of central Himalayas such as phosphate-solubilizing *P. putida* (Pandey et al. 2006); Himalayan cold desert, i.e., *Acinetobacter rhizosphaerae* (Gulati et al. 2009); North Western Himalayas, i.e., biocontrol strain of *Exiguobacterium acetylicum* (Selvakumar et al. 2009); potato fields of the Himalayas such as *Pseudomonas*, *Penicillium*, and *Bacillus* and along with yeasts and actinomycetes (Sati et al. 2013); low-temperature-adapted nodules of alfalfa, i.e., *Sinorhizobium meliloti* (Prevost et al. 2003); wheat seedlings from northern Himalayas such as *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Bordetella*, *Providencia*, *Pseudomonas*, and *Stenotrophomonas* (Verma et al. 2015); rhizosphere of *Podophyllum hexandrum* such as *Virgibacillus arenosi* PH15 (Gautam et al. 2019); *Cucurbita pepo* such as plant growth-promoting *Serratia marcescens* (Selvakumar et al. 2008a, b); Gangotri soil ecosystem such as *Pseudomonas helmanticensis*, *Pseudomonas mandelii*, *Brevibacillus invocatus*, and *Arthrobacter humicola* (Kumar et al. 2019); and cold desert of Arunachal Pradesh such as *Pseudomonas koreensis* P2 (Awasthi et al. 2019). In addition, various novel microbial species were also isolated from cold deserts of the Himalayas (Yadav et al. 2015a, b, 2016). Diverse genotypes of fungi were also reported from the Himalayan region such as *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Gangronella*, *Myrothecium*, *Paecilomyces*, *Penicillium*, and *Trichoderma* (Kushwaha et al. 2020). Few ectomycorrhizal fungal genera were also reported in the Himalayan region such as *Amanita*, *Boletus*, *Hygrophorus*, *Lactarius*, *Russula*, and *Suillus* from temperate forest (Wang et al. 2015); *T. viride*, *T. koningii*, and *T. harzianum* from soil (Ghildiyal and Pandey 2008); *Streptomyces* strains from glaciers (Malviya et al. 2009); and phosphate-solubilizing fungus, i.e., *Paecilomyces hepiali*, from rock soil (Rinu and Pandey 2011).

13.3 Adaptations of Psychrotrophic Microbes

Low temperature adversely affects the microbes by numerous means such as variation in nutritional requirements and decrease in cell number and growth rate, solute solubility, nutrient distribution, cell composition, cell density, and osmotic adjustment of the membrane (Singh et al. 2020). Psychrotrophic microbes tend to remain in the thermal equilibrium with their environments; thus all structural and functional properties of these microorganisms are adapted for low temperature existence (Casanueva et al. 2010). These adaptation strategies include various modifications in structure and physiological, biochemical, and molecular architecture (Ramana et al. 2000). Survival strategies of microbes at low temperatures aggravate a scientific interest because it is useful in unraveling the machinery of life under extreme climatic conditions (Yadav et al. 2019a). In molecular phylogenetics, high-throughput whole genome sequencing has broadened the sensitivity and resolution

of microbial ecology. However, intraregional transfers of organisms due to anthropogenic activities still remain a concern (Cowan et al. 2011).

13.3.1 Cell Membrane-Associated Changes

The cell membrane regulates cellular homeostasis by regulating the transport, ion permeability, and signaling processes. A major metabolic adaptation of psychrophilic microorganisms affecting photosynthesis and growth at cryo-temperatures is the regulation of membrane fluidity and ion permeability (Morgan-Kiss et al. 2006; Casanueva et al. 2010). Lipid unsaturation is thoroughly investigated for cold adaptation (Cossins et al. 2002) in psychrotrophic microbes, and synthesis of polyunsaturated fatty acids is imperative in their chemotaxonomic classification (Morgan-Kiss et al. 2006). With the decrease in temperature, fatty acid unsaturation, methyl branching of cyclic fatty acids, and the ratio of anteiso- to iso-branching increase, while membrane fluidity, ion permeability, membrane phase separation, and average chain length decrease (Singh et al. 2020). Higher polyunsaturated fatty acid content helps psychrotrophic microbes including sea ice diatoms, dinoflagellates, and green algae to absorb nutrients under low-temperature conditions (Cao-Hoang et al. 2010; Wei et al. 2019). It was reported earlier that fatty acid desaturase enzyme, regulated by sensory DesK kinase, causes unsaturation of fatty acids in membrane phospholipids of *Bacillus subtilis* (Singh et al. 2020). In addition, *P. fluorescens* causes structural changes in the outer membrane protein and reduces the pore size of the ion channel at cryo-temperatures (Wei et al. 2019). Similarly, *M. burtonii* produces a high proportion of unsaturated lipids through a distinct pathway reported in other bacteria and eukaryotes (Morgan-Kiss et al. 2006). Fluidity of the chloroplast membrane is directly linked with photosynthesis under low temperatures, which is linked with complex multisubunit membrane-associated proteins of electron transport chain. Membrane fluidity (fatty acyl content of the photosynthetic membranes) is also essential for electron transport to plastoquinone, gaseous diffusion, resistance to photoinhibitory damage, and photosystem II repair cycle and D1 assembly (Morgan-Kiss et al. 2006). The microorganisms transduce cold stress signal through a two-component system machinery, where the signal is received by sensors and transduced to the response regulator, leading to upregulation of genes associated with membrane fluidity (Singh et al. 2020).

13.3.2 Role of Cryoprotectants

Cryoprotectants, such as monosaccharides (glucose, fructose), disaccharides (sucrose, trehalose, etc.), polyamines, polyols (glycerol and sorbitol), and amino acids (glycine, alanine, proline), are chaperones that provide cold stress tolerance (Kawahara et al. 2008). Microorganisms such as *Lactobacillus*, *Pseudomonas*, and *Pantoea* accumulate compatible solutes specially mannitol, glycerol, glycine betaine, and trehalose for protection against freezing, desiccation, and hyperosmolality.

These compounds are secreted outside the cell or are located intracellularly. These polyhydroxylated compounds reduce the freezing point of the cytoplasmic aqueous phase, increase total internal solute concentrations, aggregate cellular proteins, stabilize cytoplasmic macromolecules such as enzymes, and modulate osmotic pressure (Chattopadhyay 2002; Bouvet and Ben 2003; Casanueva et al. 2010). Exopolysaccharides (EPS) produced by psychrophiles under cryo-temperatures have polyhydroxyls that inhibit ice nucleation of water, enzyme denaturation, and cell lysis (Feng et al. 2014). Similarly, ergosterol is a fungal sterol which increases the rigidity of lipid membranes and decreases its permeability, hence ergosterol deficiency in membrane susceptibility to cold stress (Singh et al. 2020).

13.3.3 Cold-Shock Proteins and Cold Acclimation Proteins

A sudden decline in temperature causes a "cold-shock response" in psychrotrophic microbes, resulting in differential regulation of various genes (Casanueva et al. 2010). Psychrophiles release ~20 cold acclimation proteins with a gradual decrease in temperature (Phadtare 2004) and nucleic acid-binding cold-shock proteins (65–75 aa in length) during a sudden decline in temperature (Lee et al. 2013; Czapski and Trun 2014) which help them to adapt under cold environment. Cold-shock proteins (CSPs) are a group of highly conserved small proteins that occur ubiquitously and bind to single-stranded nucleic acids via nucleic acid binding motif termed as cold-shock domain (CSD) (Casanueva et al. 2010; Yadav et al. 2019a). At low temperature, RNA structure stabilizes and becomes non-dynamic to induce early termination of transcription and translation, resulting in protein misfolding and functional hindrance in the ribosome. In mesophiles, RNA chaperone CspAs are reported as CSPs which help in the binding of ribosomes with target mRNA (Singh et al. 2020). In addition, CSPs also maintained the single-stranded state of RNA, reduce the housekeeping gene expression (Barria et al. 2013), and maintain chromosome folding (Chaikam and Karlson 2010).

13.3.4 Ice Nucleators and Antifreeze Proteins

Ice-binding proteins (IBPs) and antifreeze proteins (AFPs) bind to ice and inhibit their crystallization and growth by irreversibly binding to their surface and by inducing high thermal hysteresis activity (Gilbert et al. 2005). Several AFPs were reported from different genera such as *Ascomycetes*, *Basidiomycetes*, and *Oomycetes* which modulate extracellular freezing and mycelial growth at cryo-temperatures (Hoshino et al. 2009). Antifreeze and ice-nucleating activities of AFP are reported in mold *Typhula ishikariensis* (Cheng et al. 2016), Arctic rhizobacterium *Pseudomonas putida* GR12-2 (Muryoi et al. 2004), and *Marinomonas primoryensis* from an Antarctic lake (Gilbert et al. 2005; Singh et al. 2020). Further, *Moraxella*, isolated from Antarctica, was the first AFP-synthesizing bacteria (Yamashita et al. 2002). Ice nucleators or ice-nucleating proteins are present in the outer bacterial wall and

induce ice crystallization near to the melting point to avoid supercooling of water, thereby regulating ice crystal surface arrangement and energy required for ice formation (Casanueva et al. 2010). Bacteria, such as *Erwinia herbicola*, that have the potential of ice crystallization at cryo-temperatures are termed as "ice plus" (Singh et al. 2020).

13.3.5 Cold-Adapted Enzymes: Proteomic and Metagenomic Analysis

Under cryo-temperatures, due to inadequate kinetic energy, the enzymatic reactions become slow, which inhibited microbial growth rate (Wei et al. 2019). It was reported that with the reduction in temperature from 37°C to 0°C, the enzymatic activity of mesophiles gets reduced by 80-fold. In contrast, the growth rate of psychrophilic bacteria gets increased as psychrophilic enzymes (or cold-adapted enzymes) require reduced temperature to decrease activation energy and increase flexibility (Siddiqui et al. 2005; Collins et al. 2008) and increase specific activity (Morgan-Kiss et al. 2006). Further, it involves molecular dynamic simulations of discrete stabilizing interactions at the enzyme active site (Siddiqui and Cavicchioli 2006), besides broadening the cavities for H_2O molecules and/or ligands (Giordano et al. 2015). These enzymes include phytases, peroxidases, catalases, keratinases, pectinases, xylanases, amylases, proteases, lipases, and cellulases (Kuddus et al. 2011; Singh et al. 2020). Thus, the regulation of appropriate metabolic processes of essential enzyme-catalyzed reactions is the major challenge of cold-adapted microorganisms. One energetically inefficient strategy is to increase enzyme concentrations (Morgan-Kiss et al. 2006), while another strategy is to stabilize the enzyme/substrate complex by reducing the activation energy (Ernst et al. 2018). Further, elevated ATP and total adenylate pools governed by F1 ATPase or AMP phosphatase/deaminase compensate for lower rates of biochemical reactions at cryo-temperatures. However, reaction rates (kcat) of psychrophilic enzymes are highly temperature independent (Singh et al. 2020).

Amylases are primarily occurring and the most studied cold-adapted enzyme in microorganisms (Siddiqui and Cavicchioli 2006). However, improved content of pectinases, cellulases, and xylanases was also reported in *Aspergillus awamori* found in the Himalayan region (Anuradha et al. 2010). Another enzyme desaturase introduces a double bond postsynthetically into the fatty acyl chain via an energy-dependent aerobic desaturation pathway (Morgan-Kiss et al. 2006). Proteomic analyses have demonstrated differential regulation of several genes, cold-inducible proteins, cell surface proteins, and nucleic acid-interacting proteins at sub-zero temperatures, suggesting a remodeling of translation, transcription, protein folding, metabolic pathway, energy production, and transport processes for cold adaptation (Morgan-Kiss et al. 2006; Casanueva et al. 2010). Similarly, comparative metagenomics analysis showed psychrophilic amino acid modulations in the genome fragments of Antarctic marine bacteria (Grzymski et al. 2006) resulting in decreased hydrophobic content, proline content, and salt-bridge formations involved

in conformational entropy. Thus, metagenomics sequence data can delineate the adaptation mechanisms and is a rich source for delineating psychrophilic adaptations from psychrotrophic microbes (Casanueva et al. 2010). The structural conservation and metabolic diversity of prokaryotic microorganisms represent adaptive strategies for survival in cold environments.

13.3.6 RNA Degradosomes in Psychrotrophic Microbes

The RNA content within the cell is temporarily regulated and is degraded and reused further for nucleic acid synthesis (Singh et al. 2020). Numerous enzymes were reported for debasing RNA, such as RNA-restricting proteins, 5′-end topping and decapping catalysts, 3′-end nucleotidyltransferases, helicases, and ribonucleases. Psychrophilic microbes consist of a degradosome (multiprotein complex), which causes debasement of delivery moiety RNA and handles ribosomal RNA which is targeted through noncoding RNA. The degradosome consists of enzymes such as RNase E, polynucleotide phosphorylase, and RNA helicase B (Cho 2017; Singh et al. 2020) or exoribonuclease and ribonuclease R (Carpousis et al. 2009; Hardwick et al. 2010). RNases modulate the regulatory protein expression and protein-coding RNA by maturation and degradation. The chaperon activity of CSPs also stabilizes the mRNA under cold conditions. CspA maintains a single-stranded structure, necessary for degradation, while CspE checks RNA degradation (Khemici et al. 2008).

13.3.7 Photosynthetic Electron Transport and Energy Balance

Balancing the energy flow between photochemical and photophysical processes through photosynthesis is called photostasis. When the rate of energy absorbed through PSII and electron transport rate exceeds the metabolic electron sink capacity, an imbalance occurs. Photosynthetically active cyanobacteria are key producers of organic carbon and nitrogen sources under low-temperature conditions such as ponds and dry valleys of Arctic and Antarctic regions (Priscu et al. 2005). Chromophytes are oxygenic phototrophs, preferentially found in cryo-temperatures, such as diatom algae which predominate under sea ice and marine habitats (Morgan-Kiss et al. 2006). Green algae play various roles in low-temperature environments, which are often more likely to be dominated by prokaryotic photosynthetic microorganisms. In cyanobacteria, light-harvesting complex or phycobilisome is an extrinsic pigment-protein complex, bound to the outer cytoplasmic surface of the thylakoids (Morgan-Kiss et al. 2006). In addition, their photosynthetic electron transport chain components are shared with the respiratory chain. It was demonstrated earlier that Antarctic cyanobacterium *P. murrayi* increases the carotenoid/chlorophyll *a* ratio under cryo-temperatures, similar to the green alga *Chlorella vulgaris* under mesophilic conditions. This concluded that mesophilic organisms and psychrotrophic photoautotrophs sense and respond in the same

manner. However, the Antarctic grass *Deschampsia antarctica* acclimates under high light intensity and cold by enhancing photochemical efficiency (Perez-Torres et al. 2004) along with photosynthetic rates, rather than nonphotochemical quenching (Xiong et al. 1999).

13.4 Implications

Psychrotrophic microbes are commercially and ecologically important because of their wide applications in various areas (Soror et al. 2007; Mishra et al. 2020). At present, commercialization and exploitation of psychrotrophic microbes in biotechnology, agriculture, and industrial sector are of profound interest. A few examples of the commercial applications of these psychrotrophic microbes are discussed below.

13.4.1 Plant Growth-Promoting Bioinoculants

Low temperature significantly reduces the global agricultural productivity (Joshi et al. 2018). However, psychrotrophic microbes are very useful in sustainable agricultural and horticultural productivity by retaining their activity even at cryotemperature range (Mishra et al. 2020). In addition, microbial plant growth promoters provide an improved strategy for conventional agricultural practices by invigorating plant growth and development either directly or indirectly (Yadav et al. 2018; Singh et al. 2020). These rhizospheric strains are termed as plant growth-promoting bacteria (PGPB). Psychrotrophic PGPB solubilizes and mineralizes the less available essential complex macronutrient complexes to their simpler forms (Mishra et al. 2020). Various psychrotrophic species causing improvement in different plant growth parameters were isolated from PGPB such as *P. lurida* (Selvakumar et al. 2011), *P. fragi* (Selvakumar et al. 2009), and *P. putida* (Pandey et al. 2006). Similarly, out of 247 morphotypes of psychrotrophic bacilli isolated from soil and water samples of northwestern Himalayas, *Bacillus licheniformis*, *B. muralis*, *Desemzia incerta*, *Paenibacillus tylopili*, and *Sporosarcina globispora* were reported to be potent candidates for multiple PGP traits at low temperature (Yadav et al. 2016). Previous studies have also reported bioformulations of the psychrotrophic PGPB which increases nutrient availability in plants (Verma et al. 2017). Further, the bioformulation enriched with organic manure of biocidal value can be utilized as biocontrol agents for pest management (Mishra et al. 2020).

13.4.1.1 Phytohormone Production

Plant-associated microbes typically produce plant growth hormones such as cytokinins, auxins, and gibberellins. Seed priming with these bacterial strains considerably increased seed germination, shoot length, root growth, biomass, and nutrient uptake in wheat seedling under cold stress (Sahu and Ray 2008; Bahuguna et al. 2012; Singh et al. 2020). Auxin production in microscopic organisms is regulated by proline-dependent pentose phosphate pathway (Sahay et al. 2017).

Indole-3-acetic acid (IAA) is an auxin which exerts positive effect on plant growth (Selvakumar et al. 2008a) and is a marker tool for identification of IAA-secreting psychrophilic microorganisms (Singh et al. 2020). Psychrotolerant *Pseudomonas jesenii* was reported to enhance plant development in *Cajanus cajan*, *Cicer arietinum*, *Eleusine coracana*, *Vigna mungo*, and *Vigna radiate* by producing IAA (Kumar et al. 2014). Further, 1-aminocyclopropane-1-carboxylate (ACC) deaminase was reported to improve plant development at low temperatures and high osmotic pressure (Singh et al. 2020) by diminishing ethylene during virus infections (Mishra et al. 2020). Twenty-five cold-tolerant bacterial strains producing ACC deaminase were reported earlier (Verma et al. 2015).

13.4.1.2 Nitrogen Fixation

Several free-living or symbiotic bacterial strains provide an alternative source for environment-friendly biological nitrogen fertilization by atmospheric nitrogen fixation to improve plant nutrition and growth for sustainable agriculture (Singh et al. 2020). Several genera of nitrogen-fixing psychrotrophic bacteria were isolated earlier such as *Azospirillum*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Gluconacetobacter*, *Serratia*, *Bacillus*, *Azoarcus*, *Arthrobacter*, and *Azotobacter* (Verma et al. 2016; Yadav et al. 2018). On the other hand, nitrogen fixation in the deep Atlantic or Arctic Ocean and freshwater available at high altitudes is the only available source of nitrogen (Díez et al. 2012; Popova et al. 2012). Because of marine nitrogen fixers, nitrogen productivity in the Arctic region increases during the ice melting season with an increase in temperature (Arrigo et al. 2012). It was demonstrated earlier that in cold regions of North America, rhizobia associated with soybean root nodules produce more nodules and show higher nitrogen fixation as compared to warmer southern regions (Zhang et al. 2003).

13.4.1.3 Phosphate Solubilization

Phosphorus is the most abundant macronutrient found in all soil types, but due to its unavailability to plants, it is a major limiting factor for plant growth (Joshi et al. 2009, 2010; Singh et al. 2020). The phosphorus-solubilizing microorganisms release organic metabolites, which chelate the cations bound to inorganic phosphate to make it soluble and readily available to plant roots (Rodriduez and Fraga 1999; Yadav et al. 2018). Only a few reports are available for the psychrotrophic P-solubilizing microorganisms (Vassilev et al. 2006). Glucose dehydrogenase is a membrane-bound enzyme that causes Pi solubilization and oxidation of glucose to gluconic acid and then to 2-ketogluconic acid, which effectively solubilizes P. Similarly, phytase causes organic P mineralization and phosphorus production from organic materials, to be stored as phytate in soil (Yi et al. 2008). Phosphate-solubilizing, fluorescent pseudomonads were isolated from the cold desert of Lahaul and Trans-Himalayan region (Gulati et al. 2008). These isolates solubilize North Carolina rock phosphate much efficiently in comparison to Udaipur rock phosphate and Mussoorie rock phosphate. Similarly, organic and inorganic phosphate-solubilizing psychrotrophic bacteria with multiple plant growth-promoting activities were isolated from *Hippophae rhamnoides* rhizosphere (Vyas et al. 2010).

13.4.1.4 Biofertilizers

Global crop production primarily relies upon chemical fertilizers used for nutrient supplement, which negatively affects the environment, soil, and human health. Thus for the sustainable benefits of agriculture and to improve crop productivity and soil fertility, biofertilizers are an alternative biopotential resource to synthetic fertilizers. Biofertilizers also termed PGP microbes are microorganisms that colonize the roots and enrich the soil nutrients by enhancing the nutrient availability to the crops (Yadav et al. 2018).

13.4.1.5 Production of Siderophores

Microbes indirectly promote plant growth by producing inhibitory substances to prevent the detrimental effects of pathogens or by increasing the natural resistance of the host (Yadav et al. 2018). The pathogenic microorganisms are regulated by releasing siderophores such as chitinases and β-1,3-glucanase (Yadav et al. 2015a, b; Verma et al. 2013, 2015). Further, siderophores help in iron assimilation at low temperatures (Yadav et al. 2018), which predominantly exists in ferric state (Fe^{3+}) in the form of insoluble hydroxides and oxyhydroxides that are unavailable to plants.

13.4.2 Enzyme Production

Psychrotrophic microorganisms synthesize cold-active enzymes by degrading various polymorphic substances such as amylases, cellulases, pectinases, β-galactosidase, oxidases, protease, and lipase (Singh et al. 2020). These psychrozymes possess a high specific activity at low temperatures that prevents time, saves volatile compounds, and prevents contamination and energy loss and have wide applicability in different sectors (Zeng et al. 2003; Margesin et al. 2005; Mishra et al. 2020; Singh et al. 2020). These psychrozymes are the best alternative to bioremediation of wastewaters, marine waters, and solids polluted by lipids, oils, and hydrocarbons under low temperature (Violot et al. 2005). Previous studies have shown that psychrotolerant yeasts of *Phialophora*, *Cladosporium*, *Penicillium* and *Aspergillus* are novel candidates for the production of psychrozymes like esterase, cellulase, amylase, protease, lipase, and pectinase (Carrasco et al. 2016; Dhakar and Pandey 2016). Similarly, the quality of the whey is improved by application of casein-coagulating enzymes obtained from psychrotrophic microorganisms. In developed countries, microbial rennet is commercially available as Moelilase, Rennilase 50TL, and Marzyme® (DuPont, USA). Psychrozyme protease with a brand name Eutrase, obtained from *Bacillus subtilis*, improves the flavor by reducing the ripening period (Kumar and Bhalla 2005). In addition, psychrozymes also facilitate in beer treatment, meat tenderization, and bakeries (Mishra et al. 2020). Through genetic engineering or through random mutagenesis, strains need to be improved for large-scale quantitative as well as qualitative property enhancement (Twardowski and Małyska 2015; Mishra et al. 2020). Ectopic expression of genes from *Shewanella* (psychrotolerant strain Ac10) encoding serine alkaline protease

(*SapSh*) to *E. coli* enhanced the enzyme production up to five times (Kulakova et al. 1999). The construction of a host-vector system in psychrophilic bacteria for gene transformation prevents the formation of inclusion bodies and protects heat-sensitive gene products even at low temperatures (Singh et al. 2020). Recombinant enzymes with enhanced catalytic activity and stability are also used under cryo-temperatures (Mishra et al. 2020).

13.4.2.1 In Food Industry

To meet the daily requirement of the burgeoning population is to ensure perishable food safety by preventing microorganism growth during storage of food (Wei et al. 2019). Several psychrophilic microorganisms such as bacteria, fungi, and yeast, growing actively under low temperatures, are primarily involved in the refrigerated foods such as meat (Cavill et al. 2011; Valerie et al. 2011), milk (Ercolini et al. 2009), and seafood (Kämpfer et al. 2012). A commonly found gram-positive psychrotrophic bacterium in dairy and meat products is *Listeria monocytogenes* (Dhama et al. 2013), while gram-positive strains in raw milk are *Aerococcus urinaeequi*, *Serratia ureilytica*, and *Enterobacter kobei* (Ribeiro Júnior et al. 2018). In contrast, psychrotrophic *Brochothrix thermosphacta* and *Clostridium estertheticum* cause spoilage of meat (Pennacchia et al. 2009) and cold storage foods (Dainty et al. 1989), respectively. It is necessary to develop rapid detection and regulation methodologies for psychrotrophic microorganisms to ensure the quality and safety of refrigerated food products. However, various food industries treat their products with psychrozymes for maintaining the quality of food during their transportation and storage. Further, proteases are useful for the removal of fish skin and cellulases/pectinases in clarification of fruit juices (Singh et al. 2020).

13.4.2.2 In Bioremediation

Psychrotolerant microorganisms can also degrade several compounds at cryo-temperatures (Mishra et al. 2020) and are used to remove pollutants such as toluene, naphthalene, hexadecane, and dodecane (Banerjee et al. 2016). Cold-adapted proteases are used for wastewater treatment and environmental bioremediation at low temperatures (Kasana 2010). In addition, *Rhodococcus* is reported to be useful in degradation of small chain alkanes and diesel under cold environments. A cold-adapted fungus from Pindari Glacier, *Penicillium pinophilum*, simultaneously produces lipase and lignolytic enzymes that are beneficial for biodegradation under cold environments (Dhakar and Pandey 2016).

13.4.2.3 Detergent, Textile, and Fine Chemical Synthesis

Biodetergents have better cleaning properties than synthetic detergents and require low energy input under cold environments (Kuddus and Ramteke 2011). Psychrotrophs are ideal candidates for enzyme production as enzymes active at low temperature and stable under alkaline condition, in presence of oxidants and detergents, are in huge demand as laundry additive and in textile industries (Kasana 2010). *Flavobacterium balustinum* synthesizes cold-active serine protease (CP70) enzyme having an optimum temperature of 20°C less than the conventional

detergent protease like Savinase and efficiently removes proteinaceous stains at cryo-temperatures (Kuddus and Ramteke 2009, 2011). Enzymes such as subtilisin, glycosidases, and lipases are poorly active at room temperature and can thus be substituted by psychrotolerant enzymes (Feller and Gerday 2003). Psychrotolerant microbes produce polyhydroxyalkanoate (PHA) compounds from the polyester group, which serve as intracellular energy and carbon reserves (Mishra et al. 2020). Due to their elastomeric and thermoplastic properties, these compounds are a preferred source for fine chemical synthesis in industries. Further esterases and lipases exhibit more stereospecificity during fine chemical synthesis (Methé et al. 2005). Various psychrozymes showed better utility in production and finishing of fabrics (Mishra et al. 2020) such as cellulases for denim finishing, laccases for textile bleaching, and amylases for desizing of clothes (Araujo et al. 2008). Similarly, proteases provide varied finishing to silk and wool fabrics (Najafi et al. 2005).

13.4.3 Stress Tolerance

Plant-associated extremophilic microorganisms, also help in the promotion and adaptation of plants under extreme environmental conditions, such as high temperature, salt, pH, and drought stress (Singh et al. 2020). Plant growth-promoting bacteria show hyperparasitic activity against pathogenic fungi by excreting cell wall hydrolases such as proteases, phosphatases, lipases, β-glucanase, dehydrogenase, and chitinases. Thus, these bacteria play a significant role in plant growth promotion by protecting them from biotic stress (Yadav et al. 2017b, 2018). Ethylene acts both as a plant growth regulator and stress hormone. However, under abiotic and biotic stresses, endogenous ethylene levels increase significantly which negatively affects plant growth and development. Few bacteria reduce the ethylene levels either by cleaving its precursor, i.e., 1-aminocyclopropane-1-carboxylate (ACC), or by producing the enzyme ACC deaminase to prevent inhibition of plant. Previous researchers characterized 247 psychrotrophic bacteria from wheat from the Indian Himalayas which exhibited multifunctional PGP attributes. Among them, 15 strains showed ACC deaminase activity under cryo-temperatures (Verma et al. 2015).

13.5 Conclusion

Cold ecosystems mark up the largest biospheres globally. Psychrotrophic microbes widely occur in the agroecosystem and are involved in multitudes of growth-promoting activities associated with cold tolerance among different agricultural crops. Recent advancements in inter-valley comparative studies demonstrate that interaction between different trophic levels and abiotic factors is the key driving force in their diversity and survival under these extreme habitats. However, high-throughput analysis will provide new insight into our understanding of microbial diversity, colonizing capability, and screening of potential microbes that retain various functional traits in the field under cryo-temperatures. In addition, their

potential to grow under a wide range of temperatures makes them multi-utility organisms for agricultural, industrial, and allied sectors. These psychrotrophic microbes with myriads of plant growth-promoting mechanisms could have immense direct and indirect potential to improve high-altitude agricultural systems. Therefore, targeted research activities are required to identify and functional cataloguing of psychrotrophic microbe interaction with plant and the extreme environmental conditions such as cold stress, heavy metal toxicity, and agro-waste decomposition. Global strategies are required for field application of these microorganisms to widen their applicability in newer areas of agriculture and industries and easily accessible resource for poor farmers in high altitudes. The potentially beneficial psychrotrophic microbes have varied applications in industrial, agricultural, and allied sectors. Detailed genomic and metagenomics studies linked with expression profiling of these psychrotrophic microbes are required for identification of new photosynthetic models, evolution pattern, diversity, and survival mechanism under low temperatures conditions.

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References

Aislabie JM, Jordan S, Barker GM (2008) Relation between soil classification and bacterial diversity in soils of the Ross Sea region, Antarctica. Geoderma 144:9–20

Anuradha K, Padma PN, Venkateshwar S, Reddy G (2010) Fungal isolates from natural pectic substrates for polygalacturonase and multienzyme production. Indian J Microbiol 50 (3):339–344

Araujo R, Casal M, Cavaco-Paulo A (2008) Application of enzymes for textile fibres processing. Biocatal Biotransformation 26(5):332–349

Arrigo KR, Perovich DK, Pickart RS, Brown ZW, Van Dijken GL, Lowry KE, Mills MM, Palmer MA, Balch WM, Bahr F, Bates NR (2012) Massive phytoplankton blooms under Arctic sea ice. Science 336(6087):1408

Awasthi S, Sharma A, Saxena P, Yadav J, Pandiyan K, Kumar M, Singh A, Chakdar H, Bhowmik A, Kashyap PL, Srivastava AK, Saxena AK (2019) Molecular detection and *in silico* characterization of cold shock protein coding gene (cspA) from cold adaptive *Pseudomonas koreensis*. J Plant Biochem Biotechnol 28:405–413

Bahuguna RN, Joshi R, Shukla A, Pandey M, Kumar J (2012) Thiamine primed defense provides reliable alternative to systemic fungicide carbendazim against sheath blight disease in rice (*Oryza sativa* L.). Plant Physiol Biochem 57:159–167

Banerjee R, Halder A, Natta A (2016) Psychrophilic microorganisms: habitats and exploitation potentials. Eur J Biotechnol Biosci 4:16–24

Barria C, Malecki M, Arraiano CM (2013) Bacterial adaptation to cold. Microbiol Soc 159:2437–2443

Bouvet V, Ben RN (2003) Antifreeze glycoproteins. Cell Biochem Biophys 39(2):133–144

Cai Q, Ye X, Chen B, Zhang B (2017) Complete genome sequence of *Exiguobacterium* sp. Strain N4-1P, a psychrophilic bioemulsifier producer isolated from a cold marine environment in North Atlantic Canada. Genome Announc 5:e01248–e01217

Cao-Hoang L, Dumont F, Marechal PA, Gervais P (2010) Inactivation of *Escherichia coli* and *Lactobacillus plantarum* in relation to membrane permeabilization due to rapid chilling followed by cold storage. Arch Microbiol 192(4):299–305

Carpousis AJ, Luisi BF, McDowall KJ (2009) Endonucleolytic initiation of mRNA decay in *Escherichia coli*. Prog Mol Biol Transl Sci 85:91–135

Carrasco M, Villarreal P, Barahona S, Alcaíno J, Cifuentes V, Baeza M (2016) Screening and characterization of amylase and cellulase activities in psychrotolerant yeasts. BMC Microbiol 16(1):21

Cary SC, McDonald I, Barrett JE, Cowan DA (2010) On the rocks: microbial ecology of Antarctic cold desert soils. Nat Rev Microbiol 8:129–138

Casanueva A, Tuffin M, Cary C, Cowan DA (2010) Molecular adaptations to psychrophily: the impact of 'omic' technologies. Trends Microbiol 18(8):374–381

Cavill L, Renteria-Monterrubio AL, Helps CR, Corry JE (2011) Detection of cold-tolerant clostridia other than Clostridium estertheticum in raw vacuum-packed chill-stored meat. Food Microbiol 28(5):957–963

Chaikam V, Karlson DT (2010) Comparison of structure, function and regulation of plant cold shock domain proteins to bacterial and animal cold shock domain proteins. BMB Rep 43(1):1–8

Chattopadhyay MK (2002) The cryoprotective effects of glycine betaine on bacteria. Trends Microbiol 10(7):311

Cheng J, Hanada Y, Miura A, Tsuda S, Kondo H (2016) Hydrophobic ice-binding sites confer hyperactivity of an antifreeze protein from a snow mold fungus. Biochem J 473(21):4011–4026

Cho KH (2017) The structure and function of the gram-positive bacterial RNA degradosome. Front Microbiol 8:154

Collins T, Roulling F, Piette F, Marx JC, Feller G, Gerday C, D'Amico S (2008) Fundamentals of cold-adapted enzymes. In: Margesin R, Schinner F, Gerday C, Marx JC (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, pp 211–227

Cossins AR, Murray PA, Gracey AY, Logue J, Polley S, Caddick M, Brooks S, Postle T, Maclean N (2002) The role of desaturases in cold-induced lipid restructuring. Biochem Soc Trans 30:1082–1086

Cowan DA, Chown SL, Convey P, Tuffin M, Hughes K, Pointing S, Vincent WF (2011) Non-indigenous microorganisms in the Antarctic: assessing the risks. Trends Microbiol 19(11):540–548

Czapski TR, Trun N (2014) Expression of csp genes in *E. coli* K-12 in defined rich and defined minimal media during normal growth, and after cold-shock. Gene 547(1):91–97

Dainty RH, Edwards RA, Hibbard CM (1989) Spoilage of vacuum-packed beef by a Clostridium sp. J Sci Food Agric 49(4):473–486

Dhakar K, Pandey A (2016) Extracellular laccase from a newly isolated psychrotolerant strain of Cladosporium tenuissimum (NFCCI 2608). Proc Natl Acad Sci India Sect B Biol Sci 86:685–690

Dhama K, Rajagunalan S, Chakraborty S, Verma AK, Kumar A, Tiwari R, Kapoor S (2013) Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. Pak J Biol Sci 16(20):1076–1085

Dhar H, Swarnkar MK, Rana A, Kaushal K, Singh AK, Kasana RC, Gulati A (2016) Complete genome sequence of a low-temperature active and alkaline-stable Endoglucanase-producing *Paenibacillus* sp. strain IHB B 3084 from the Indian Trans-Himalayas. J Biotechnol 230:1–2

Díez B, Bergman B, Pedrós-Alió C, Antó M, Snoeijs P (2012) High cyanobacterial nifH gene diversity in Arctic seawater and sea ice brine. Environ Microbiol Rep 4(3):360–366

Du Y, Yuan B, Zeng Y, Meng J, Li H, Wang R, Li G, Feng F (2015) Draft genome sequence of the cellulolytic bacterium *Clavibacter* sp. CF11, a strain producing cold-active cellulase. Genome Announc 3:e01304–e01314

Ercolini D, Russo F, Ferrocino I, Villani F (2009) Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. Food Microbiol 26(2):228–231
Ernst FG, Erber L, Sammler J, Jühling F, Betat H, Mörl M (2018) Cold adaptation of tRNA nucleotidyltransferases: a tradeoff in activity, stability and fidelity. RNA Biol 15(1):144–155
Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 1(3):200
Feng S, Powell SM, Wilson R, Bowman JP (2014) Extensive gene acquisition in the extremely psychrophilic bacterial species Psychroflexus torquis and the link to sea-ice ecosystem specialism. Genome Biol Evol 6(1):133–148
Gautam N, Sharma P, Rana JC, Singh M (2019) Plant growth promoting traits of a novel psychrotrophic bacterium Virdibacillus arenosi PH15 isolated from rhizosphere of *Podophyllum hexandrum* Royle. Acad J Med Plants 7(1):013–019
Ghildiyal A, Pandey A (2008) Isolation of cold tolerant antifungal strains of *Trichoderma* sp. from glacial sites of Indian Himalayan region. Res J Microbiol 3(8):559–564
Gilbert JA, Davies PL, Laybourn-Parry J (2005) A hyperactive, Ca^{2+}-dependent antifreeze protein in an Antarctic bacterium. FEMS Microbiol Lett 245(1):67–72
Giordano D, Pesce A, Boechi L, Bustamante JP, Caldelli E, Howes BD, Riccio A, Prisco G, Nardini M, Estrin D, Smulevich G (2015) Structural flexibility of the heme cavity in the cold adapted truncated hemoglobin from the Antarctic marine bacterium *Pseudoalteromonas haloplanktis* TAC125. FEBS J 282(15):2948–2965
Grzymski JJ, Carter BJ, DeLong EF, Feldman RA, Ghadiri A, Murray AE (2006) Comparative genomics of DNA fragments from six Antarctic marine planktonic bacteria. Appl Environ Microbiol 72(2):1532–1541
Gulati A, Rahi P, Vyas P (2008) Characterization of phosphate-solubilizing fluorescent pseudomonads from rhizosphere of seabuckthorn growing in cold deserts of Himalayas. Curr Microbiol 56:73–79
Gulati A, Vyas P, Rahi P, Kasana RC (2009) Plant growth-promoting and rhizosphere-competent Acinetobacter rhizosphaerae strain BIHB 723 from the cold deserts of the Himalayas. Curr Microbiol 58:371–377
Hardwick SW, Chan VS, Broadhurst RW, Luisi BF (2010) An RNA degradosome assembly in Caulobacter crescentus. Nucleic Acids Res 39(4):1449–1459
Hoshino T, Xiao N, Tkachenko OB (2009) Cold adaptation in the phytopathogenic fungi causing snow molds. Mycoscience 50(1):26–38
Jiang F, Danzeng W, Zhang Y, Zhang Y, Jiang L, Liu J, Lu L, Fan W, Peng F (2018) *Hymenobacter rubripertinctus* sp. nov., isolated from Antarctic tundra soil. Int J Syst Evol Microbiol 68:663–668
Johnson LF, Bernard EC, Qian P (1987) Isolation of *Trichoderma* spp. at low temperatures from Tennessee and Alaska soils. Plant Dis 71:137–140
Joshi R, Shukla A, Kumar P (2009) *In vitro* flowering in hill maize: a novel technique for future. Indian J Plant Physiol 16:299–302
Joshi R, Shukla A, Kumar P (2010) *In vitro* selection of hill maize (*Zea mays* L.) hybrids for low phosphate tolerance. Indian J Plant Physiol 15(2):159–163
Joshi R, Singh B, Chinnusamy V (2018) Genetically engineering cold stress-tolerant crops: approaches and challenges. In: Cold tolerance in plants. Springer, Cham, pp 179–195
Kämpfer P, Lodders N, Martin K, Avendaño-Herrera R (2012) Flavobacterium chilense sp. nov. and Flavobacterium araucananum sp. nov., isolated from farmed salmonid fish. Int J Systemat Evol Microbiol 62(6):1402–1408
Kasana RC (2010) Proteases from psychrotrophs: an overview. Crit Rev Microbiol 36(2):134–145
Kawahara H, Omori N, Obata H (2008) Cryoprotective activity of mannoprotein from the cell membrane of Pichiaanomala. CryoLetters 29(5):437–445
Khan N (2008) Hypolithic communities in the Miers Valley, Eastern Antarctica. Thesis, University of Western Cape, South Africa

Khemici V, Poljak L, Luisi BF, Carpousis AJ (2008) The RNase E of *Escherichia coli* is a membrane-binding protein. Mol Microbiol 70(4):799–813

Kuddus M, Ramteke PW (2009) Cold-active extracellular alkaline protease from an alkaliphilic *Stenotrophomonas maltophilia*: production of enzyme and its industrial applications. Can J Microbiol 55(11):1294–1301

Kuddus M, Ramteke PW (2011) Production optimization of an extracellular cold-active alkaline protease from *Stenotrophomonas maltophilia* MTCC 7528 and its application in detergent industry. Afr J Microbiol Res 5(7):809–816

Kuddus M, Roohi AJ, Ramteke PW (2011) An overview of cold-active microbial α-amylase: adaptation strategies and biotechnological potentials. Biotechnology 10(3):246–258

Kulakova L, Galkin A, Kurihara T, Yoshimura T, Esaki N (1999) Cold-active serine alkaline protease from the psychrotrophic bacterium *Shewanella* strain Ac10: gene cloning and enzyme purification and characterization. Appl Environ Microbiol 65(2):611–617

Kumar D, Bhalla TC (2005) Microbial proteases in peptide synthesis: approaches and applications. Appl Microbiol Biotechnol 68(6):726–736

Kumar A, Dixit S, Ram T, Yadaw RB, Mishra KK, Mandal NP (2014) Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. J Exp Bot 65:6265–6278

Kumar S, Suyal DC, Yadav A, Shouche Y, Goel R (2019) Microbial diversity and soil physiochemical characteristic of higher altitude. PLoS One 14(3):e0213844

Kumar V, Thakur V, Kumar V, Kumar R, Singh D (2020) Genomic insights revealed physiological diversity and industrial potential for *Glaciimonas* sp. PCH181 isolated from Satrundi glacier in Pangi-Chamba Himalaya. Genomics 112(1):637–646

Kushwaha P, Kashyap PL, Kuppusamy P (2020) Microbes for cold stress resistance in plants: mechanism, opportunities, and challenges. In: Microbiological advancements for higher altitude agro-ecosystems and sustainability. Springer, Singapore, pp 269–292

Lee J, Jeong KW, Jin B, Ryu KS, Kim EH, Ahn JH, Kim Y (2013) Structural and dynamic features of cold-shock proteins of Listeria monocytogenes, a psychrophilic bacterium. Biochemistry 52 (14):2492–2504

Malviya MK, Pandey A, Trivedi P, Gupta G (2009) Chitinolytic activity of cold tolerant antagonistic species of Streptomyces isolated from glacial sites of Indian himalaya. Curr Microbiol 59:502–508

Margesin R, Collins T (2019) Microbial ecology of the cryosphere (glacial and permafrost habitats): current knowledge. Appl Microbiol Biotechnol 103:2537

Margesin R, Dieplinger H, Hofmann J, Sarg B, Lindner H (2005) A cold-active extracellular metalloprotease from P cryoconitis production and properties. Res Microbiol 156:499–505

Margesin R, Zhang D-C, Frasson D, Brouchkov A (2016) Glaciimonas frigoris sp. nov., a psychrophilic bacterium isolated from ancient Siberian permafrost sediment, and emended description of the genus Glaciimonas. Int J Syst Evol Microbiol 66:744–748

Methé BA, Nelson KE, Deming JW, Momen B, Melamud E, Zhang X, Moult J (2005) The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. Proc Natl Acad Sci U S A 102:10913–10918

Mishra PK, Joshi S, Gangola S, Khati P, Bisht JK, Pattanayak A (2020) Psychrotolerant microbes: characterization, conservation, strain improvements, mass production, and commercialization. In: Microbiological advancements for higher altitude agro-ecosystems and sustainability. Springer, Singapore, pp 227–246

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NP (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70(1):222–252

Muryoi N, Sato M, Kaneko S, Kawahara H, Obata H, Yaish MW, Griffith M, Glick BR (2004) Cloning and expression of afpA, a gene encoding an antifreeze protein from the arctic plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. J Bacteriol 186 (17):5661–5671

Najafi MF, Deobagkar D, Deobagkar D (2005) Potential application of protease isolated from *Pseudomonas aeruginosa* PD100. Electron J Biotechnol 8(2):79–85

Negi YK, Kumar J, Garg SK (2005) Cold-tolerant fluorescent *Pseudomonas* isolates from Garhwal Himalayas as potential plant growth promoting and biocontrol agents in pea. Curr Sci 89:2151–2156

Niederberger TD, McDonald IR, Hacker AL, Soo RM, Barrett JE, Wall DH, Cary SC (2008) Microbial community composition in soils of Northern Victoria Land, Antarctica. Environ Microbiol 10(7):1713–1724

Pandey A, Trivedi P, Kumar B, Palni LM (2006) Characterization of a phosphate solubilizing and antagonistic strain of Pseudomonas putida (B0) isolated from a sub-alpine location in the Indian central Himalaya. Curr Microbiol 53(2):102–107

Pennacchia C, Ercolini D, Villani F (2009) Development of a real-time PCR assay for the specific detection of Brochothrix thermosphacta in fresh and spoiled raw meat. Int J Food Microbiol 134 (3):230–236

Pérez-Torres E, García A, Dinamarca J, Alberdi M, Gutiérrez A, Gidekel M, Ivanov AG, Hüner NP, Corcuera LJ, Bravo LA (2004) The role of photochemical quenching and antioxidants in photoprotection of *Deschampsia antarctica*. Funct Plant Biol 31(7):731–741

Phadtare S (2004) Recent developments in bacterial cold-shock response. Curr Issues Mol Biol 6 (2):125–136

Popova EE, Yool A, Coward AC, Dupont F, Deal C, Elliott S, Hunke E, Jin M, Steele M, Zhang J (2012) What controls primary production in the Arctic Ocean? Results from an intercomparison of five general circulation models with biogeochemistry. J Geophys Res Oceans 117(C8): C00D12

Prevost D, Drouin P, Antoun H (1999) The potential use of cold-adapted rhizobia to improve symbiotic nitrogen fixation in legumes cultivated in temperate regions. In: Biotechnological applications of cold-adapted organisms, pp 161–176

Prevost D, Drouin P, Laberge S, Bertrad A, Cloutier J, Levesque G (2003) Cold-adapted rhizobia for nitrogen fixation in temperate regions. Can J Bot 81(12):1153–1161

Priscu JC, Adams EE, Paerl HW, Fritsen CH, Dore JE, Lisle JT, Wolf CF, Mikucki JA (2005) Perennial Antarctic lake ice: a refuge for cyanobacteria in an extreme environment. In: Castello JD, Rogers SO (eds) Life in ancient ice. Princeton Press, Princeton, NJ, pp 22–49

Qin Y, Fu Y, Kanga W, Li H, Gao H, Vitalievitch KS, Liu H (2017) Isolation and identification of a cold-adapted bacterium and its characterization for biocontrol and plant growth-promoting activity. Ecol Eng 105:362–369

Ramana KV, Singh L, Dhaked RK (2000) Biotechnological application of psychrophiles and their habitat to low-temperature. J Sci Ind Res 59:87–101

Ribeiro Júnior J, De Oliveira AM, Silva FD, Tamanini R, De Oliveira AL, Beloti V (2018) The main spoilage-related psychrotrophic bacteria in refrigerated raw milk. J Dairy Sci 101 (1):75–83

Rinu K, Pandey A (2011) Slow and steady phosphate solubilisation by a psychrotolerant strain of *Paecilomyces hepiali* (MTCC 9621). World J Microbiol Biotechnol 27:1055–1062

Rodriduez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Sahay H, Yadav AN, Singh AK, Singh S, Kaushik R, Saxena AK (2017) Hot springs of Indian Himalayas: potential sources of microbial diversity and thermostable hydrolytic enzymes. 3Biotech 7:1–11

Sahu B, Ray MK (2008) Auxotrophy in natural isolate: minimal requirements for growth of the Antarctic psychrotrophic bacterium *Pseudomonas syringae* Lz4W. J Basic Microbiol 48 (1):38–47

Sati P, Dhakar K, Pandey A (2013) Microbial diversity in soil under potato cultivation from Cold Desert Himalaya, India. ISRN Biodiversity 2013: 9

Saxena AK, Yadav AN, Rajawat M, Kaushik R, Kumar R, Kumar M, Prasanna R, Shukla L (2016) Microbial diversity of extreme regions: an unseen heritage and wealth. Indian J Plant Genet Resour 29:246–248

Selvakumar G, Kundu S, Joshi P, Nazim S, Gupta AD, Mishra PK, Gupta HS (2008a) Characterization of a cold tolerant plant growth-promoting bacterium *Pantoea dispersa* 1A isolated from a sub-alpine soil in the North Western Indian Himalayas. World J Microbiol Biotechnol 24 (7):955–960

Selvakumar G, Mohan M, Kundu S, Gupta AD, Joshi P, Nazim S, Gupta HS (2008b) Cold tolerance and plant growth promotion potential of *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash (*Cucurbita pepo*). Lett Appl Microbiol 46 (2):171–175

Selvakumar G, Joshi P, Nazim S, Mishra P, Bisht J, Gupta H (2009) Phosphate solubilisation and growth promotion by *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium isolated from a high altitude Himalayan rhizosphere. Biologia 64(2):239–245

Selvakumar G, Joshi P, Suyal P, Mishra PK, Joshi GK, Bisht JK, Bhatt JC, Gupta HS (2011) *Pseudomonas lurida* M2RH3 (MTCC 9245), a psychrotolerant bacterium from the Uttarakhand Himalayas, solubilize phosphate and promotes wheat seedling growth. World J Microbiol Biotechnol 27:1129–1135

Shen L, Liu Y, Gu Z, Xu B, Wang N, Jiao N, Liu H, Zhou Y (2015) *Massilia eurypsychrophila* sp. nov. a facultatively psychrophilic bacteria isolated from the ice core. Int J Syst Evol Microbiol 65:2124–2129

Siddiqui KS, Cavicchioli R (2006) Cold-adapted enzymes. Annu Rev Biochem 75:403–433

Siddiqui KS, Feller G, D'Amico S, Gerday C, Giaquinto L, Cavicchioli R (2005) The active site is the least stable structure in the unfolding pathway of a multidomain cold-adapted α-amylase. J Bacteriol 187(17):6197–6205

Singh RN, Gaba S, Yadav AN, Gaur P, Gulati S, Kaushik R, Saxena AK (2016) First, high-quality draft genome sequence of plant growth promoting and cold active enzymes producing psychrotrophic Arthrobacter agilis strain L77. Stand Genomic Sci 11:54

Singh H, Sinha N, Bhargava P (2020) Understanding cold-adapted plant growth-promoting microorganisms from high-altitude ecosystems. In: Microbiological advancements for higher altitude agro-ecosystems and sustainability. Springer, Singapore, pp 247–268

Soror SH, Verma V, Rao R, Rasool S, Koul S, Qazi GN, Cullum J (2007) A cold-active esterase of Streptomyces coelicolor A3 (2): from genome sequence to enzyme activity. J Ind Microbiol Biotechnol 34(8):525–531

Srivastava AK, Kumar S, Kaushik R, Saxena AK, Padaria JC, Gupta A, Pal KK, Gujar GT, Sharma A, Singh P (2013) Diversity analysis of Bacillus and other predominant genera in extreme environments and its utilization in agriculture. Tech Rep 414402

Thakur A, Kumar S, Kumari S, Kumar R, Kumar S (2020) Investigation of a night soil compost psychrotrophic bacterium *Glutamicibacter arilaitensis* LJH19 for its safety, efficient hydrolytic and plant growth-promoting potential. bioRxiv.

Twardowski T, Małyska A (2015) Uninformed and disinformed society and the GMO market. Trends Biotechnol 33(1):1–3

Valerie DJ, An C, Koenraad VH, Winy M, Anita VL, Paul DV et al (2011) Influence of storage conditions on the growth of Pseudomonas species in refrigerated raw milk. Appl Environ Microbiol 77(2):460–470

Vassilev N, Vassileva M, Nikolaeva I (2006) Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl Microbiol Biotechnol 71(2):137–144

Verma P, Yadav AN, Kazy SK, Saxena AK, Suman A (2013) Elucidating the diversity and plant growth promoting attributes of wheat (*Triticum aestivum*) associated acidotolerant bacteria from southern hills zone of India. Natl J Life Sci 10:219–227

Verma P, Yadav AN, Khannam KS, Panjiar N, Kumar S, Saxena AK, Suman A (2015) Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the northern hills zone of India. Ann Microbiol 65:1885–1899

Verma P, Yadav AN, Khannam KS, Kumar S, Saxena AK (2016) Molecular diversity and multifarious plant growth promoting attributes of bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. J Basic Microbiol 56:44–58

Verma P, Yadav AN, Kumar V, Singh DP, Saxena AK (2017) Beneficial plant-microbes interactions: biodiversity of microbes from diverse extreme environments and its impact for crops improvement. In: Singh DP, Singh HB, Prabha R (eds) Plant-microbe interactions in agroecological perspectives. Springer, Singapore, pp 543–580

Violot S, Aghajari N, Czjzek M, Feller G, Sonan GK, Gouet P, Gerday C, Haser R, Receveur-Brechot V (2005) Structure of a full length psychrophilic cellulase from Pseudoalteromonas haloplanktis revealed by X-ray diffraction and small angle X-ray scattering. J Mol Biol 348 (5):1211–1224

Vyas P, Joshi R, Sharma KC, Rahi P, Gulati A, Gulati A (2010) Cold-adapted and rhizosphere competent strain of Rahnella sp. with broad-spectrum plant growth-promotion potential. J Microbiol Biotechnol 20(12):1724–1734

Wang M, Jiang X, Wu W, Hao Y, Su Y, Cai L, Xiang M, Liu X (2015) Psychrophilic fungi from the world's roof. Persoonia 34:100–112

Wei Q, Wang X, Sun DW, Pu H (2019) Rapid detection and control of psychrotrophic microorganisms in cold storage foods: a review. Trends Food Sci Technol 86:453–464

Xiong FS, Ruhland CT, Day TA (1999) Photosynthetic temperature response of the Antarctic vascular plants Colobanthus quitensis and Deschampsia antarctica. Physiologia Plantarum 106 (3):276–286

Yadav AN, Sachan SG, Verma P, Saxena AK (2015a) Prospecting cold deserts of North-Western Himalayas for microbial diversity and plant growth promoting attributes. J Biosci Bioeng 119:683–693

Yadav AN, Sachan SG, Verma P, Tyagi SP, Kaushik R, Saxena AK (2015b) Culturable diversity and functional annotation of psychrotrophic bacteria from cold desert of Leh Ladakh (India). World J Microbiol Biotechnol 31(1):95–108

Yadav AN, Sachan SG, Verma P, Saxena AK (2016) Bioprospecting of plant growth promoting psychrotrophic Bacilli from cold desert of north western Indian Himalayas. Indian J Exp Biol 52 (2):142–150

Yadav AN, Verma P, Sachan SG, Saxena AK (2017a) Biodiversity and biotechnological applications of psychrotrophic microbes isolated from Indian Himalayan regions. EC Microbiol Eco 1:48–54

Yadav AN, Verma P, Kumar V, Sachan SG, Saxena AK (2017b) Extreme cold environments: a suitable niche for selection of novel psychrotrophic microbes for biotechnological applications. Adv Biotechnol Microbiol 2(2):1–4

Yadav AN, Verma P, Sachan SG, Kaushik R, Saxena AK (2018) Psychrotrophic microbiomes: molecular diversity and beneficial role in plant growth promotion and soil health. In: Microorganisms for green revolution. Springer, Singapore, pp 197–240

Yadav AN, Kour D, Sharma S, Sachan SG, Singh B, Chauhan VS, Sayyed RZ, Kaushik R, Saxena AK (2019a) Psychrotrophic microbes: biodiversity, mechanisms of adaptation, and biotechnological implications in alleviation of cold stress in plants. In: Plant growth promoting rhizobacteria for sustainable stress management. Springer, Singapore, pp 219–253

Yadav AN, Yadav N, Sachan SG, Saxena AK (2019b) Biodiversity of psychrotrophic microbes and their biotechnological applications. J Appl Biol Biotechnol 7:99–108

Yamashita Y, Nakamura N, Omiya K, Nishikawa J, Kawahara H, Obata H (2002) Identification of an antifreeze lipoprotein from Moraxella sp. of Antarctic origin. Biosci Biotechnol Biochem 66 (2):239–247

Yi Y, Huang W, Ge Y (2008) Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. World J Microbiol Biotechnol 24(7):1059–1065

Zachariah S, Kumari P, Das SK (2017) *Psychrobacter pocilloporae* sp. nov., isolated from a coral, Pocillopora eydouxi. Int J Syst Evol Microbiol 66:5091–5098

Zeng R, Zhang R, Zhao J, Lin N (2003) Cold-active serine alkaline protease from the psychrophilic bacterium Pseudomonas strain DY-A: enzyme purification and characterization. Extremophiles 7(4):335–337

Zhang H, Prithiviraj B, Charles TC, Driscoll BT, Smith DL (2003) Low temperature tolerant Bradyrhizobium japonicum strains allowing improved nodulation and nitrogen fixation of soybean in a short season (cool spring) area. Eur J Agron 19:205–213

Zhang C, Guo W, Wang Y, Chen X (2018) Draft genome sequences of two psychrotolerant strains, Colwellia polaris MCCC 1C00015T and *Colwellia chukchiensis* CGMCC 1.9127 T. Genome Announc 6:e01575–e01517

Zhou MY, Zhang YJ, Zhang XY, Yang XD, He HL, Ning D, Du Z (2018) Flavobacterium phocarum sp. nov., isolated from soils of a seal habitat in Antarctica. Int J Syst Evol Microbiol 68:536–541

Significance of Belowground Microbial-Rhizosphere Interactions

14

C. M. Mehta and Kanak Sirari

Abstract

Soil is considered as a reservoir for microorganisms. A small fraction of soil consists millions of microbes, and microbial activity is referred as maximum near the root zone simply referred as rhizosphere zone. The rhizosphere is defined as the soil zone that exists near the plant roots. Due to plant roots and soil interaction, microbial activity is found maximum in the area. As they are referred as the most active, microbes play a defined role in soil and plant health. This chapter aimed to focus on microbial diversity in the rhizosphere zone and its significance to the crop and soil. A diverse population of microbes associated with different activities in the rhizosphere zone is discussed in detail in this chapter.

Keywords

Soil · Rhizosphere · Microorganisms · Functional diversity · Plant roots

14.1 Introduction

The word "rhizosphere" was coined by Hiltner in the year 1904 to explain the particular relation among bacteria and legume roots. Since then there have been different approaches to define the term. According to Hinsinger et al. (2005) owing

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to a variety of biological, biochemical, chemical, and physical methods that take place as a result of root growth, water and nutrient uptake, respiration, and rhizodeposition, the rhizosphere is unlike to the bulk soil. Darrah (1993) defined rhizosphere as a "zone of soil surrounding the root." Rhizosphere size varies spatially and temporally depending on the cause regarded as follows: for microbial populations and immobile nutrients, it is a portion of a millimeter; for mobile nutrients and water, it is tens of millimeters; and for volatile compounds and gases released from roots, it is numerous tens of millimeters.

In the rhizosphere there is a coexistence of broad variety of organisms including bacteria, fungi, actinomycetes, virus, algae, protozoa, nematodes, and arthropods that have a diversity of connections among themselves as well as with the plant. Plants and most of the microbes are in symbiotic association. Rhizodeposits (root exudates containing lysates, mucilages) are a food source for microorganisms, and in turn microbes help the plants by secreting organic acids, growth-promoting hormones, and siderophores that improve the availability and uptake of nutrients by plants. Rhizosphere microflora have a significant impact on plant growth by several mechanisms including atmospheric nitrogen fixation by diverse classes of proteobacteria, existence of endophytic microbes for improved biotic and abiotic stress tolerance, and presence of plant growth-promoting rhizobacteria.

A complex system of microbial interactions that involves both root-infecting and free-living microbes and associated food webs of microbial grazers is stimulated by the roots of living plants. These microbes are influenced by plant growth, and they affect plant growth. A huge fraction of photosynthetically synthesized carbon of plants is provided to root-infecting symbionts (Lynch and Whipps 1990), for example, mycorrhizal fungi and small fraction are freed as exudates largely to free-living rhizobacteria. Rhizobacteria are robustly synchronized by microfaunal grazers, chiefly protozoa, and mainly the protozoan grazers determine the effect of rhizobacteria on root architecture.

14.2 Rhizosphere

The region of soil that surrounds plant roots and one of the most diverse habitats on earth is rhizosphere (van der Heijden et al. 1998; Torsvik and Øvreås 2002; Jones and Hinsinger 2008). It is a thin zone (1–2 mm thick) that grasps a huge soil volume, differing with the plant, soil, root structure, and mainly the technique employed to establish it since it does not have a definite boundary (Hinsinger et al. 2005). In the rhizosphere microbes affect the host plants in different manners that are either useful consequences like enhancement of plant health and growth or injurious outcomes, i.e., pathogenic behavior. Ahead of making use of the rhizosphere microflora for sustainable agricultural methods, we have to know the composition, ecology, dynamics, and behavior of rhizospheric microbial communities. Rhizosphere is a Greek word ("rhiza"=root and "sphere"=field of influence), originally given by

German scientist Hiltner (1904) and defined as "the zone of soil immediately adjacent to legume roots that supports high levels of bacterial activity." It has been redefined several times to comprise the volume of soil affected by the root and root tissue parts and the soil adjacent to the root in which physical, chemical, and biological properties have been changed by root growth and activity (Pinton 2001).

The rhizosphere is classified into three zones (Clark 1949; Lynch 1987; Pinton 2001):

1. Endorhizosphere: It is the root tissue containing the endodermis and cortical layers.
2. Rhizoplane: Soil particles and microbes stick on this root surface. It is made up of the epidermis, cortex, and mucilaginous polysaccharide layer.
3. Ectorhizosphere: It is made up of soil adjoining the root.

In plants having mycorrhizal relationship, there is an additional zone, mycorrhizosphere (Linderman 1988), and in a few plants an intense layer, rhizosheath, is present that comprises root hairs, mucoid material, microorganisms, and soil particles (Curl and Truelove 1986). Since endophytic microbes inhabit the inner root tissues, the root is also a component of the rhizosphere (Bowen and Rovira 1999). Bulk soil is the volume of the soil that is included in the rhizosphere but not affected by the root (Gobat et al. 2004). Through rhizospheric action the dead root is converted into soil, but it is unlike the bulk soil.

14.3 Diversity of Organisms in the Rhizosphere

The rhizosphere holds a huge diversity of organisms specifically bacteria, fungi, oomycetes, actinomycetes, algae, viruses, protozoa, nematodes, archaea, and arthropods. While comparing to genome size of organisms, plants hold the maximum genome size followed by nematodes, fungi, protozoa, arthropods, algae, bacteria, archaea, and viruses. However, while comparing populations of organisms in similar fraction of soil, bacteria are most abundant in soil followed by viruses, fungi, archaea, algae, protozoa, nematode, and arthropods. Rhizosphere diversity of organisms plays an important role to maintain soil fertility, crop health, and other factors. These organisms are directly involved in manipulating soil's physical, chemical, and biological properties. Rhizosphere soil has an abundance of both beneficial and harmful microorganisms. These can be categorized in beneficial microorganisms, plant pathogens, and human pathogens. A fraction of these microorganisms decides the soil health. In healthy soil, the major fraction of the rhizosphere is covered by beneficial microorganisms followed by a small fraction of plant and human pathogens. When soil is sick, the status of these organisms become vice versa, i.e., the population of pathogens increases tremendously as compared to that of beneficial microorganisms (Figs. 14.1, 14.2 and 14.3).

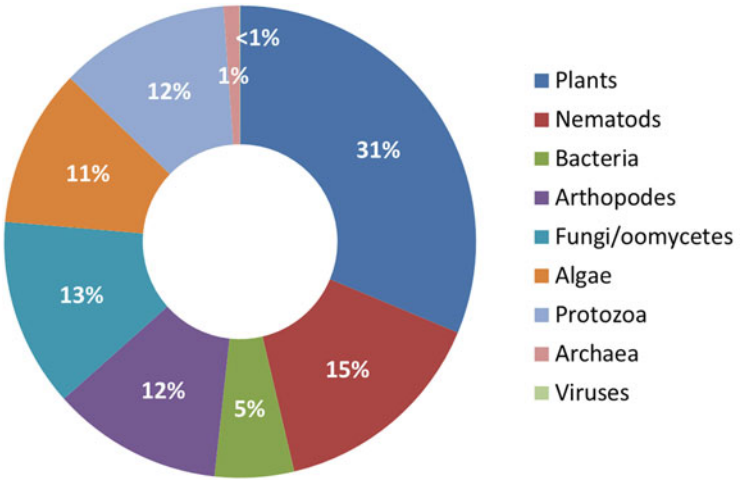

Fig. 14.1 Average number of genes in the genomes of representative species of each group of organisms existing in the rhizosphere (highly modified from Mendes et al. 2013)

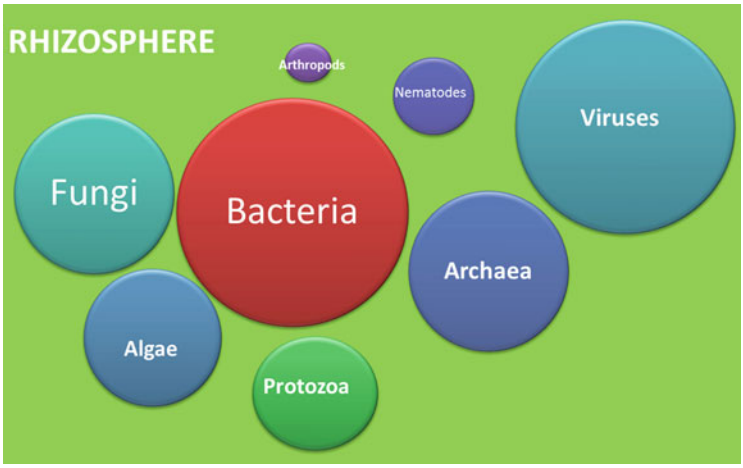

Fig. 14.2 Abundance (population in similar fraction of soil) of organisms in the rhizosphere (highly modified from Mendes et al. 2013)

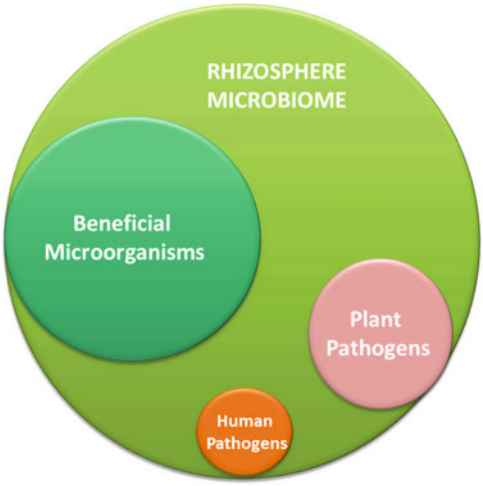

Fig. 14.3 Types of microorganisms and their abundance in the rhizosphere (highly modified from Mendes et al. 2013)

14.4 Significance of Rhizosphere Microorganisms

14.4.1 Decomposition of Organic Matter

In soil, organic matter is mainly present as the uppermost layer of soil profile. Organic matter present up to 20–30 cm of soil depth, and this is also the active zone for rhizosphere microorganisms. Organic matter is a major source of carbon, and microorganisms rely on carbon source for their food. When crop residue returned to the soil, various organic compounds undergo degradation/decomposition. This decomposition is mainly facilitated by soil microorganisms. It is a biological process that includes physical breakdown and biochemical transformation of organic molecules into simpler organic or inorganic molecules. A continuous deposition of biological residue to the soil surface adds a huge amount of organic matter on the upper surface of soil. The major microflora involved in degradation of biological residue, i.e., mainly crop and plant residue (leaf litter), are bacteria, fungi, and actinomyces. These microbes depend on carbon source for their food. Cellulose and polysaccharides are the major constituents of plant litter, and microbes growing on the surface of this litter produce enzymes that degrade this complex molecule to simpler molecules. Due to huge availability of litter, a rapid increase in microbial population occurs at the initial phase. This microbial population mainly belongs to the mesophilic category (Mehta et al. 2012, 2016), and a rapid increase in the population of these microbes occurs due to rapid multiplication. The major microbial population specifically includes *Pseudomonas*, *Bacillus*, *Flavobacterium*, and *Clostridium*. It is reported by many authors including the author of this chapter that there is clear incidence of microbial succession from mesophilic to thermophilic phase. During thermophilic phase, temperature is one of the most important factors for the succession of composting microorganisms.

Enzymes deteriorating structural polysaccharides (like cellulose) that leads to softening of leaf structure and boost in food worth for shredders are produced by fungi and bacteria growing on the leaf surface and inside the mesophyll tissue (Kaushik 1971). After 1 or 2 weeks of immersing in temperate streams, there is an increase in fungal biomass and reproduction (Gessner and Chauvet 1994). Similarly in tropical streams like 10–20 days in Columbia (Mathuriau and Chauvet 2002), or in less time like within 7 days in Costa Rica (Stallcup et al. 2006) has been reported. Comminution and consumption of the litter and linked microbes are completed by invertebrate shredders and huge benthic omnivores (decapods, crabs, and fish), and these invertebrates decrease the leaf particles to minute fragments and fibers with the help of physical degradation by the water current. In several tropical headwaters, most of the coarse litter remains usually elevated (Mathooko 1995; Morara et al. 2003), but all through spates and high-flow actions, a big quantity of leaf matter and fine remains of organic matter are transported to the lesser route and floodplains. In the deposition zone of rivers, this organic substance creates a huge buildup frequently covered as sandy-loamy coating inside "sand/debris dunes" (Fittkau 1982; Wantzen et al. 2005).

In the soil exterior to the biological activity and the carbon cycling, there is addition of the recurring accumulation of decomposing plant deposits. To these courses there is further addition of collapse of soil organic substance and root growth and decomposition. Among the plants, soil, and the atmosphere, there is nonstop conversion of organic and inorganic carbon compounds through plants and micro- and macroorganisms, and this process is carbon cycling. A naturally ongoing biological process is organic matter decomposition. The pace of organic matter decomposition is controlled by soil organisms, the physical environment, and the quality of the organic matter (Brussaard 1994). Carbon dioxide (CO_2), energy, water, plant nutrients, and resynthesized organic carbon compounds are produced throughout this decomposition. A further intricate organic matter known as humus is produced due to consecutive disintegration of dead material and modified organic matter (Juma 1998). Soil characteristics are influenced by humus like soil becomes darker in color, raise in soil aggregation and aggregate stability, and augmentation of the capability to draw and keep hold of the supply of N, P, and other nutrients, because of slow decomposition of humus.

14.4.2 Nutrient Cycling

The rhizospheric microorganisms, both mutualistic symbionts and saprophytic ones, are known to have a crucial role in the cycling of nutrients and their availability to plants. Arbuscular mycorrhizal (AM) fungi are the important mutualistic symbionts present in the soil that increase plant nutrient uptake after creating the AM symbiosis with the majority of plant species. Saprophytic microbes are known to increase nitrogen (N) fixation and/or phosphorus (P) mobilization. After formation of mycorrhiza, the biological and physicochemical properties of the rhizosphere are altered that leads to mycorrhizosphere creation that is crucial in mycorrhizosphere of

legume plants as it too includes the symbiosis with N_2-fixing nodulating rhizobial bacteria.

14.4.2.1 Nitrogen-Fixing Bacteria

As plants are not capable to utilize nitrogen, thus this form of N has to be changed into an available form to plants, i.e., ammonia, through the process called nitrogen fixation. Only a group of prokaryotes (bacteria and archaea) are able to do this nitrogen fixation as they contain the enzyme nitrogenase (Olivares et al. 2013; De Bruijn 2015).

Nitrogen-fixing bacteria include free-living, associative, and symbiotic bacteria (Olivares et al. 2013). Studies show that different free-living bacterial genera can fix nitrogen but with small direct N transport to the plant. On the other hand, mutualistic symbiotic bacteria forming root nodules with the plants can transport the fixation derived ammonium to them. The word "rhizobia" is used for bacterial genera that are able to fix nitrogen in mutualistic symbiosis with legume plants, whereas the bacterial genus *Frankia* (*Actinomycetes*) makes nitrogen-fixing nodules on the actinorhizal plant species roots. The associative bacteria are known to colonize plant root surfaces and can also penetrate intercellular tissues, but no specialized nitrogen-fixing structure formation takes place (Olivares et al. 2013). *Azospirillum*, a free-living nitrogen-fixing rhizobacteria residing most intimately with plant roots as compared to all other free-living bacteria, can make diazotrophic rhizocenosis. *Azospirillum* increases plant's N supply by enhanced N uptake potential of their roots but not as nitrogen-fixing bacteria (Dobbelaere et al. 2001). The most significant and proficient nitrogen-fixing system certainly is the rhizobial legume symbiosis.

14.4.2.2 Phosphate-Mobilizing Microorganisms

In several arable soils throughout the world, P availability is the main restraining issue for crop yield. Hence, plant P nutrition can be facilitated by a few microbes that can mobilize P from scantily accessible resources (Barea and Richardson 2015). Microbial activities improve the discharge of available P from scarcely available soil P forms via two mechanisms, i.e., inorganic (solubilization) and organic (mineralization).

Phosphate Solubilization

In vitro inorganic phosphate resources that are present in an insoluble form (beneath the form of calcium, aluminum, or iron salts) can be proficiently solubilized by the soil bacterial isolates like *Bacillus*, *Enterobacter*, *Rhizobium*, *Bradyrhizobium*, *Pantoea*, *Erwinia*, and *Pseudomonas*, and fungal isolates such as *Aspergillus*, *Trichoderma*, and *Penicillium* (Marschner 2008). Mechanisms of solubilization are chiefly dependent on the proton release and medium acidification but also on the chelation method in the scarcely soluble calcium phosphate forms. While in the case of iron or aluminum phosphates, solubilization involves the chelating organic acid formation. Successful chelation procedure leads to the sequestration of calcium, iron, or aluminum, with the consequent P discharge to the soil solution. Citrate, oxalate,

lactate, succinate, gluconate, and 2-ketogluconate chelating organic acids are from the microbial C metabolism. Studies have shown that the solubilization of Fe phosphates also includes siderophore production (Marschner 2008).

Phosphate Mineralization

Through the method called mineralization of organic P that discharges orthophosphate into the soil solution, bacterial and fungal soil isolates are capable to hydrolyze organic P substrates (Richardson et al. 2009). P-mineralizing microbes include bacteria (which are chiefly *Bacillus* and *Pseudomonas*) and fungi (which are mainly *Aspergillus* and *Penicillium*) (Marschner 2008). Brought by the action of phosphatase enzymes, organic P mineralization is essentially a solubilization or hydrolytic procedure. P-mineralizing microbes are known to make a varied kind of nonspecific enzymes like acid and alkaline phosphatases or the specific ones like phytases that are recognized to discharge orthophosphate from phytate and further inositol phosphates (Jorquera et al. 2008).

14.4.2.3 Arbuscular Mycorrhizal Fungi

These fungi whose origin and divergence are over 500 million years are omnipresent soilborne fungi (Bonfante and Genre 2008; Honrubia 2009; Schüßler and Walker 2011; Barea and Azcón-Aguilar 2013). They are classified under phylum *Glomeromycota* of true fungi (Schüßler et al. 2001). AM fungal associations are known to be present in most of the terrestrial agroecosystems globally and in all soil types and biomes (Brundrett 2009). AM fungi have an obligate symbiotic association with their host plants. They provide mineral nutrients (mainly P) to the host plant roots that their mycelia absorb from the soil solution, and in exchange they get the C compounds required for their growth and metabolism (Kiers et al. 2011).

AM mycelium can be believed as an expansion of the root system as they uptake P from the identical pool of soluble ions than roots (Azcón-Aguilar and Barea 2015). The majority of the P taken by mycorrhizal plants was through the fungal associate as shown by isotopic studies (Smith and Smith 2012). AM fungus can possibly utilize different P sources in soil through enzymatic activities because various research findings specify that phosphatase enzyme activity is higher in soil associated to AM-colonized roots.

AM fungi can be capable of plant N nutrition by taking it up from the soil as findings suggest (Barea et al. 2005; Veresoglou et al. 2012). AM symbiosis was also found to increase the ammonium uptake under all conditions as well as nitrate uptake under drought stress situations through the isotopic studies (Tobar et al. 1994a, b). The uptake of other nutrients like that of K, Ca, Zn, Cu, or Fe can also be increased by AM fungi (Liu et al. 2000). The capability of the AM mycelium to make proficient use of huge soil volume and the existence of nutrient transporters precise of the AM symbiosis are the factors that define the AM fungal ability to uptake these nutrients from the soil solution (Gianinazzi-Pearson et al. 2012).

14.4.3 Altering the Availability of Nutrients to Plants

The important function of nutrient cycling is played by soil rhizospheric microorganisms that are beneficial for plants (Kumar et al. 2017a; Verma et al. 2017). For processes such as nutrient solubilization, mobilization, mineralization, and nutrient uptake, these microorganisms are vital (Verma et al. 2015; Meena et al. 2015; Nath et al. 2017).

Up to 65–95% of the total nitrogen (N) supply of legume crops is through beneficial nitrogen-fixing microbes (Rakshit et al. 2015; Kumar et al. 2017a). There are a variety of these beneficial nitrogen-fixing microorganisms like *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia*. Due to the enhanced N supply to legumes by these beneficial nitrogen fixers, when in a temperate area legumes are used as cover crops, there was about 28% increase in the growth and development of trees as compared to the trees grown in monospecies planted forests (Kumar et al. 2017b).

The main prevailing P-solubilizing bacteria are *Pseudomonas*, *Bacillus*, *Rhizobium*, and *Enterobacter* and fungi are *Penicillium* and *Aspergillus* (Verma et al. 2014a, b). The phosphate rocks are also known to be solubilized by a nematode fungus *Arthrobotrys oligospora* (Cordero et al. 2012; Verma et al. 2014a, b). *Rhizobium* (Verma et al. 2017) and *Azotobacter* are also able of P-solubilization (Kumar et al. 2017b). During both pot experiments and field conditions, an enhancement in plant P availability has been found by inoculation of P-solubilizing microbes (Zaidi et al. 2009; Kumar et al. 2014; Meena et al. 2014).

Potassium (K)-solubilizing bacteria can repress pathogens and enhance soil nutrients and structure, thus offering better plant growth (Pattanayak et al. 2017). Some bacteria are capable to liberate potassium, silicon, and aluminum and produce bioactive substances to improve plant growth through weathering silicate minerals, and therefore these bacteria act as biological K fertilizers and also have a role in biological leaching (Lian et al. 2006; Zhang and Kong 2014; Ma et al. 2016; Nath et al. 2017; Sarkar et al. 2017).

Zinc (Zn) is chiefly absorbed by plants in Zn^{2+} form, whereas in calcareous and high pH soils, it is known to be taken up in $ZnOH^{+}$ form (Gontia-Mishra et al. 2016b). In contrast to chemical fertilizers, Zn solubilization by beneficial microbes is superior. Studies suggest that rhizobacteria bring about the solubilization of insoluble Zn compounds like ZnO, $ZnCO_3$, and $Zn_3\ (PO_4)_2$ (Sarathambal et al. 2010; Rokhbakhsh-Zamin et al. 2011; Kumar et al. 2012; Sharma et al. 2011; Krithika and Balachandar 2016; Gontia-Mishra et al. 2016a). By assisting in solubilization of the insoluble form of Zn and augmenting the Zn uptake, the zinc-solubilizing bacteria result in Zn enrichment of grains (Barbagelata and Mallarino 2013).

Plants discharge phytosiderophores to improve their Fe uptake, but these phytosiderophores have a lesser affinity for iron as compared to microbial siderophores (Li et al. 2016a). Consequently adequate quantity of iron is not taken up by these plants. In plant tissues heavy metal buildup can alter different essential growth functions and also hinder their iron nutrition. Siderophore-producing

rhizosphere bacteria that are able to chelate with Fe^{3+} make Fe accessible to plant roots during these conditions (Rajkumar et al. 2010).

14.4.4 Support Plant Growth Under Biotic and Abiotic Stress

Abiotic and biotic stresses cause a significant decline in global agriculture production (Shinwari et al. 1998). Exposure of plants to abiotic stresses (like water scarcity, high/low temperature, heavy metal toxicity, soil salinity) as well as biotic stresses (insects, pests, or pathogens) can lead to the development of tolerant or resistant plants and also may trigger plant defense mechanisms because of the useful characters of microorganisms (Gómez-Merino and Trejo-Téllez 2018).

14.4.4.1 Biotic Stress

Bacterial species, fungal species, oomycetes, viruses, nematodes, and parasitic plants are known to cause plant diseases (Berg et al. 2017). Employment of microorganisms in the form of biological control tools is well-recognized, and they are able to manage plant diseases and other stress factors as well as encourage plant growth (Dodd and Pérez-Alfocea 2012; Egamberdieva et al. 2013). Biological control agents repress plant disease development by means of methods classified as indirect and direct antagonism, for example, production of antifungal metabolites, proteolytic enzyme formation for plant cell wall biodegradation, induced host resistance, and competition for habitat and nutrients (Li et al. 2016b). Host plant systemic resistance towards pathogens can be influenced by bacterial or fungal plant pathogens through subsequent colonization and entrance into the host plant, causing plant morphology and physiology modification, or by stimulation of the bioactive component production (Melnick et al. 2008).

Direct antagonism methods include the inhibition of injurious plant pathogens through their direct physical contact with the biocontrol bacteria (Pundir and Jain 2015). This can be accomplished by the biocontrol microorganisms through production of extracellular enzymes like β-1,3-glucanase, proteases, and chitinase, as well as antibiotics, siderophores, and hydrogen cyanide (HCN) (Bhatia et al. 2005; Dutta and Khurana 2015). In addition, these microorganisms might as well compete for nutrient attainment and root colonization with the plant pathogens (Haas and Défago 2005). Antibiosis, competition, and hyperparasitism are the mechanisms deployed by the biocontrol microorganisms (Laatsch 2010).

Measures that are not concerned with pathogen recognition through the biological control microbes come under indirect antagonism (Pundir and Jain 2015). Improvement of host plant defense system by a nonpathogenic biological control agent is an example of indirect antagonism leading to mechanisms of stimulation and effort for host plant resistance (Pal and Gardener 2006). Induced systemic resistance (ISR) and systemic acquired resistance (SAR) are the two mechanisms of antagonism. These two mechanisms through the means of inducers and monitoring of pathogens interpret host plant mechanisms of chemical or physical resistance (Singh and Pathak 2015).

14.4.4.2 Abiotic Stress

Temperature, water, salts, nutrients, and pH are the fundamental abiotic or nonliving stress factors affecting plant growth in agriculture (Enebe and Babalola 2018). To deal with abiotic stresses, microorganisms from diverse ecosystems possess huge possibilities (Meena et al. 2017). From stress-tolerant wild plants, plant growth-promoting bacterial strains have been procured that act as successful inoculants for the agricultural crops (Coleman-Derr and Tringe 2014).

Through maintenance of plant antioxidant enzyme concentration, plant growth-promoting bacteria (PGPB) assist in providing plant tolerance against abiotic stresses (Ghosh et al. 2018). Improvement of drought situation is carried by plant growth-promoting rhizobacteria through modification of host plant's biochemical and physiological processes like regulation of phytohormones and antioxidant, production of exopolysaccharides and identical organic solutes (amino acids, sugars, and polyamines), and production of volatile organic compounds (dehydrins) and heat shock protein (Kaushal and Wani 2016a).

Salinity stress has been shown to be lessened by plant growth-promoting bacteria (PGPB). Manufacture of phytohormones and siderophores and uptake of nutrients and nitrogen fixation are some of the direct mechanisms engaged in reducing salinity stress by PGPB. Through induced systemic tolerance (IST), plant growth-promoting rhizobacteria (PGPR) are known to enhance plant tolerance against salinity stress (Kaushal and Wani 2016b; Kumar and Verma 2018).

Particular enzymes assist microorganisms to acclimatize to altering temperature and therefore to sustain their membrane integrity and enzyme stability. In such conditions there is overexpression of heat and cold shock proteins (Alam et al. 2017; Kumar and Verma 2018). Cold-adapted microorganisms can aid plants to survive in difficult climatic conditions in high altitude. In an Indian Himalayan cold desert region, psychrophilic and psychrotolerant bacteria like *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Staphylococcus*, *Burkholderia*, *Brevundimonas*, *Methylobacterium*, *Pantoea*, *Plantibacter*, *Variovorax*, *Rhodococcus*, and others have been found to have plant growth-stimulating characters (Yadav et al. 2015). Likewise at elevated temperature, wheat-isolated heat-tolerant plant-associated bacteria (*Arthrobacter*, *Alcaligenes*, *Bacillus*, *Methylobacterium*, *Delftia*, and several pseudomonads) have been found to have plant growth- and development-promoting qualities (Verma et al. 2019).

For improvement of high-pH stress conditions, an attractive substitute is the application of bioinoculants. Through enhancement of nitrogenase enzyme action (for efficient nitrogen fixation), augmentation in nodule formation in plants via plant growth-promoting bacteria (PGPB) has been found (Abd-Alla et al. 2014).

14.5 Conclusion

There is a huge importance to understand rhizosphere biology and its significance to the soil and plant health. It is a very diverse and broad area of research to understand the significance of different microbes living in the rhizosphere. A rapid change in the

rhizosphere is always reported depending on the environmental and ecological factors, and several researchers have also reported crop-specific rhizosphere diversity. Therefore, it is further needed to explore this area since soil microorganisms have a huge potential and significant importance in crop and soil health that may lead to sustainable agriculture practices.

References

Abd-Alla MH, El-Enany AWE, Nafady NA, Khalaf DM, Morsy FM (2014) Synergistic interaction of Rhizobium leguminosarum bv. viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (Vicia faba L.) in alkaline soil. Microbiol Res 169 (1):49–58

Alam MA, Seetharam K, Zaidi PH, Dinesh A, Vinayan MT, Nath UK (2017) Dissecting heat stress tolerance in tropical maize (Zea mays L.). Field Crop Res 204:110–119

Azcón-Aguilar C, Barea JM (2015) Nutrient cycling in the mycorrhizosphere. J Soil Sci Plant Nutr 15(2):372–396

Barbagelata PA, Mallarino AP (2013) Field correlation of potassium soil test methods based on dried and field-moist soil samples for corn and soybean. Soil Sci Soc Am J 77(1):318–327

Barea JM, Azcón-Aguilar C (2013) Evolution, biology and ecological effects of arbuscular mycorrhizas. Symbiosis: evolution, biology and ecological effects. 1–34

Barea JM, Richardson AE (2015) Phosphate mobilisation by soil microorganisms. In: Principles of plant-microbe interactions. Springer, Cham, pp 225–234

Barea JM, Azcón R, Azcón-Aguilar C (2005) Interactions between mycorrhizal fungi and bacteria to improve plant nutrient cycling and soil structure. In: Microorganisms in soils: roles in genesis and functions. Springer, Berlin, pp 195–212

Berg G, Köberl M, Rybakova D, Müller H, Grosch R, Smalla K (2017) Plant microbial diversity is suggested as the key to future biocontrol and health trends. FEMS Microbiol Ecol 93(5)

Bhatia S, Dubey RC, Maheshwari DK (2005) Enhancement of plant growth and suppression of collar rot of sunflower caused by Sclerotium rolfsii through fluorescent Pseudomonas. Indian Phytopathol 58(1):17–24

Bouffant P, Genre A (2008) Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. Trends Plant Sci 13(9):492–498

Bowen GD, Rovira AD (1999) The rhizosphere and its management to improve plant growth. In: Advances in agronomy, vol 66. Academic Press, pp 1–102

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320(1–2):37–77

Brussaard L (1994) Interrelationships between biological activities, soil properties and soil management. In: Soil resilience and sustainable land use. pp 309–329

Clark FE (1949) Soil microorganisms and plant roots. In: Advances in agronomy, vol 1. Academic Press, pp 241–288.

Coleman-Derr D, Tringe SG (2014) Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. Front Microbiol 5:283

Cordero OX, Ventouras LA, DeLong EF, Polz MF (2012) Public good dynamics drive evolution of iron acquisition strategies in natural bacterioplankton populations. Proc Natl Acad Sci 109 (49):20059–20064

Curl EA, Truelove B (1986) The rhizosphere. In: Advanced series in agricultural science, vol 15. Springer, Berlin

Darrah PR (1993) The rhizosphere and plant nutrition: a quantitative approach. Plant Soil 155 (1):1–20

De Bruijn FJ (2015) Biological nitrogen fixation. In: Principles of plant-microbe interactions. Springer, Cham, pp 215–224

Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Aguirre JF, Kapulnik Y, Brener S (2001) Responses of agronomically important crops to inoculation with Azospirillum. Funct Plant Biol 28(9):871–879

Dodd IC, Pérez-Alfocea F (2012) Microbial amelioration of crop salinity stress. J Exp Bot 63 (9):3415–3428

Dutta S, Khurana SP (2015) Plant growth-promoting rhizobacteria for alleviating abiotic stresses in medicinal plants. In: Plant-growth-promoting rhizobacteria (PGPR) and medicinal plants. Springer, Cham, pp 167–200

Egamberdieva D, Jabborova D, Wirth S (2013) Alleviation of salt stress in legumes by co-inoculation with Pseudomonas and Rhizobium. In: Plant microbe symbiosis: fundamentals and advances. Springer, New Delhi, pp 291–303

Enebe MC, Babalola OO (2018) The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. Appl Microbiol Biotechnol 102(18):7821–7835

Fittkau EJ (1982) Structure, function and diversity of central Amazonian ecosystems. Archiv fur Hydrobiologie. Stuttgart 95(1):29–45

Gessner MO, Chauvet E (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75(6):1807–1817

Ghosh D, Sen S, Mohapatra S (2018) Drought-mitigating Pseudomonas putida GAP-P45 modulates proline turnover and oxidative status in Arabidopsis thaliana under water stress. Ann Microbiol 68(9):579–594

Gianinazzi-Pearson V, van Tuinen D, Wipf D, Dumas-Gaudot E, Recorbet G, Liu Y, et al. (2012) Exploring the genome of Glomeromycotan fungi

Gobat JM, Aragno M, Matthey W (2004) The living soil: fundamentals of soil science and soil biology. Science Publishers

Gómez-Merino FC, Trejo-Téllez LI (2018) The role of beneficial elements in triggering adaptive responses to environmental stressors and improving plant performance. In: Biotic and abiotic stress tolerance in plants. Springer, Singapore, pp 137–172

Gontia-Mishra I, Sapre S, Sharma A, Tiwari S (2016a) Amelioration of drought tolerance in wheat by the interaction of plant growth-promoting rhizobacteria. Plant Biol 18(6):992–1000

Gontia-Mishra I, Sapre S, Sharma A, Tiwari S (2016b) Alleviation of mercury toxicity in wheat by the interaction of mercury-tolerant plant growth-promoting rhizobacteria. J Plant Growth Regul 35(4):1000–1012

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3(4):307–319

Hiltner L (1904) Uber nevere Erfahrungen und Probleme auf dem Gebiet der Boden Bakteriologie und unter besonderer Beurchsichtigung der Grundungung und Broche. Arbeit Deut Landw Ges Berlin 98:59–78

Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW (2005) Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytol 168(2):293–303

Honrubia M (2009) The Mycorrhizae: a plant-fungus relation that has existed for more than 400 million years. Anales del Jardín Botánico de Madrid 66(S1):133–144

Jones DL, Hinsinger P (2008) The rhizosphere: complex by design

Jorquera MA, Hernández MT, Rengel Z, Marschner P, de la Luz Mora M (2008) Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biol Fertil Soils 44(8):1025

Juma NG (1998) The pedosphere and its dynamics: a systems approach to soil science, vol 1. Edmonton, Canada

Kaushal M, Wani SP (2016a) Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. Ann Microbiol 66(1):35–42

Kaushal M, Wani SP (2016b) Rhizobacterial-plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. Agric Ecosyst Environ 231:68–78

Kaushik K (1971) The fate of the dead leaves that fall into streams. Arch Hydrobiol 68:465–515
Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E et al (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333(6044):880–882
Krithika S, Balachandar D (2016) Expression of zinc transporter genes in rice as influenced by zinc-solubilizing Enterobacter cloacae strain ZSB14. Front Plant Sci 7:446
Kumar A, Verma JP (2018) Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52
Kumar V, Sarma MVRK, Saharan K, Srivastava R, Kumar L, Sahai V et al (2012) Effect of formulated root endophytic fungus Piriformospora indica and plant growth promoting rhizobacteria fluorescent pseudomonads R62 and R81 on Vigna mungo. World J Microbiol Biotechnol 28(2):595–603
Kumar A, Kumar A, Pratush A (2014) Molecular diversity and functional variability of environmental isolates of Bacillus species. Springerplus 3(1):312
Kumar A, Maurya BR, Raghuwanshi R, Meena VS, Islam MT (2017a) Co-inoculation with Enterobacter and Rhizobacteria on yield and nutrient uptake by wheat (Triticum aestivum L.) in the alluvial soil under indo-gangetic plain of India. J Plant Growth Regul 36(3):608–617
Kumar A, Meena VS, Maurya BR, Raghuwanshi R, Bisht JK, Pattanayak A (2017b) Towards the biological nitrogen fixation and nitrogen management in legume under sustainable agriculture
Laatsch H (2010) A data base for rapid structural determination of microbial natural products, and annual updates. http://wwwuser.gwdg.de/~ucoc/laatsch/AntiBase.htm
Li M, Ahammed GJ, Li C, Bao X, Yu J, Huang C et al (2016a) Brassinosteroid ameliorates zinc oxide nanoparticles-induced oxidative stress by improving antioxidant potential and redox homeostasis in tomato seedling. Front Plant Sci 7:615
Li N, Alfiky A, Vaughan MM, Kang S (2016b) Stop and smell the fungi: fungal volatile metabolites are overlooked signals involved in fungal interaction with plants. Fungal Biol Rev 30 (3):134–144
Lian X, Wang S, Zhang J, Feng Q, Zhang L, Fan D et al (2006) Expression profiles of 10,422 genes at early stage of low nitrogen stress in rice assayed using a cDNA microarray. Plant Mol Biol 60 (5):617–631
Linderman RG (1988) Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology 78:366–371
Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (Zea mays L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9(6):331–336
Lynch JM (1987) The rhizosphere. Wiley Interscience, Chichester
Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129(1):1–10
Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manag 174:14–25
Marschner P (2008) The role of rhizosphere microorganisms in relation to P uptake by plants. In: The ecophysiology of plant-phosphorus interactions. Springer, Dordrecht, pp 165–176
Mathooko JM (1995) The retention of plant coarse particulate organic matter (CPOM) at the surface of the wet-store and dry-store zones of the Njoro River, Kenya. Afr J Ecol 33(2):151–159
Mathuriau C, Chauvet E (2002) Breakdown of leaf litter in a neotropical stream. J N Am Benthol Soc 21(3):384–396
Meena VS, Maurya BR, Verma JP (2014) Does a rhizospheric microorganism enhance K+ availability in agricultural soils? Microbiol Res 169(5–6):337–347
Meena VS, Verma JP, Meena SK (2015) Towards the current scenario of nutrient use efficiency in crop species
Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A et al (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:172
Mehta CM, Gupta V, Singh S, Srivastava R, Sen E, Romantschuk M, Sharma AK (2012) Role of microbiologically rich compost in reducing biotic and abiotic stresses. In: Microorganisms in environmental management. Springer, Dordrecht, pp 113–134

Mehta CM, Yu D, Srivastava R, Sinkkonen A, Kurola JM, Gupta V et al (2016) Microbial diversity and bioactive substances in disease suppressive composts from India. Compost Sci Util 24 (2):105–116

Melnick RL, Zidack NK, Bailey BA, Maximova SN, Guiltinan M, Backman PA (2008) Bacterial endophytes: Bacillus spp. from annual crops as potential biological control agents of black pod rot of cacao. Biol Control 46(1):46–56

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37 (5):634–663

Morara GO, Mathooko JM, Leichtfried M (2003) Natural leaf litter transport and retention in a second-order tropical stream: the Njoro River, Kenya. Afr J Ecol 41(3):277–279

Nath D, Maurya BR, Meena VS (2017) Documentation of five potassium-and phosphorus-solubilizing bacteria for their K and P-solubilization ability from various minerals. Biocatal Agric Biotechnol 10:174–181

Olivares J, Bedmar EJ, Sanjuán J (2013) Biological nitrogen fixation in the context of global change. Mol Plant-Microbe Interact 26(5):486–494

Pal KK, Gardener BM (2006) Biological control of plant pathogens

Pattanayak A, Bisht JK, Mishra PK, Meena VS (eds) (2017) Agriculturally important microbes for sustainable agriculture. Springer

Pinton R (2001) The rhizosphere as a site of biochemical interactions among soil components, plants and microorganisms. In: The rhizosphere biochemistry and organic substances at the soil-plant interface, pp 1–17

Pundir RK, Jain P (2015) Mechanism of prevention and control of medicinal plant-associated diseases. In: Plant-growth-promoting rhizobacteria (PGPR) and medicinal plants. Springer, Cham, pp 231–246

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28(3):142–149

Rakshit A, Singh HB, Sen A (eds) (2015) Nutrient use efficiency: from basics to advances, vol 417. Springer, New Delhi

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321 (1–2):305–339

Rokhbakhsh-Zamin F, Sachdev D, Kazemi-Pour N, Engineer A, Pardesi KR, Zinjarde S et al (2011) Characterization of plant-growth-promoting traits of Acinetobacter species isolated from rhizosphere of Pennisetum glaucum. J Microbiol Biotechnol 21(6):556–566

Sarathambal C, Thangaraju M, Paulraj C, Gomathy M (2010) Assessing the Zinc solubilization ability of Gluconacetobacter diazotrophicus in maize rhizosphere using labelled 65 Zn compounds. Indian J Microbiol 50(1):103–109

Sarkar D, Meena VS, Haldar A, Rakshit A (2017) Site-specific nutrient management (SSNM): a unique approach towards maintaining soil health. In: Adaptive soil management: from theory to practices. Springer, Singapore, pp 69–88

Schüßler A, Walker C (2011) Evolution of the 'plant-symbiotic' fungal phylum, glomeromycota. In: Evolution of fungi and fungal-like organisms. Springer, Berlin, pp 163–185

Schüβler A, Gehrig H, Schwarzott D, Walker C (2001) Analysis of partial Glomales SSU rRNA gene sequences: implications for primer design and phylogeny. Mycol Res 105(1):5–15

Sharma, S. K. M. P., Ramesh, A., & Joshi, O. P. (2011). Characterization of zinc-solubilizing Bacillus isolates and their potential to influence zinc assimilation in soybean seeds

Shinwari ZK, Nakashima K, Miura S, Kasuga M, Seki M, Yamaguchi-Shinozaki K, Shinozaki K (1998) An Arabidopsis gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. Biochem Biophys Res Commun 250(1):161–170

Singh SK, Pathak R (2015) Ecological manipulations of rhizobacteria for curbing medicinal plant diseases. In: Plant-growth-promoting rhizobacteria (PGPR) and medicinal plants. Springer, Cham, pp 217–230

Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. Mycologia 104(1):1–13

Stallcup LA, Ardon M, Pringle CM (2006) Does nitrogen become limiting under high-P conditions in detritus-based tropical streams? Freshw Biol 51(8):1515–1526

Tobar R, Azcón R, Barea JM (1994a) Improved nitrogen uptake and transport from 15N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. New Phytol 126(1):119–122

Tobar RM, Azcón R, Barea JM (1994b) The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae. Mycorrhiza 4(3):105–108

Torsvik V, Øvreås L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5(3):240–245

Van Der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T et al (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396(6706):69–72

Veresoglou SD, Chen B, Rillig MC (2012) Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol Biochem 46:53–62

Verma JP, Yadav J, Tiwari KN, Jaiswal DK (2014a) Evaluation of plant growth promoting activities of microbial strains and their effect on growth and yield of chickpea (Cicer arietinum L.) in India. Soil Biol Biochem 70:33–37

Verma R, Maurya BR, Meena VS (2014b) Integrated effect of bio-organics with chemical fertilizer on growth, yield and quality of cabbage (Brassica oleracea var capitata). Indian J Agric Sci 84 (8):914–919

Verma JP, Jaiswal DK, Meena VS, Kumar A, Meena RS (2015) Issues and challenges about sustainable agriculture production for management of natural resources to sustain soil fertility and health

Verma R, Maurya BR, Meena VS, Dotaniya ML, Deewan P, Jajoria M (2017) Enhancing production potential of cabbage and improves soil fertility status of Indo-Gangetic Plain through application of bio-organics and mineral fertilizer. Int J Curr Microbiol App Sci 6(3):301–309

Verma P, Yadav AN, Khannam KS, Mishra S, Kumar S, Saxena AK, Suman A (2019) Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India. Saudi J Biol Sci 26(7):1882–1895

Wantzen KM, Drago E, Da Silva CJ (2005) Aquatic habitats of the Upper Paraguay River-Floodplain-System and parts of the Pantanal (Brazil). Int J Ecohydrol Hydrobiol 5(2):107–126

Yadav AN, Sachan SG, Verma P, Saxena AK (2015) Prospecting cold deserts of north western Himalayas for microbial diversity and plant growth promoting attributes. J Biosci Bioeng 119 (6):683–693

Zaidi A, Khan M, Ahemad M, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol Immunol Hung 56(3):263–284

Zhang C, Kong F (2014) Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. Appl Soil Ecol 82:18–25

Functional Niche Under Abiotic Stress

15

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Abstract

The ecosystem functioning of any functional niche is largely dependent on the structure and organization of the inhabiting microbial communities. The microbial communities often display dynamic organization which is under constant exposure to diverse abiotic stress conditions. Such stress conditions play a major role in shaping and influencing the microbial community organization and its diversity. Microorganisms, which represent the earliest life forms, have undergone the longest evolutionary period resulting in the acquisition and development of capabilities to withstand extreme stress conditions. Besides their resilience nature against various abiotic stress conditions, microbes also display adaptability by rapid mutation to counter the stress conditions. As different microbes have different capabilities to tolerate any stress condition, the stress condition often favors the enrichment of microbes which display tolerance to the exposed condition. In this chapter, we summarized various abiotic stress conditions, including temperature, salt, drought, waterlogging, and metal toxicity stress, and how they influence the structure and diversity of the inhabiting microbial community structure and diversity. Various mechanisms employed by microorganisms to withstand these abiotic stress conditions are also described in the chapter.

Keywords

Niche · Abiotic stress · Salt · Temperature · Drought · Waterlogging · Heavy metal toxicity

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15.1 Introduction

Niche is the position or role of a species within a given community or the distributional relation of a species to a range of environments and communities. The three metrics were employed for describing the pattern of functional niche occupation and infer the processes of community assembly (Li et al. 2018a). Environmental gradients including stress and disturbances potentially cause changes in both species richness and trait variations (Loranger et al. 2016; Le Bagousse-Pinguet et al. 2014).

Abiotic stresses include low or high temperature, deficient or excessive water, high salinity, heavy metals, and exposure to radiations, adversely affecting the microbial diversity and growth. Different abiotic stresses can provoke common cellular disorder and secondary stresses, including membrane injury, reactive oxygen species (ROS) damage, protein denaturation, and osmotic stress, and further they all are interrelated with each other. Microbes exhibit a large number of adaptation mechanisms through which they can alleviate the abiotic stresses and successfully thrive in these stress conditions. Microbes when exposed to abiotic stresses undergo rapid metabolic, physicochemical, as well as adaptive changes. Sudden onset of abiotic stress can damage microbial richness of the habitat while chronic exposure of abiotic stressor induces resilience in the individuals of the native microbial community. Microorganisms growing in presence of abiotic stresses actively induce the synthesis and increase in the levels of antioxidant enzymes, accumulation of osmolytes, and expression of different stress-responsive genes. Microbes even show interaction with plants and also enhance germination and establishment of juvenile seedlings under abiotic stress conditions.

In this chapter, we will discuss in detail abiotic stresses and their types and impact on microbial community structure as well as function and adaptive mechanisms shown by the microbes to mitigate the abiotic stresses.

15.2 Functional Niche Under Temperature Stress

The extreme diversity of the soil microbial communities (Tringe et al. 2005) is considered as the dominant driving force of biogeochemical cycles in terrestrial ecosystems (Fierer et al. 2012). The critical roles of microbial communities in regulating the cycling of micro- and macronutrients are the basis of soil fertility, plant health, and the maintenance of macroscopic life (Fierer et al. 2012; Mandakovic et al. 2018). Although microbial communities are highly adaptable and resilience in nature, their structure and co-occurrence are primarily dependent upon various abiotic stress conditions and environmental factors such as temperature (Stomeo et al. 2012), pH of soil (Fierer and Jackson 2006; Lauber et al. 2009), humidity (Angel et al. 2010; Neilson et al. 2017), presence of toxic compounds, salinity, etc. This section will cover the influence of the temperature on the functional niche of microbial communities.

15.2.1 The Temperature Stress

The temperature stress in the microbial communities can be defined as an alteration in the thermal state of the community as a result of a transient or prolonged exposure to elevated or low-temperature conditions. The uncontrolled and ever-increasing anthropogenic activities releasing the greenhouse gases are leading to the variation in rainfall patterns, climate change, and global warming. The global increase in temperature is a serious concern among the scientific communities, and therefore, it is crucial to understand how diversity, abundance, and the structure of the soil microbial community are influenced by the temperature stress.

15.2.2 Influence of Temperature on the Abundance of Microbial Community

The temperature has long been known as an important factor influencing the physiological activity in the soil such as respiration of soil microbes, the plant roots, and rhizosphere (Lundegårdh 1927) and the growth of microorganisms (Ratkowsky et al. 1982). The temperature can influence soil microbial communities by direct or indirect pathways (Shaver et al. 2000), where direct effects are the consequence due to change in temperature and indirect effect is a complicated cascade of effects as a result of interactions among various processes influenced by the direct effect.

Warming has been shown to have a season-dependent impact on the microbial community. The experimental warming of the ecosystem of tallgrass prairie in winter and spring seasons resulted in the increase in the microbial biomass along with an alteration in the efficiency of C and N usage by microbes suggesting a possible shift to a fungi-predominant microbial population, which could favor soil C storage (Belay-Tedla et al. 2009). The warming in summer and early fall, however, displayed a negative effect on the bacterial biomass (Liu et al. 2009).

Besides direct effect, the high temperature also influences soil microbial community indirectly (Shaver et al. 2000; Wan et al. 2005, 2007; Norby and Luo 2016) suggesting that different abiotic factors often work in combination to influence the functional niche of soil microbes. The negative effect on microbial community through the water stress stimulated by the warming condition in the summer was found to be stronger than any positive effects of elevated temperature (Liu et al. 2009). Similarly, in a study on the effects of temperature (15–35°C) and elevation (600–1800 m) on the microbial communities in bamboo plantation soils in Taiwan, an increase in the soil respiration was observed. However, the relative abundance of *Acidobacteria* and *α-Proteobacteria* decreased upon prolonged exposure of 112 days at 35°C, indicating a decrease in bacterial diversity due to the prolonged exposure at 35°C. In contrast to α-*Proteobacteria*, the relative abundance of *γ-Proteobacteria* collected from the elevation of 600–1200 m increased after prolonged incubation at 35°C, while samples collected from 1800 m elevation displayed overall decreases in the abundance (Lin et al. 2017). Phospholipid fatty

acid profiles (PLFA) analysis, which is used for analyzing the relative abundance of Gram-negative, Gram-positive, actinobacteria, and fungi, suggests that the composition of the bacterial community in grassland soil is resilient to the combined effect of water and temperature changes (Balser and Firestone 2005).

The low temperature appears to have a negative effect on the microbial community. In a study on three zonal forests with distinguished climatic conditions including a subtropical, a warm, and a cool temperate forest in China, the temperature among all the treatment factors displayed the most profound effect on C mineralization, which decreased with the drop in temperature from 25°C to 5°C (Tang et al. 2018). In the temperate forest ecosystem, with annual mean temperature and mean precipitation 15.1 mm and 900 mm, respectively (Luan et al. 2011), the soil water and the temperature were identified as the predominant factors in shaping soil microbial community structure (You et al. 2014). The soil water was found to positively influence the abundance of Gram-negative bacterial community, while the soil temperature was found to increase the relative abundance of the saprophytic fungal community and decreased the abundance of the bacterial communities (You et al. 2014).

A metagenome study on the impact of decade-long warming stress on soil microbial community in grassland soil ecosystem revealed that the *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Proteobacteria* are the most abundant bacterial phyla, whereas the most dominant fungal phyla—*Ascomycota and Basidiomycota*—exhibited decreased abundance under the warming conditions (Luo et al. 2014). In a similar study on the microbial community of the Arctic soils, the long-term warming resulted in the notable decrease and significant increase in the evenness of bacterial communities and fungal communities, respectively (Deslippe et al. 2012). The microbial communities in the low-Arctic tundra soils are usually quite stable; however, long-term warming-induced changes in the nutrient cycling consequently result in the change in the dominance level of the microbial communities. Among bacterial composition, the slog-growing Gram +ve *Actinobacteria* displayed a considerable increase in their abundance, while the dominance of *Gemmatimonadaceae* and the *Proteobacteria* decreased significantly. In contrast to the bacterial species, the fungal community exhibited a significant increase in the abundance of *Russula* spp., ectomycorrhizal fungi, *Cortinarius* spp., and *Helotiales* (Deslippe et al. 2012).

15.2.3 Influence of Temperature on Genomic Adaptation

The abiotic factors not only shape the complex structure and assembly of the microbial communities but also trigger their genomic adaptation against the stress conditions such as elevated temperature and carbon dioxide (Heimann and Reichstein 2008; Bond-Lamberty and Thomson 2010; He et al. 2010; Deng et al. 2012). A decade of soil warming of grassland soil in the Midwestern USA displayed a significant increase in the G+C content of microbial community indicating a

selection pressure in favor of genome stability under temperature stress (Luo et al. 2014).

15.2.4 Influence of Temperature on Metabolic Pathways of Microbial Community

Moreover, temperature stress was also found to influence the overall changes in the metabolic pathways. The 2°C warming for 10 years displayed enrichment in the metabolic pathways involved in respiratory pathways, sporulation-related pathways, denitrification, and labile carbon source metabolism including cellulose degradation, glycerate metabolism, and β-glucuronide utilization. In contrast, the fermentation pathways and metabolic pathways for recalcitrant carbon sources such as chitin and lignin are less abundant under warming stress conditions (Luo et al. 2014). Such shifts in the abundance of metabolic pathways indicate that warming promotes higher primary production, higher microbial respiration rates, higher sporulation, and decreased inorganic nitrogen content (Luo et al. 2014). The overall effect of temperature on the functional niche of microorganisms has been summarized in Table 15.1.

15.3 Functional Niche Under Heavy Metal Toxicity Stress

In the early 1980s and 1990s, a series of laboratory-based studies on soil microbes (McGrath et al. 1988; Chaudri et al. 1992; Smith and Giller 1992; Smith 1997) established a new line of research on "effects of heavy metals on soil microorganism." Although heavy metals are naturally produced on the earth's surface by natural activities such as geothermal and volcanic eruptions (Pirrone et al. 2010), the anthropogenic activities such as the uncontrolled use of pesticides and fertilizers, improper and illegal release of industrial wastes, and chemical manufacturing have increased its level tremendously (Nwuche and Ugoji 2008; Selin 2009; Gómez-Sagasti et al. 2012). The problem of heavy metal pollution is rapidly growing and has affected various parts of the world (Imperato et al. 2003; Morton-Bermea et al. 2009; Su 2014; Kou et al. 2018; Tang et al. 2019).

15.3.1 Heavy Metal Toxicity Stress

Heavy metals in trace amounts are vital for living organisms, including microbes, as they are involved in biologically important redox reactions and also serve as metallic cofactors for a wide range of enzymes (Silver and Phung 2005). However, their accumulation in excess leads to the condition known as metal toxicity, which is life-threatening to the living organisms. The heavy metal stress conditions are caused by the excessive accumulation of a variety of heavy metals such as arsenic (Ar), mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), vanadium (V), etc. in

Table 15.1 Effect of temperature on the functional niche of microbes

Abiotic factor	Niche	Species, phyla, metagenome, pathways	Effect	References
Warming in winter and spring seasons	Tallgrass prairie	Fungi	Increase in abundance	Belay-Tedla et al. (2009)
Warming in summer	Terrestrial ecosystem	Bacterial community	Decrease in abundance	Liu et al. (2009)
Elevated temperature (35°C)	Bamboo plantation soil at 600 m elevation	*Acidobacteria*, and *α-Proteobacteria*	Decrease in the abundance	Lin et al. (2017)
		γ-Proteobacteria	Increase in abundance	
	Bamboo plantation soil at 1200 m elevation	*Acidobacteria*, and *α-Proteobacteria*	Decrease in the abundance	
		γ-Proteobacteria	Increase in abundance	
	Bamboo plantation soil at 1800 m elevation	*Acidobacteria*, and *α-Proteobacteria*, and *γ-Proteobacteria*	Decrease in the abundance	
Drop in temperature from 25°C to 5°C	Three zonal forests with distinguished climatic conditions including a subtropical, a warm, and a cool temperate forest	Carbon mineralization	Decrease in carbon mineralization	Tang et al. (2018)
Annual mean temperature of 15°C	Temperate forest	Gram-negative bacterial community	Increase in the abundance	You et al. (2014)
Annual mean precipitation of 900 mm		Saprophytic fungal community	Increase in the abundance	
		Bacterial community	Decrease in the abundance	
Decade long temperature stress (ambient +2°C)	Grassland soil in the Midwestern USA	*Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Proteobacteria*	Increase in abundance	Luo et al. (2014)
		Ascomycota and *Basidiomycota*	Decrease in abundance	
		Metagenome of all microbial communities in the sample	Increase in G +C content	

(continued)

Table 15.1 (continued)

Abiotic factor	Niche	Species, phyla, metagenome, pathways	Effect	References
Long-term warming	Low-Arctic tundra soils	*Actinobacteria*	Increase in the abundance	Deslippe et al. (2012)
		Gemmatimonadaceae and *Proteobacteria*	Decrease in the abundance	
		Russula spp., ectomycorrhizal fungi, *Cortinarius* spp. and *Helotiales*	Increase in the abundance	

soils and other ecological niches. Being recalcitrant and nonbiodegradable, heavy metals can persist for many years affecting soil health and the ecological environment (Khan et al. 2007; Yang et al. 2016; Xiao et al. 2017).

The heavy metal toxicity has been shown to cause protein dysfunction and damage to cell membrane integrity (Leita et al. 1995), damage to the DNA structure (McEntee et al. 1986; Mergeay 1991), and replacement of biologically important elements from cells (Göhre and Paszkowski 2006). Therefore, heavy metal stress is known to shape the structure and diversities of the microbial communities particularly bacteria and fungi (Frey and Rieder 2013; Frossard et al. 2017).

15.3.2 Microbial Mechanism to Deal with Heavy Metal Toxicity Stress

The microbial mechanisms to deal with metal toxicity have a strong evolutionary connection. It is evident that life originated in an anoxic environment rich in toxic metals; therefore, it is plausible that the evolution of early life forms including archean ancestors must have evolved the mechanisms to deal with prevalent toxic heavy metals such as Hg, Ar, Cd, etc. The metal resistance in bacterial system evolved way earlier than the emergence of antibiotic resistance (Hughes and Datta 1983), and therefore it is a misconception that anthropogenic activity causing excessive release of the heavy metals has caused the evolution of heavy metal resistance system. The theory that these toxic metal resistance systems were evolved billions of years ago with the early life forms is further reaffirmed by the abundance of such systems in a wide range of microorganisms irrespective of their place of isolation ranging from heavily polluted sites to the pristine nature (Rodríguez-Rojas et al. 2016; Wang et al. 2016). Moreover evolutionary analysis of microbial genes involved in the detoxification of heavy metals suggests the ancient origin of such genes (Mukhopadhyay et al. 2002; Lebrun et al. 2003; Bhattacharjee and Rosen 2007). However, a prolonged exposure to the heavy metal toxicity would reduce the population of sensitive microbial species, while enriching the resistant species. With time microorganisms have evolved and developed several strategies including

sequestration, active export, enzymatic detoxification, and extracellular precipitation.

15.3.2.1 Extracellular Sequestration

A wide range of microorganisms often use extracellular sequestration as a mechanism to combat metal toxicity. For extracellular sequestration, microbes use different extracellular structures including siderophores (Schalk et al. 2011), glutathione (Lima et al. 2006), bio-surfactants (Banat et al. 2000), ionic functional groups on bacterial cell wall (Naik and Dubey 2013), and extracellular polymeric substances (EPS) (Gupta and Diwan 2017). Siderophores secreted by bacteria and fungi have the ability to bind to several heavy metals including zinc, nickel, copper, manganese, cobalt, mercury, and iron and protect them from heavy metal toxicity by sequestration (Chaturvedi et al. 2012; Yin et al. 2014, 2016; Sharma et al. 2018; Patel et al. 2018). Some microbes such as *Rhizobium leguminosarum* secrete glutathione which has been shown to sequester cadmium (Lima et al. 2006). Microbial surfactants, such as fatty acids, glycolipids, lipopeptides, and phospholipids, are surface-active metabolites, which possess both the hydrophilic moieties (such as amino acids, peptides, carbohydrates, and ions) and the hydrophobic moieties (such as fatty acids) (Ayangbenro and Babalola 2018). The amphiphilic nature of these compounds enables them to form micelles and thereby reducing the interfacial surface tension between fluids of different polarities (Mazaheri Assadi and Tabatabaee 2010). Strains of *Pseudomonas aeruginosa* produces rhamnolipid biosurfactant, which preferentially forms a complex with toxic cationic metal ions such as Cd, Pb, and Zn than with normal cations such as Ca and Mg (Torrens et al. 1998; Herman et al. 2002; Singh and Cameotra 2004). The cell wall of some microbes carries a variety of cationic and anionic functional groups including hydroxyl, carboxyl, amine, and phosphate, which sequester metallic ions and prevent them from entering the cell (Naik and Dubey 2013). EPS offers another means of extracellular sequestration in heavy metal resistant microbes (Table 15.2).

15.3.2.2 Extracellular Precipitation

Sulfate-reducing bacteria such as *Desulfovibrio desulfuricans* have evolved an interesting mechanism to counter the heavy metal toxicity. It produces and secretes hydrogen sulfide in the extracellular environment, which causes precipitation of metallic ions such as Cd, Ni, and Cr, thereby protecting the living cell from heavy metals (Voordouw 1995; Kieu et al. 2011; Joo et al. 2015).

15.3.2.3 Intracellular Sequestration

The mechanism of intracellular sequestration of heavy metal helps microbes prevent the exposure of intracellular components to toxic heavy metals. Some microbes have devised a way to detoxify heavy metal ions by transforming them with the help of sulfides (De Freitas Lima et al. 2011) and cytosolic polyphosphates (De Freitas Lima et al. 2011). The fungus *Trichoderma harzianum* and thermoacidophilic archaeon *Sulfolobus metallicus* have been shown to utilize their polyphosphates to sequester cadmium (De Freitas Lima et al. 2011) and copper (Remonsellez et al. 2006),

Table 15.2 Microbial mechanisms for detoxification of heavy metals

SN	Mechanism	Heavy metals	Species or genus	References
1	Extracellular sequestration by siderophores	Zn, Ni, Cu, Mn, Co, Hg, Fe, Al, Pb, Sn	*Pseudomonades*	Pattus and Abdallah (2000), Braud et al. (2009), Chaturvedi et al. (2012), Yin et al. (2014, 2016), Sharma et al. (2018), Patel et al. (2018)
2	Extracellular sequestration by glutathione	Cd	*Rhizobium leguminosarum*	Lima et al. (2006)
3	Extracellular sequestration by microbial surfactants	Cd, Pb, and Zn	*Pseudomonas aeruginosa*	Torrens et al. (1998), Herman et al. (2002), Singh and Cameotra (2004)
4	Extracellular sequestration by EPS	Cu, Pb	*Methylobacterium organophilum*	Kim et al. (1996)
		As	*Herminiimonas arsenicoxydans*	Marchal et al. (2010)
			Thiomonas sp.	Marchal et al. (2011)
5	Extracellular precipitation of heavy metals by hydrogen sulfide	Cd, Ni, and Cr	*Desulfovibrio desulfuricans*	Voordouw (1995), Kieu et al. (2011), Joo et al. (2015)
6	Intracellular sequestration using metallothionein	Cd, Zn	*Synechococcus* sp.	Blindauer et al. (2008)
7	Intracellular sequestration by biomineralization	Cd	*Bacillus cereus*	Li et al. (2018b)
		Ni, Cu, Pb, Co, Zn, and Cd	Urease-producing bacteria	Li et al. (2013), Khadim et al. (2019)
8	Intracellular sequestration by polyphosphates	Cd	*Trichoderma harzianum*	De Freitas Lima et al. (2011)
		Cu	*Sulfolobus metallicus*	Remonsellez et al. (2006)
9	Efflux pumps	Cd	*S. aureus*	Smith and Novick (1972), Nies (1992)
10	Enzymatic detoxification	Hg	*Bacillus* sp.	Noroozi et al. (2017)
		As	*Micrococcus* sp., *Acinetobacter* sp.	Nagvenkar and Ramaiah (2010)

respectively. Biomineralization—the natural process of forming minerals by microorganisms—offers an efficient way to sequester toxic heavy metals (Li et al. 2013, 2018b). Cysteine-rich protein metallothioneins function as a sink for toxic metals, and their expression elevates during the metal stress (Blindauer et al. 2008).

15.3.2.4 Enzymatic Redox Reactions and Export Pumps

Enzymatic redox reactions and export pumps are widely used mechanisms by Hg- and As-resistant bacteria. The toxicity of Hg is attributed to its high affinity for the sulfhydryl ligands (-SH group) in the amino acids causing structural changes in the protein often leading to the loss of functions (Nies 2003). The microbial response to the Hg(II) is extensively studied in the last three decades. A wide range of Gram-negative and Gram-positive bacterial isolates from environmental, industrial, and clinical isolates harbor mercury resistance *mer* operon, which encodes for proteins involved in detoxification of mercury (Silver and Phung 2005; Dash and Das 2012). These genes may be located either on chromosomal DNA as in *Bacillus* isolates or on extrachromosomal plasmid DNA (Narita et al. 2003; Silver and Phung 2005; Dash and Das 2012), and also the number of genes in the "*mer* operon" and the identity of genes vary from species to species (Wilson et al. 2000).

The components of *mer* operon includes MerA, MerB, MerC (or MerT or MerF), MerD, MerP, and MerR. The bacteria first bring the toxic mercury compound from outside to its cytoplasm, where cytoplasmic enzymes can detoxify it. In the first step, the periplasmic protein MerP acts as a mercuric ion scavenging protein. The Cys-X-X-Cys motif of MerP protein first binds to the Hg(II) in the periplasmic space in a linear S-Hg-S manner (Steele and Opella 1997; Ballou and Miller 1999). The MerP protein then transfers the Hg(II) to the inner membrane protein MerT, which drives the transport of mercuric ion into the cytoplasm via membrane potential. In addition to MerT, bacterial species may possess other divergent mercuric ion transporters including MerC, MerF, and MerE. Among these mercuric ion transporters, MerC appeared as the most efficient Hg(II) transporter compared to MerT, MerE, and MerF. These mercuric ion transporters are broad-spectrum mercury transporters and are capable of transporting Hg(II) and phenylmercury ($C_6H_5Hg(I)$) (Sone et al. 2013a, b). As all these three membrane proteins are mercuric ion transporters, it is conceivable that they share some similarities despite their different structures and divergent sequences. A pair of cysteine residues is present on the inner membrane, while another pair of cysteine residues is located on the cytoplasmic face of these ion transporters. Moreover, these proteins also display similarity in the presence of a proline residue and one charged residue in the second helix (Wilson et al. 2000). The mercuric ion transporter transfers the Hg (II) to the MerA protein, which is a large homodimer of mercuric reductase, which is a flavoprotein containing FAD as the cofactor. The electron transfer from the FAD cofactor at the active site of MerA reduces the Hg(II) to volatile, relatively inert, monoatomic Hg(0) vapor (Schiering et al. 1991; Engst and Miller 1999; Barkay et al. 2003).

The *mer* operon is regulated by a metal responsive regulatory protein MerR. In the absence of metal, the apo MerR acts as a repressor protein and binds to the promoter/operator region of the *mer* operon. However, the binding of Hg(II) to the MerR protein results in the conformational transition causing the activation of the *mer* operon (Chang et al. 2015). Many *mer* operons also encodes for MerB protein that is involved in detoxification of organomercurials by protonolysis (Barkay et al. 2003). MerB is a monomeric lyase that cleaves the Hg-C covalent bond. The resultant Hg(II) then can be reduced by MerA protein (Silver and Phung 2005).

Arsenic is primarily found in four different oxidation states including arsenate (As(V)), arsenite (As(III), elemental arsenic (As(0)), and arsenide (As(-III)). Microorganisms have developed different strategies to deal with As toxicity. Microbial enzymes As(III) oxidase and methyltransferase can oxidize and methylate As (III), respectively. As can also be utilized by microorganisms as a terminal electron acceptor or an electron donor in As(V) respiration and in chemoautotrophic As(III) oxidation, respectively (Wang et al. 2016). However, the ars operon system is by far the most extensively characterized and most widely found As-resistance mechanism (Oremland and Stolz 2003).

A wide range of Gram-negative and Gram-positive bacteria harbor *ars* operon to detoxify both arsenite and arsenate (Carlin et al. 1995; Bhattacharjee and Rosen 2007). The *ars* operon is comprised of at least three genes including *arsR* (encodes for the repressor protein), *arsB* (encodes for membrane efflux pump), and *arsC* (encodes for arsenate reductase). In some Gram-negative bacteria, two additional genes *arsA* and *arsD* are also found, which encode for an intracellular ATPase and a corepressor, respectively (Tisa and Rosen 1990; Li et al. 2002). The membrane efflux pump, ArsB can independently work as anion efflux channel. Moreover, it provides a membrane-binding site for ArsA, which then can convert the energy coupling of ArsB from membrane potential to ATP (Tisa and Rosen 1990). The efflux pumps are also used by microbes to export Cd, Cu, and Zn (Smith and Novick 1972; Nies 1992; Maynaud et al. 2014). *Mesorhizobium metallidurans* isolated from a zinc-rich mining soil uses P-type efflux ATPase to export Zn and Cd (Maynaud et al. 2014).

15.3.3 Microbial Diversity on Heavy Metal-Contaminated Niche

The abundance and type of heavy metal in the soil influence the soil ecosystem and the microbial diversity. A metagenomic study of different soils with varying concentrations of Pb and Zn contamination identified *Solirubrobacter*, *Geobacter*, *Edaphobacter*, *Pseudomonas*, *Gemmatimonas*, *Nitrosomonas*, *Xanthobacter*, *Sphingomonas*, *Pedobacter*, and *Ktedonobacter*, as the 10 most abundant bacteria in all the samples. With the increased concentration of Zn and Pb, the abundance of resistant species increased (Hemmat-Jou et al. 2018). Comparative study of the microbial communities on the pristine and contaminated mangrove forests identified that the contaminated samples had reduced microbial metabolism including nitrogen-fixing capability, with an increase in metal resistance encoding genes indicating enrichment of heavy metal-resistant species (Li et al. 2019). In a similar study on the bacterial and fungal community structure on long-term metal exposure from six different sites in Canada, *Ascomycota* and *Basidiomycota* fungi were found to have the highest relative abundance in the metal-contaminated and reference soils, respectively. Among the bacterial genera, the metal-contaminated soils were enriched with *Geobacillus* and *Thioalkalispira* (Narendrula-Kotha and Nkongolo 2017). The species from the genus *Geobacillus* are known to detoxify heavy metals, and moreover, they denitrify nitrate to nitrogen (Chatterjee et al. 2010). The majority

of bacterial families (around 62%) and fungal families (around 58%) were common to the contaminated and the reference sites (Narendrula-Kotha and Nkongolo 2017).

15.4 Functional Niche Under Salt Stress

A soil with electrical conductivity of a saturated paste extract equal to more than 4 decisiemens per meter (dS/m) is considered as saline soil by the United States Department of Agriculture. Cations like Na^+ (Sodium), Ca^{2+} (Calcium), and K^+ (Potassium) as well as anions such as Cl^- (Chloride) and No^{3-} (Nitrate) contribute to salinity by increasing the electrical conductivity of soils (Sairam et al. 2016). A natural activity like low precipitation as well as human activities such as high surface irrigation and poor agricultural management contributes towards soil salinization, which further suppresses growth and linearly decreases microbial species diversity mainly through osmotic effect and ion toxicity (Kumar and Verma 2018).

15.4.1 Microbial Community Under Salt Stress

Halophiles include microbes of all three domains of archaea, bacteria, and eukarya and possess the ability to thrive and multiply in ecological niches with high concentration of salts (Todhar et al. 2012). A common habitat for halophilic microbes includes saline soil, saline ocean water, salt lakes, soda lakes, salterns, salt foods, etc. Salinity niche preference has been exhibited by microbial phylotypes belonging to *Nitriliruptoria*, *Alphaproteobacteria*, *Halobacteria*, *Gammaproteobacteria*, *Actinobacteria*, *Thermoleophilia*, *Bacilli*, and *Acidimicrobiia* (Zhang et al. 2019). *Halobacterium*, *Rhodothermaeota*, *Balneolaeota*, *Nanohaloarchaeota*, and *Bacteroidetes* were found to be the dominant prokaryotic groups in soil samples from salt marshes (Vera-Gargallo and Ventosa 2018). Niche in rhizosphere soils with high saline condition mainly comprise of taxonomic groups *Alphaproteobacteria* and *Gammaproteobacteria* and especially *deltaproteobacterial* which are close relatives to *Myxobacteria*, together with *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Gemmatimonadetes* (Philippot et al. 2013). Arbuscular mycorrhizal fungi belonging to the order *Glomerales* (Carvalho et al. 2003; Bencherif et al. 2015), *Archeosporales* (Wilde et al. 2009), and *Diversisporales* (Sonjak et al. 2009) are reported to exist in natural saline soils (Evelin et al. 2009; Bencherif et al. 2015). Five halotolerant endophytic fungi *Aspergillus terreus*, *Acremonium sclerotigenum* strain CCTU1171, *Paecilomyces formosus*, *Monosporascus ibericus*, and *Microascus pyramidus* were isolated from halotolerant plants, and they exhibited survival on 3.5 M NaCl concentration (Jalili et al. 2020).

15.4.2 Mechanisms for Tolerance of Salt Stress

Saline stress significantly reduces activities of various beneficial microbes present in soil niche and rhizosphere and also decreases organic matter accumulation and can result in low crop productivity (Waskiewicz et al. 2013). Salt stress can upset the balance between different cellular processes, leading to generation of reactive oxygen species which can further cause oxidative damage to proteins, DNA, and lipids (Miller et al. 2010).

Microorganisms adapt to variations in osmolarity or salt via mechanisms which include—by accruing low molecular weight organic compatible solutes (Shamseldin et al. 2006; Hagemann 2011)—exclusion of Na^+ ion from cells through the action of a Na^+/H^+ antiporter and Na^+-ATPase (Waditee et al. 2002), alteration in the composition of membrane via changes in fatty acid saturation or phospholipid composition to better manage with the changed turgor pressure (Romantsov et al. 2009), scavenging of reactive oxygen species in order to prevent the oxidative degradation of lipids (Waditee et al. 2002), employing molecular chaperons for the restoration of the native folding of proteins (Brígido et al. 2012), exopolysaccharide production (Sandhya et al. 2009), expressions of salt-responsive genes (Diby et al. 2005), and differentially expressed stress-related proteins (Paul and Lade 2014).

Microbial cell survival during salt stress is dependent over maintenance of correct fluidity of the bilayer in the membrane. Compatible solutes comprise a restricted range of highly water-soluble, osmotically active, low molecular weight amino acids like proline, serine, and glutamate (TeChien et al. 1992) and their derivatives, sugars such as trehalose or sugar alcohols, other alcohols (Ventosa et al. 1998) and inorganic cations such as K^+ (Smith and Smith 1989), and quaternary ammonium compounds such as glycine betaine and proline betaine (Tsuzuki et al. 2011), polyamines, and organic solutes (TeChien et al. 1992). Compatible solutes are amassed in the cytoplasm by the halophiles to maintain osmotic pressure, and their accumulation does not affect normal metabolism and neither cellular processes of the organism (Pastor et al. 2010). Till date many halotolerant genes and their products in the form of proteins as well as enzymes play a vital role in the survival of microbes in salt stress niche (Table 15.3). A functional metagenomic strategy was utilized to retrieve novel salt-resistant genes like endonuclease III, proton pumps, glycerol transporters, and DNA/RNA helicases in rhizospheric microorganisms present in both hyper and moderately saline environments (Mirete et al. 2015).

15.5 Functional Niche Under Waterlogging Stress

Agriculture around the world is constantly challenged by different environmental changes as a consequence of global warming. The environmental changes that alter water availability include mainly two conditions, flood (waterlogging) and drought. This section describes the functional niche under the waterlogging condition; the functional niche under the drought conditions is covered in Sect. 15.6.

Table 15.3 Various mechanisms in microbes to tolerate salt stress

S. N.	Mechanism	Microbial species/genus	References
1. Compatible solutes			
1.1	Ecotin	*Ectothiorhodospira halochloris*	Galinski et al. (1985)
1.2	Hydroxyecotin	*Halomonas elongata*	Margesin and Schinner (2001)
1.3	Trehalose	*Rhizobium etli*	Reina et al. (2012)
1.4	Proline	*Halobacillus halophilus*	Saum and Müller (2007)
1.5	Glycine betaine	*Tistlia consotensis*	Rubiano et al. (2015)
1.6	Glycine betaine	*Methanosacrina*	Roessler et al. (2002)
1.7	Glomalin	*Rhizophagus irregularis*	Giri and Varma (2019), Estrada et al. (2013)
2. Halotolerant genes			
2.1	betS	*Sinorhizobium meliloti*	Boscari et al. (2002)
2.2	katE	*Escherichia coli* K12	Islam et al. (2013)
2.3	proH, proJ, and proA	*Halobacillus halophilus*	Saum and Müller (2007)
2.4	codA	*Arthrobacter globiformis*	Goel et al. (2011)
2.5	ostA/ostB, mpgS/mpgP	*Thermus thermophilus*	Alarico et al. (2005)
2.6	HAL1	*Saccharomyces cerevisiae*	Serrano and Gaxiola (1994)
3. Enzymes			
3.1	Alkaline proteases	*Bacillus halodurans* CAS6	Annamalai et al. (2013)
3.2	Haloalkaline proteases	*Bacillus horikoshii*	Joo and Choi (2012)
3.3	Halophilic extracellular protease	*Halobacillus karajensis*	Karbalaei-Heidari et al. (2009)
3.4	Halotolerant alpha-amylase	*Bacillus licheniformis* shahed-07	Rasooli et al. (2008)
3.5	Halotolerant alpha-amylase	*Bacillus amyloliquefaciens* IIB-14	Zar et al. (2013)
3.6	Choline sulfatases	*Cyclobacterium qasimii*	Cregut et al. (2014)

Flooding is generally caused by intensive and/or extensive rainfall over a period of time (Fukao et al. 2019). Flooding produces an important type of abiotic stress like soil waterlogging. Flooding can be classified as waterlogging or as submergence (Sasidharan et al. 2017). When soil is covered with an excess amount of water which limits the rate of gas diffusion especially of oxygen in soil, it is known as waterlogging soil. This soil remains covered with water for a longer time duration, so it is fully saturated with water. The oxygen diffusion rate in waterlogged soil is approximately 320,000 times less than the unsaturated soil (Nishiuchi et al. 2012; Colmer and Flowers 2008). So, waterlogging interferes the O_2 movement in soil and creates hypoxia (sub-optimal O_2) and anoxia (absence of O_2) condition and favors anaerobic microbial community. In waterlogging condition, a number of physical, chemical, and biological changes may occur in soil like soil fertility due to changes in soil physicochemical and microbiological properties (Sahrawat 2005). The main

driving force for such changes is primarily a decrease in redox potential (Olmedo et al. 2015). When soil becomes anoxic, remineralization rate decreases, and organic matter accumulate in the soil. Due to differences in O_2 availability, pH, and availability of different ions like Fe and Mn in unsaturated normal and waterlogged soil, the types of microorganisms are also varying in both types.

In waterlogging soils, which are generally anoxic, O_2 usually diffuses from the surface into the soil and creates a thin (1–5 mm deep) oxic soil layer which becomes a habitat for aerobic bacteria which use oxygen as a terminal electron acceptor. When all the available oxygen in the soil has been utilized in respiration by aerobic bacteria, anaerobic bacteria, which live in oxygen-free environment inside the soil aggregates, respire by using other compounds like NO_3^-, Mn^{+4}, Fe^{+3}, SO_4^{-2}, and CO_2 as alternative electron acceptors and transformed them into various reduced toxic compounds like Fe^{+2}, H_2S, CH_4, etc. (Hamilton 1979). An anaerobic bacterium decomposes the organic matter and releases CO_2. The sequence of reduction would be O_2, NO_3^-, Mn^{+4}, Fe^{+3}, SO_4^{-2}, CO_2, and H^+, and their reduced counterparts H_2O, N_2, Mn^{+2}, Fe^{+2}, H_2S, CH_4, and H_2 are produced, respectively (Bhaduri et al. 2017). Sometimes, facultative and obligate anaerobes use the dissimilation products of carbohydrates and proteins as electron acceptors in their respiration.

Oxygen is depleted as the depth increase in soil, and as a result the redox potential (E_h) of the soil declines gradually which creates redox stratification (Drew and Lynch 1980). This stratification theoretically creates relatively well-defined habitats for the different groups of microorganisms according to which terminal electron acceptor used by microorganisms. The different redoxzones are characterized by the dominance of the electron acceptors O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2}, and CO_2 (Fig. 15.1). At the few centimeters depth, no other electron accepters are available except for CO_2 and H^+. Therefore, this zone is dominated by fermentation and methanogenesis. Methanogens used most of the H_2 as an electron donor for the production of CH_4, which is produced during the fermentation. H_2 is also utilized by sulfate reducers, iron reducers, and denitrifiers in their metabolism in decreasing order, respectively, and the remaining H_2 is released in the atmosphere (Achtnich et al. 1995).

15.5.1 Microbial Community Involved in Waterlogging Stress

The functional niche under waterlogging conditions comprises various microorganisms which can be broadly grouped as denitrifying bacteria, manganese reducers, iron reducers, sulfate reducers, and methanogenic bacteria.

15.5.1.1 Denitrifying Bacteria

As soon as O_2 is depleted in the soil, nitrate (NO_3^-) is used by soil microorganisms as a terminal electron acceptor. The transformation of other nitrogen compounds including ammonium (NH_4^+) and nitrite (NO_2^-) through the process of nitrification can result in the formation of nitrate (NO_3^-). NO_3^- is reduced to NH_4^+, or stepwise

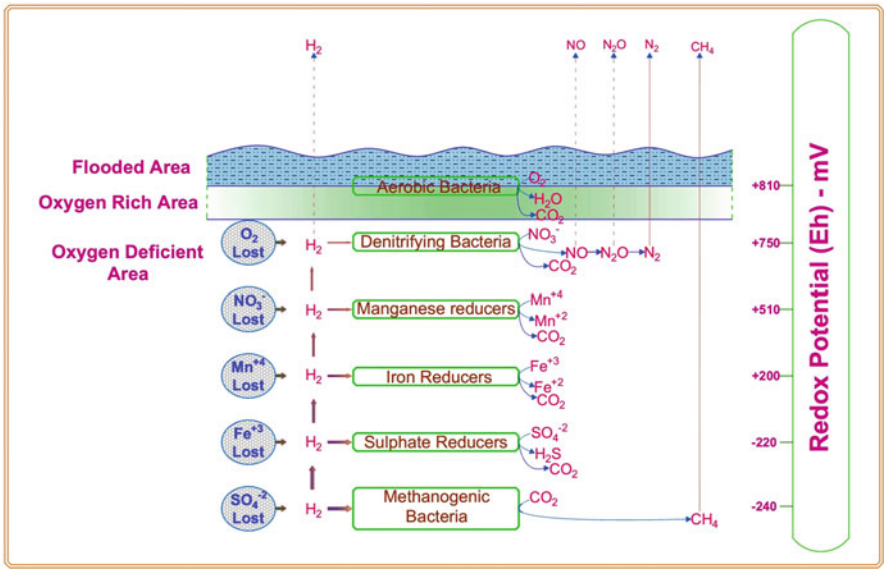

Fig. 15.1 Schematic diagram of distribution of different groups of microorganisms according to redox reactions in a waterlogged soil

NO_3^- is reduced to nitrite (NO_2^-) and then the gases, nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), by denitrification. This process is mediated by several facultative bacteria, such as *Pseudomonas*, *Achromobacter*, *Bacillus*, *Micrococcus*, *Agrobacterium*, *Alcaligenes*, and *Paracoccus*. Denitrification may cause significant N loss from the soil and emission of the potent greenhouse gas NO and N_2O which are responsible for global warming and ozone depletion (Hamont et al. 2012; Ravishankara et al. 2009). But the flux of NO and N_2O into the atmosphere is comparatively low, because most of the produced NO and N_2O are further reduced to N_2. Other bacteria like *Thiobacillus denitrificans* also reduce nitrate to nitrogen using sulfur or thiosulfate as a source of energy. The process of nitrification in waterlogged soil is inhibited because anoxic environments inhibit the activity of nitrifying communities, resulting in depletion of soil N availability (Nguyen et al. 2018).

15.5.1.2 Manganese Reducers

Manganese (Mn^{4+}) oxide (MnO_2) which is reduced to Mn^{2+} ions is the second most energy-releasing electron acceptor following nitrate. The Mn^{2+} forms of manganese are very insoluble in water and deposit in soils. This process is carried out by several anaerobic microorganisms like *Metallogenium*, *Bacillus*, *Geobacter*, *Pseudomonas*, and *Shewanella*. Interestingly, manganese reducers are also capable of reducing iron (Bhaduri et al. 2017). During waterlogging, the reduction of Mn oxides (MnO_2) occurs faster at a higher temperature like 20°C and 30°C than at 10°C or 4°C, which

suggests that manganese reducers are mesophilic organisms (Sparrow and Uren 2014).

15.5.1.3 Iron Reducers

Microorganisms use ferric iron ions (Fe^{3+}) as electron acceptor which is further reduced to ferrous iron ion (Fe^{2+}), following utilization of Mn^{4+}(Gotoh and Patrick 1974). Fe^{3+} ions sources in soil include metal deposits and minerals present in the soil. Gleying (a process in which waterlogged soils appears bluish-gray due as a result of reduction of iron and manganese due to low oxygen conditions) is a result of accumulation of ferrous iron (Fe^{2+}). This reduction is mediated by different anaerobic microbes, such as *Clostridium*, *Desulfovibrio*, *Pseudomonas*, *Geothrix*, *Shewanella*, and *Thiobacillus.* The elevated concentration of Mn^{2+} and Fe^{2+} is toxic in the soil. The dominant forms of iron waterlogged soil are goethite, hematite, ferrihydrite, etc. (Annisa and Nursyamsi 2016).

15.5.1.4 Sulfate Reducer

Further decrease in the redox potential results in the reduction of SO_4^{2-} to H_2S, which is also potentially toxic. This reaction is carried out generally by sulfate reducers (obligate anaerobes) such as *Desulfovibrio*, *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, and *Desulfosporosinus*. H_2S is susceptible and reacts with Fe^{2+} to form iron sulfide (FeS) which causes corrosion in underground iron pipes. Soil flooding leads to the emergence of hydrogen sulfide which is also known as "swamp gas" (Conrad 1996).

15.5.1.5 Methanogenic Bacteria

As SO_4^{2-} is exhausted, soil microorganisms use CO_2 in their respiration. Methanogenesis is a process of formation of methane (CH_4) by utilizing carbon dioxide (CO_2) as a terminal electron acceptor. Methane is the end product of the microbial metabolism in anaerobic conditions, and waterlogged soil is the largest natural source of atmospheric CH_4, an important contributor to global warming (Kotsyurbenko et al. 2019). The lower reduction potential of CO_2 is attributed to be the foremost reason for methanogenesis which is occurring in waterlogged soils. Methanogens generally exhibit a slow growth rate as they use CO_2 as an electron acceptor in respiration, and this produces lower energy when compared with the reactions where NO_3^-, Mn^{+4}, Fe^{+3}, and SO_4^{-2} act as electron acceptors. Methanogens, which perform methanogenesis, include *Methanobacterium*, *Methanosarcina*, *Methanobrevibacter*, *Methanoculleus*, *Methanogenium*, *Methanosaeta*, and *Methanospirillum* and also a group of anaerobic archaea (Bhaduri et al. 2017). Methanogenesis is the dominant pathway for the decomposition of organic matter in waterlogged soil. Methanogens also produced CH_4 by acetate (CH_3COOH) fermentations.

15.6 Functional Niche Under Drought Stress

Water and life are inseparable, and yet, mainly due to drought and related calamities, about 20% of soil is presently estimated to be severely degraded with a decline in productivity (United Nations Convention to Combat Desertification 2017). Many soil regions globally are threatened severely due to drought, in both natural and agricultural settings, acutely getting affected as documented regularly. Soils possess a diversity of microorganisms which play a vital role in their function (Vries et al. 2018). The microbial communities present in the soil differ greatly due to climate, plant community, and chemical nature of the soil at different geographic locations of the world. The taxonomic diversity may get perturbed due to stress posed by water-related issues with both reduction and increase of diversity. The soil microbial community and drought have variation in natural and experimental environments due to the duration of the event or treatment or on the timings (Hoover and Rogers 2016; Mengtian et al. 2018), the amount of change in available water, historical precipitation regime, and the legacy effects (Preece et al. 2019). The effect elicited by drought may or not follow a straight curve (Knapp et al. 2017). It is very difficult to draw a conclusion due to all these variable factors about the impact of drought on microbial community present in the soil (Preece et al. 2019). To keep the overall ecosystem intact especially in terms of ensuring food security, continuation of nutrient cycle, and production of timber along with regulation of climate, the knowledge of diversity and structure of microbes by drought will help develop strategies to maintain healthy soils because soil forms a core part for various ecosystems.

15.6.1 Impact of Drought on Microbial Community Composition

Investigations into the drought effects on various microbial communities suggest a varied response, with both fungal and bacterial communities showing very different susceptibility for drought. The soil fungi are generally more resistant, but less resilient, than bacteria (Vries et al. 2018). The observations have suggested that microbial biomass decreases as a result of drought (Bastida et al. 2017) with only a little fraction of soil microbes showing adaptation to drought-like situations (Kaisermann et al. 2017). Moreover, the duration of drought had a notable effect on the fungal/bacterial ratio which enlarges with intense drought (Preece et al. 2019). There are contrasting findings indicating that drought stress may not always be tolerated by fungal species but it can respond sensitively or opportunistically the same as bacteria (Meisner et al. 2018). The changes in the soil moisture can be reported by some fungal species as they are sensitive bioindicators for moisture changes (Kaisermann et al. 2015). Therefore, composition of fungal communities may differ between drought and irrigation (Barnes et al. 2018), and in dry and wet conditions, their biomass may remain unaffected, increase, or decrease (Hartmann et al. 2017). Responding fungal OTUs as observed in *Ascomycota*, particularly, *Sordariomycetes* and *Dothideomycetes* appeared to be the major answerers to

drought history (Meisner et al. 2018). Some fungal species can be affected by very little change in the moisture content of the soil because they can use an opportunistic strategy, thereby changing the composition of the fungal community (Kaisermann et al. 2015). Increased resistance of fungal networks to environmental fluctuations is likely attributable to the chitinous cell walls of fungi, and their extended and exploratory hyphal structures allow them to cross the small areas of dry soil (Bapiri et al. 2010; De Vries et al. 2012; Barnard et al. 2013). Some findings report that among fungi, yeasts may have a high tolerance to drought as they have adapted to extreme environmental conditions and also have the ability to reproduce by budding, which is generally a more stress tolerant strategy of reproduction (Treseder and Lennon 2015).

Recent findings suggest that droughts have a much stronger impact on bacterial than on fungal networks and bacterial communities might not be as resilient as previously thought (Vries et al. 2018). The external environment is tolerated by the bacterial community by employing several physiological changes such as production of exopolysaccharides, sporulation, and balancing internal water potential. Bacteria develop this adaptation as they assemble low molecular weight osmoregulators within their cytoplasm and release them as soil moisture increases (Hueso et al. 2012). There is also an important association between the drought-induced shifts in a plant community's composition and bacterial communities, the latter being affected lastingly due to vegetation changes (Vries et al. 2018).

The composition of bacterial community is clearly affected as drought reduces the proportion of bacterial biomass more compared to fungi (Preece et al. 2019). The Gram-negative lineages are more susceptible to drought compared to Gram-positive bacterial lineages, possibly due to their thinner cell walls (Schimel et al. 2007). The character, however, may be correlated to the increase in root exudation during prolonged drought (Preece et al. 2019) because Gram-positive bacteria generally consume more recalcitrant carbon sources (Naylor and Coleman-Derr 2017) compared to Gram-negative bacteria that, on the other hand, consume labile carbon source. Some bacterial communities are known to have a higher abundance in areas which had history of drought. The group of *Archaea* and *Thaumarchaeota* found in extreme environments shows an increase in the relative abundance in soil with a drought history (Stieglmeier et al. 2014). These species could be more present in the desert soil (Shi et al. 2016). The *Alpha-*, *Beta-*, and *Gammaproteobacteria*, which are basically operational taxonomic units, are studied as copiotrophs with more growth rates (Meisner et al. 2018). They have shown an increase in relative richness in the soil with a drought past by having an opportunistic strategy. They are the quick responders as they showed their presence in more amounts after rewetting (Placella et al. 2012; Meisner et al. 2018). Cyanobacteria are very susceptible to drought and have shown decreased abundance in the soil during drought conditions and also show a low recovery rate on rewetting of soils (Hagemann et al. 2017). Moreover, *Cyanobacteria* and *Acidobacteria* may have responded via the microbial facilitation mechanisms which means during the drought conditions, they can synthesize extracellular polysaccharides and can create micro-niches which benefit other bacteria (Kielak et al. 2016). The endophytic *Actinomycetes* are widely known for their

outstanding ability to survive in unfavorable environments (Chukwuneme et al. 2020). *Actinobacteria* abundance relatively increased during drought as suggested in previous reports that similar phenomenon is observed in different soil types and also in many species of plants including rhizosphere and endosphere (Naylor and Coleman-Derr 2017; Preece et al. 2019). These results may be observed due to formation of spores by *Actinobacteria*, which enables them to be in a dormant state during the extreme stress conditions of the environment such as drought (Naylor and Coleman-Derr 2017; Preece et al. 2019).

Bacteria adapt to drought also by secreting exopolysaccharide (EPS), a structural component of the extracellular matrix in high quantities (Susilowati et al. 2018). Microbial EPS possesses unique water retention and cementing properties, which protect bacteria against desiccation. EPS also helps bacteria in absorption of water and nutrients and to help them colonize plant roots by attaching on roots via a network of fibrillar materials that permanently connects bacteria to the surface and prevents removal from the site (Niu et al. 2018). Moreover in sandy soils, EPS can protect plants from stress and lack of water and contribute to the formation of soil aggregates (Susilowati et al. 2018). Certain bacterial species like *Pseudomonas* sp., *Bacillus* sp., *Bacillus licheniformis*, *Bacillus megaterium*, and *Bacillus pumilus* are found to increase the production of EPS during the dry season (Susilowati et al. 2018). The increase in EPS production was deemed responsible for protection under extreme desiccation conditions in *A. brasilense* Sp245 (Konnova et al. 2001). The high tolerance of the four *Rhizobacteria*, *Pseudomonas*, *Fluorescens*, *Enterobacter hormaechei*, and *Pseudomonas migulae* to drought stress could be explained by production of EPS. Production of EPS enables them to grow at a minimum water potential and appears to enhance their ability to increase the growth of plants possibly by improving soil structure and colonization (Niu et al. 2018). The siderophore production is checked for drought resistance ability. Siderophores not only forage iron from the surroundings to create mineral which is very important and accessible to the microbes, but they also form complexes with other metals like molybdenum, manganese, cobalt, and nickel in the environment and enhance availability to microbial cells (Pahari et al. 2017). They also play an important role in the biological control against certain phytopathogens (Patel et al. 2018).

The growth of mycorrhiza in roots and soils are strongly affected by the stress imposed by drought. Three AMF *Glomus* species, *G. macrocarpum*, *G. clarum*, and *G. etunicatum*, exhibited substantial endurance to drying of soil. Glomalin, an immunoreactive glycoprotein, is a soil protein released exclusively by mycorrhizal hyphae and spores of AMF and improves soil structure (Wu and Zou 2017); GRSP causes a reduction in water loss in the soil aggregated because it generally coats on fungal hyphae forming hydrophobic layers and hence regulating water relations of plant/soil. Drought tolerance of host plants is also improved by AMF using physiological mechanisms of nutrient uptake and biochemical mechanisms regarding hormones, osmotic adjustment, and antioxidant systems (Wu and Zou 2017).

Some rare biosphere members may be very active in contributing in various soil phenomena in spite of them having low-ranked abundance (Pedros-Alio 2012). Moreover, a growth rate is almost similar in low- and high-abundant microbes

(Kurm et al. 2017). This results in changed microbial community composition as rare members of the community exhibit their effects without apparent consequences on the total uniformity indices (Meisner et al. 2018).

15.6.2 Impact of Drought on Microbial Community Diversity

The more diversity in microbial communities, especially those with diversity in the function, may be more tolerant to drought (and to different stress conditions) albeit with the fact that is type of tolerance is in strong conjunction with soil biotic and abiotic elements (Griffiths and Philippot 2013).

The studies conducted earlier suggest that drought results in a negative effect on bacterial community alpha diversity (Bouskill et al. 2013), although diversity generally remains similar (Tóth et al. 2017; Preece et al. 2019).

Compared to control the low and high-level of drought treatment on various fungus showed positive effect on all over growth of the fungus. High fungal diversity has also been reported previously subjecting drought conditions (Schmidt et al. 2018) indicating even if bacteria are negatively affected, they can thrive themselves as these organisms have higher tolerance to drought. The findings reported that drought affects both fungal and bacterial diversity (Bouskill et al. 2013; Preece et al. 2019). Moreover, the presence of plants could have positive effects on bacterial diversity mostly due to the existence of microbes on the roots (Hortal et al. 2015; Preece et al. 2019).

Microbial community-level physiological profile (CLPP) which is based on the checking of the strength of soil microbial communities to metabolize different organic carbon substrates that vary in structural complexity was used for the assessment of site-specific differences in bacteria of soil and the evaluation of the relationship between biodiversity and site conditions. The microbial community's physiology was more acutely affected due to water stress than due to its structure (Hueso et al. 2012). The investigation suggests that highly active component of the microbes will not able to survive in drought stress as drought exhibits a deleterious effect on the size and activity of the microbial community. The microbial community present in the natural samples were unable to recover as prolonged drought might have inhibited the growth of active population (Griffiths et al. 2003; Braun et al. 2010). The gradient in substrate utilization rate during various treatments shows the soil microbial communities have diverse metabolic capabilities (Chakraborty et al. 2011).

Authors' Contribution

AKS, **VN**, **AN**, **KH**, and **BS** wrote the chapter on functional niche under "salt stress," "heavy metal toxicity stress," "temperature stress," "waterlogging stress," and "drought stress," respectively. Introduction and Abstract were written by **AKS** and **VN**, respectively.

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References

Achtnich C, Bak F, Conrad R (1995) Competition for electron donors among nitrate reducers, ferric iron reducers, sulphate reducers, and methanogens in anoxic paddy soil. Biol Fertil Soils 19:65–72

Alarico S, Empadinhas N, Simões C, Silva Z, Henne A, Mingote A, Santos H, Da Costa MS (2005) Distribution of genes for synthesis of trehalose and mannosylglycerate in Thermus spp. and direct correlation of these genes with halotolerance. Appl Environ Microbiol 71:2460–2466

Angel R, Soares MIM, Ungar ED, Gillor O (2010) Biogeography of soil archaea and bacteria along a steep precipitation gradient. ISME J 4:553–563. https://doi.org/10.1038/ismej.2009.136

Annamalai N, Rajeswari MV, Thavasi R, Vijayalakshmi S, Balasubramanian T (2013) Optimization, purification and characterization of novel thermostable, halo alkaline, solvent stable protease from *Bacillus halodurans* CAS6 using marine shellfish wastes: a potential additive for detergent and antioxidant synthesis. Bioprocess Biosyst Eng 36(7):873–883

Annisa W, Nursyamsi D (2016) Iron dynamics and its relation to soil redox potential and plant Growth in acid sulphate soil of South Kalimantan, Indonesia. Indian J Agric Sci 17(1):1–8

Ayangbenro AS, Babalola OO (2018) Metal(loid) bioremediation: strategies employed by microbial polymers. Sustainability 10:3028. https://doi.org/10.3390/su10093028

Ballou DP, Miller SM (1999) Bacterial detoxification of Hg (II) and organomercurials. Essays Biochem 34:17–30

Balser TC, Firestone MK (2005) Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73:395–415. https://doi.org/10.1007/s10533-004-0372-y

Banat IM, Makkar RS, Cameotra SS (2000) Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 53:495–508. https://doi.org/10.1007/s002530051648

Bapiri A, Baath E, Rousk J (2010) Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microb Ecol 60:419–428

Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 27:355–384. https://doi.org/10.1016/S0168-6445(03)00046-9

Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J 7:2229–2241

Barnes CJ, Van der Gast CJ, Mcnamara NP, Rowe R, Bending GD (2018) Extreme rainfall affects assembly of the root associated fungal community. New Phytol 220(4):1172–1184

Bastida F, Torres IF, Andres AM, Baldrian P, Lopez Mondejar R, Vetrovsky T, Richnow HH, Starke R, Ondono S, Garcia C, Lopez Serrano FR, Jehmlich N (2017) Differential sensitivity of total and active soil microbial communities to drought and forest management. Glob Chang Biol 23:4185–4203

Belay-Tedla A, Zhou X, Su B et al (2009) Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. Soil Biol Biochem 41:110–116. https://doi.org/10.1016/j.soilbio.2008.10.003

Bencherif K, Boutekrabta A, Fontaine J, Laruelle F, Dalpé Y, Lounès-Hadj Sahraoui A (2015) Impact of soil salinity on arbuscular mycorrhizal fungi biodiversity and microflora biomass associated with Tamarix articulata Vahll rhizosphere in arid and semi-arid Algerian areas. Sci Total Environ 533:488–494

Bhaduri D, Mandal A, Chakraborty K, Chatterjee D, Dey R (2017) Interlinked chemical-biological processes in anoxic waterlogged soil – a review. Indian J Agric Sci 87(12):1587–1599

Bhattacharjee H, Rosen BP (2007) Arsenic metabolism in prokaryotic and eukaryotic microbes. In: Nies DH, Silver S (eds) Molecular microbiology of heavy metals. Springer, Berlin, pp 371–406

Blindauer C, Harrison M, Parkinson J et al (2008) Isostructural replacement of zinc by cadmium in bacterial metallothionein. Metal Ions Biol Med 10:167–173
Bond-Lamberty B, Thomson A (2010) Temperature-associated increases in the global soil respiration record. Nature 464:579–582. https://doi.org/10.1038/nature08930
Boscari A, Mandon K, Dupont L, Poggi MC, Rudulier DL (2002) BetS Is a major glycine betaine/proline betaine transporter required for early osmotic adjustment in *Sinorhizobiummeliloti*. J Bacteriol 184(10):2654–2663
Bouskill NJ, Lim HC, Borglin S, Salve R, Wood TE, Silver WL, Brodie EL (2013) Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. ISME J 7:384–394
Braud A, Hannauer M, Mislin GLA, Schalk IJ (2009) The Pseudomonas aeruginosa pyochelin-iron uptake pathway and its metal specificity. J Bacteriol 191:3517–3525. https://doi.org/10.1128/JB.00010-09
Braun B, Bockelmann U, Grohmann E, Szewzyk U (2010) Bacterial soil communities affected by water-repellency. Geoderma 158:343–351
Brígido C, Alexandre A, Oliveira S (2012) Transcriptional analysis of major chaperone genes in salt-tolerant and salt-sensitive mesorhizobia. Microbiol Res 167:623–629
Carlin A, Shi W, Dey S, Rosen BP (1995) The ars operon of Escherichia coli confers arsenical and antimonial resistance. J Bacteriol 177:981–986. https://doi.org/10.1128/jb.177.4.981-986.1995
Carvalho LM, Correia PM, Caçador I, Martins-Louçao MA (2003) Effects of salinity and flooding on the infectivity of salt marsh arbuscular mycorrhizal fungi in Aster tripolium L. Biol Fertil Soils 38:137–143
Chakraborty A, Chakrabarti K, Chakraborty A, Ghosh S (2011) Effect of long-term fertilizers and manure application on microbial biomass and microbial activity of a tropical agricultural soil. Biol Fertil Soils 47:227–233
Chang C-C, Lin L-Y, Zou X-W et al (2015) Structural basis of the mercury (II)-mediated conformational switching of the dual-function transcriptional regulator MerR. Nucleic Acids Res 43:7612–7623. https://doi.org/10.1093/nar/gkv681
Chatterjee SK, Bhattacharjee I, Chandra G (2010) Biosorption of heavy metals from industrial waste water by *Geobacillus thermodenitrificans*. J Hazard Mater 175:117–125. https://doi.org/10.1016/j.jhazmat.2009.09.136
Chaturvedi KS, Hung CS, Crowley JR et al (2012) The siderophore yersiniabactin binds copper to protect pathogens during infection. Nat Chem Biol 8:731–736. https://doi.org/10.1038/nchembio.1020
Chaudri AM, McGrath SP, Giller KE (1992) Metal tolerance of isolates of Rhizobium leguminosarum biovar Trifolii from soil contaminated by past applications of sewage sludge. Soil Biol Biochem 24:83–88. https://doi.org/10.1016/0038-0717(92)90262-V
Chukwuneme CF, Babalola OO, Kutu RF, Ojuederie OB (2020) Characterization of *actinomycetes* isolates for plant growth promoting traits and their effects on drought tolerance in maize. J Plant Interact 15(1):93–105
Colmer TD, Flowers TJ (2008) Flooding tolerance in halophytes. New Phytol 179:964–974
Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiol Rev 60(4):609–640
Cregut M, Durand MJ, Thouand G (2014) The diversity and functions of choline sulphatases in microorganisms. Microb Ecol 67:350–357. https://doi.org/10.1007/s00248-013-0328-7
Dash HR, Das S (2012) Bioremediation of mercury and the importance of bacterial mer genes. Int Biodeterior Biodegradation 75:207–213. https://doi.org/10.1016/j.ibiod.2012.07.023
De Freitas LA, Ferreira de Moura G, Barbosa de Lima MA et al (2011) Role of the morphology and polyphosphate in *Trichoderma harzianum*related to cadmium removal. Molecules 16:2486–2500. https://doi.org/10.3390/molecules16032486
De Vries FT, Liiri ME, Bjornlund L, Bowker MA, Christensen S, Setala HM, Richard DB (2012) Land use alters the resistance and resilience of soil food webs to drought. Nat Clim Chang 2:276–280

De Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, Hallin S, Kaisermann A, Keith AM, Kretzschmar M, Lemanceau P, Lumini E, Mason K, Oliver A, Ostle N, Posser JI, Thion C, Thomson B, Badgett RD (2018) Soil bacterial networks are less stable under drought than fungal networks. Nat Commun 9:3033

Deng Y, He Z, Xu M et al (2012) Elevated carbon dioxide alters the structure of soil microbial communities. Appl Environ Microbiol 78:2991–2995. https://doi.org/10.1128/AEM.06924-11

Deslippe JR, Hartmann M, Simard SW, Mohn WW (2012) Long-term warming alters the composition of Arctic soil microbial communities. FEMS Microbiol Ecol 82:303–315. https://doi.org/10.1111/j.1574-6941.2012.01350.x

Diby P, Sarma YR, Srinivasan V, Anandaraj M (2005) Pseudomonas fluorescens mediated vigour in black pepper (piper nigrum L.) under greenhouse cultivation. Ann Microbiol 5:171–174

Drew MC, Lynch JM (1980) Soil anaerobiosis, microorganisms, and root function. Annu Rev Phytopathol 18:37–66

Engst S, Miller SM (1999) Alternative routes for entry of HgX2 into the active site of mercuric ion reductase depend on the nature of the X ligands. Biochemistry 38:3519–3529. https://doi.org/10.1021/bi982680c

Estrada B, Aroca MFJM et al (2013) Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. Plant Cell Environ 36:1771–1782

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci 103:626–631

Fierer N, Leff JW, Adams BJ et al (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. PNAS 109:21390–21395. https://doi.org/10.1073/pnas.1215210110

Frey B, Rieder SR (2013) Response of forest soil bacterial communities to mercury chloride application. Soil Biol Biochem 65:329–337. https://doi.org/10.1016/j.soilbio.2013.06.001

Frossard A, Hartmann M, Frey B (2017) Tolerance of the forest soil microbiome to increasing mercury concentrations. Soil Biol Biochem 105:162–176. https://doi.org/10.1016/j.soilbio.2016.11.016

Fukao T, Barrera-Figueroa BE, Juntawong P, Pena-Castro JM (2019) Submergence and waterlogging stress in plants: a review highlighting research opportunities and understudied aspects. Front Plant Sci 10:340. https://doi.org/10.3389/fpls.2019.00340

Galinski EA, Pfeiffer HP, Truper HG (1985) 1, 4, 5, 6-Tetrahydro-2-methyl-4-pyrimidinecarboxylic acid. A novel cyclic amino acid from halophilic phototrophic bacteria of the genus Ectothiorhodospira. Eur J Biochem 149:135–139

Giri B, Varma A (2019) Microorganisms in saline environments: strategies and functions. Soil Biol 56. https://doi.org/10.1007/978-3-030-18975-4_16

Goel D, Singh AK, Yadav V, Babbar SB, Murata N, Bansal KC (2011) Transformation of tomato with a bacterial codA gene enhances tolerance to salt and water stresses. Plant Physiol 168 (11):1286–1294

Göhre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115–1122. https://doi.org/10.1007/s00425-006-0225-0

Gómez-Sagasti MT, Alkorta I, Becerril JM et al (2012) Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation. Water Air Soil Pollut 223:3249–3262. https://doi.org/10.1007/s11270-012-1106-8

Gotoh S, Patrick WH (1974) Transformation of iron in a waterlogged soil as influenced by redox potential and pH. Soil Sci Soc Am J 38(1):66–71

Griffiths BS, Philippot L (2013) Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol Rev 37:112–129

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ (2003) Physiological and community response of established grassland bacterial populations to water stress. Appl Environ Microbiol 69:6961–6968

Gupta P, Diwan B (2017) Bacterial exopolysaccharide mediated heavy metal removal: a review on biosynthesis, mechanism and remediation strategies. Biotechnol Rep 13:58–71. https://doi.org/10.1016/j.btre.2016.12.006

Hagemann M (2011) Molecular biology of cyanobacterial salt acclimation. FEMS Microbiol Rev 35:87–123

Hagemann M, Henneberg M, Felde VJ, Berkowicz SM, Raanan H, Pade N et al (2017) Cyanobacterial populations in biological soil crusts of the northwest Negev Desert, Israel – effects of local conditions and disturbance. FEMS Microbiol Ecol 93(6):1–9

Hamilton WA (1979) Microbial energetics and metabolism. In: Lynch JM, Poole NJ (eds) Microbial ecology. A conceptual approach. Blackwell, Oxford, pp 22–44. 266 pp

Hartmann M, Brunner I, Hagedorn F, Bardgett RD, Stierli B, Herzog C, Chen X, Zingg A, Graf-Pannatier E, Rigling A, Frey B (2017) A decade of irrigation transforms the soil microbiome of a semi-arid pine forest. Mol Ecol 26:1190–1206

He Z, Xu M, Deng Y et al (2010) Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2. Ecol Lett 13:564–575. https://doi.org/10.1111/j.1461-0248.2010.01453.x

Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature 451:289–292. https://doi.org/10.1038/nature06591

Hemmat-Jou MH, Safari-Sinegani AA, Mirzaie-Asl A, Tahmourespour A (2018) Analysis of microbial communities in heavy metals-contaminated soils using the metagenomic approach. Ecotoxicology 27:1281–1291. https://doi.org/10.1007/s10646-018-1981-x

Herman DC, Artiola JF, Miller RM (2002) Removal of cadmium, lead, and zinc from soil by a rhamnolipid biosurfactant. https://pubs.acs.org/doi/pdf/10.1021/es00009a019

Hoover DL, Rogers BM (2016) Not all droughts are created equal: the impacts of interannual drought pattern and magnitude on grassland carbon cycling. Glob Chang Biol 22:1809–1820

Hortal S, Bastida F, Moreno JL, Armas C, García C, Pugnaire FI (2015) Benefactor and allelopathic shrub species have different effects on the soil microbial community along an environmental severity gradient. Soil Biol Biochem 88:48–57

Hueso S, Garcia C, Hernandez T (2012) Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biol Biochem 50:167–173

Hughes VM, Datta N (1983) Conjugative plasmids in bacteria of the 'pre-antibiotic' era. Nature 302:725–726. https://doi.org/10.1038/302725a0

Imperato M, Adamo P, Naimo D et al (2003) Spatial distribution of heavy metals in urban soils of Naples city (Italy). Environ Pollut 124:247–256. https://doi.org/10.1016/S0269-7491(02)00478-5

Islam MS, Azam MS, Sharmin S, Sajib AA, Alam MM, Reza MS, Ahmed R, Khan H (2013) Improved salt tolerance of jute plants expressing the *katE gene from Escherichia coli*. Turk J Biol 37(2):206–211

Jalili B, Bagheri H, Azadi S et al (2020) Identification and salt tolerance evaluation of endophyte fungi isolates from halophyte plants. Int J Environ Sci Technol 17:3459–3466. https://doi.org/10.1007/s13762-020-02626-y

Joo HS, Choi JW (2012) Purification and characterization of a novel alkaline protease from *Bacillushorikoshii*. J Microbiol Biotechnol 22(1):58–68

Joo J, Choi J-H, Kim IH et al (2015) Effective bioremediation of Cadmium (II), nickel (II), and chromium (VI) in a marine environment by using *Desulfovibrio desulfuricans*. Biotechnol Bioprocess Eng 20:937–941. https://doi.org/10.1007/s12257-015-0287-6

Kaisermann A, Maron PA, Beaumelle L, Lata JC (2015) Fungal communities are more sensitive indicators to non-extreme soil moisture variations than bacterial communities. Appl Soil Ecol 86:158–164

Kaisermann A, de Vries FT, Griffiths RI, Bardgett RD (2017) Legacy effects of drought on plant–soil feedbacks and plant–plant interactions. New Phytol 215:1413–1424

Karbalaei-Heidari HR, Amoozegar MA, Hajighasemi M, Ziaee AA, Ventosa A (2009) Production, optimization and purification of a novel extracellular protease from the moderately halophilic bacterium *Halobacillus karajensis*. J Ind Microbiol 36:21–27

Khadim HJ, Ammar SH, Ebrahim SE (2019) Biomineralization based remediation of cadmium and nickel contaminated wastewater by ureolytic bacteria isolated from barn horsessoil. Environ Technol Innov 14:100315. https://doi.org/10.1016/j.eti.2019.100315

Khan S, Cao Q, AE-L H et al (2007) Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb. J Environ Sci 19:834–840. https://doi.org/10.1016/S1001-0742(07)60139-9

Kielak AM, Barreto CC, Kowalchuk GA, Van Veen JA, Kuramae EE (2016) The ecology of *Acidobacteria*: moving beyond genes and genomes. Front Microbiol 7:744

Kieu HTQ, Müller E, Horn H (2011) Heavy metal removal in anaerobic semi-continuous stirred tank reactors by a consortium of sulfate-reducing bacteria. Water Res 45:3863–3870. https://doi.org/10.1016/j.watres.2011.04.043

Kim S-Y, Kim J-H, Kim C-J, Oh D-K (1996) Metal adsorption of the polysaccharide produced from *Methylobacterium organophilum*. Biotechnol Lett 18:1161–1164. https://doi.org/10.1007/BF00128585

Knapp AK, Ciais P, Smith MD (2017) Reconciling inconsistencies in precipitation– productivity relationships: implications for climate change. New Phytol 214:41–47

Konnova SA, Brykova OS, Sachkova OA, Egorenkova IV, Ignatov VV (2001) Protective role of the polysaccharide containing capsular components of *Azospirillum brasilense*. Microbiology 70:436–440

Kotsyurbenko OR, Glagolev MV, Merkel AY, Sabrekov AF, Terentieva IE (2019) Methanogenesis in soils, wetlands, and peat. biogenesis of hydrocarbons, pp 211–228

Kou S, Vincent G, Gonzalez E et al (2018) The response of a 16S ribosomal RNA gene fragment amplified community to lead, zinc, and copper pollution in a Shanghai Field Trial. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.00366

Kumar A, Verma J (2018) Does plant-microbe interaction confer stress tolerance in plants. A review. Microbiol Res 207:41–52

Kurm V, Van Der Putten WH, De Boer W, Naus-Wiezer S, Hol WHG (2017) Low abundant soil bacteria can be metabolically versatile and fast growing. Ecology 98:555–564

Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol 75:5111–5120. https://doi.org/10.1128/AEM.00335-09

Le Bagousse-Pinguet Y, de Bello F, Vandewalle M, Leps J, Sykes MT, Wardle D (2014) Species richness of limestone grasslands increases with trait overlap: evidence from within- and between-species functional diversity partitioning. J Ecol 102:466–474

Lebrun E, Brugna M, Baymann F et al (2003) Arsenite oxidase, an ancient bioenergetic enzyme. Mol Biol Evol 20:686–693. https://doi.org/10.1093/molbev/msg071

Leita L, De Nobili M, Muhlbachova G et al (1995) Bioavailability and effects of heavy metals on soil microbial biomass survival during laboratory incubation. Biol Fertil Soils 19:103–108. https://doi.org/10.1007/BF00336144

Li S, Rosen BP, Borges-Walmsley MI, Walmsley AR (2002) Evidence for cooperativity between the four binding sites of dimeric ArsD, an As(III)-responsive transcriptional regulator. J Biol Chem 277:25992–26002. https://doi.org/10.1074/jbc.M201619200

Li M, Cheng X, Guo H (2013) Heavy metal removal by biomineralization of urease producing bacteria isolated from soil. Int Biodeterior Biodegradation 76:81–85. https://doi.org/10.1016/j.ibiod.2012.06.016

Li F, Wang W, Li C et al (2018a) Self-mediated pH changes in culture medium affecting biosorption and biomineralization of Cd^{2+} by *Bacillus cereus* Cd01. J Hazard Mater 358:178–186. https://doi.org/10.1016/j.jhazmat.2018.06.066

Li Y, Shipley B, Price JN, Dantas VL, Tamme R, Westoby M, Siefert A, Schamp BS, Spasojevic MJ, Jung V, Laughlin DC, Richardson SJ et al (2018b) Habitat filtering determines the functional niche occupancy of plant communities worldwide. J Ecol 106:1001–1009

Li Y, Zheng L, Zhang Y et al (2019) Comparative metagenomics study reveals pollution induced changes of microbial genes in mangrove sediments. Sci Rep 9:5739. https://doi.org/10.1038/s41598-019-42260-4

Lima AIG, Corticeiro SC, de Almeida Paula Figueira EM (2006) Glutathione-mediated cadmium sequestration in Rhizobium leguminosarum. Enzym Microb Technol 39:763–769. https://doi.org/10.1016/j.enzmictec.2005.12.009

Lin Y-T, Jia Z, Wang D, Chiu C-Y (2017) Effects of temperature on the composition and diversity of bacterial communities in bamboo soils at different elevations. Biogeosciences 14:4879–4889

Liu W, Zhang Z, Wan S (2009) Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Glob Chang Biol 15:184–195. https://doi.org/10.1111/j.1365-2486.2008.01728.x

Loranger J, Violle C, Shipley B, Lavorel S, Bonis A, Cruz P, Louault F, Loucougaray G, Mesléard F, Yavercovski N, Garnier É, Piper F (2016) Recasting the dynamic equilibrium model through a functional lens: the interplay of trait-based community assembly and climate. J Ecol 104:781–791

Luan J, Liu S, Wang J et al (2011) Rhizospheric and heterotrophic respiration of a warm-temperate oak chronosequence in China. Soil Biol Biochem 43:503–512. https://doi.org/10.1016/j.soilbio.2010.11.010

Lundegårdh H (1927) Carbon dioxide evolution of soil and crop growth. Soil Sci 23:417–453

Luo C, Rodriguez-R LM, Johnston ER et al (2014) Soil microbial community responses to a decade of warming as revealed by comparative metagenomics. Appl Environ Microbiol 80:1777–1786. https://doi.org/10.1128/AEM.03712-13

Mandakovic D, Rojas C, Maldonado J et al (2018) Structure and co-occurrence patterns in microbial communities under acute environmental stress reveal ecological factors fostering resilience. Sci Rep 8:5875. https://doi.org/10.1038/s41598-018-23931-0

Marchal M, Briandet R, Koechler S et al (2010) Effect of arsenite on swimming motility delays surface colonization in *Herminiimonas arsenicoxydans*. Microbiology 156:2336–2342. https://doi.org/10.1099/mic.0.039313-0

Marchal M, Briandet R, Halter D et al (2011) Subinhibitory arsenite concentrations lead to population dispersal in Thiomonas sp. PLoS One 6:e23181. https://doi.org/10.1371/journal.pone.0023181

Margesin R, Schinner F (2001) Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles 5:73–83

Maynaud G, Brunel B, Yashiro E et al (2014) CadA of *Mesorhizobium metallidurans* isolated from a zinc-rich mining soil is a PIB-2-type ATPase involved in cadmium and zinc resistance. Res Microbiol 165:175–189. https://doi.org/10.1016/j.resmic.2014.02.001

Mazaheri Assadi M, Tabatabaee MS (2010) Biosurfactants and their use in upgrading petroleum vacuum distillation residue: a review. Int J Environ Res 4:549–572. https://doi.org/10.22059/ijer.2010.242

McEntee JD, Woodrow JR, Quirk AV (1986) Investigation of cadmium resistance in an *Alcaligenes spp*. Appl Environ Microbiol 51:515–520

McGrath SP, Brookes PC, Giller KE (1988) Effects of potentially toxic metals in soil derived from past applications of sewage sludge on nitrogen fixation by *trifolium repens* L. Soil Biol Biochem 20:415–424. https://doi.org/10.1016/0038-0717(88)90052-1

Meisner A, Jacquiod S, Snoek BL, Ten Hooven FC, Van Der Putten WH (2018) Drought legacy effects on the composition of soil fungal and prokaryote communities. Front Microbiol 9:294

Mengtian H, Xuhui W, Trevor FK, Shilong P (2018) Drought timing influences the legacy of tree growth recovery. Glob Chang Biol 24:3546–3559

Mergeay M (1991) Towards an understanding of the genetics of bacterial metal resistance. Trends Biotechnol 9:17–24. https://doi.org/10.1016/0167-7799(91)90007-5

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 33:453–467

Mirete S, Mora-Ruiz MR, Lamprecht-Grandío M, De Figueras CG, Rosselló-Móra R, González-Pastor J (2015) Salt resistance genes revealed by functional metagenomics from brines and moderate-salinity rhizosphere within a hypersaline environment. Front Microbiol 6:1121

Morton-Bermea O, Hernández-Álvarez E, González-Hernández G et al (2009) Assessment of heavy metal pollution in urban topsoils from the metropolitan area of Mexico City. J Geochem Explor 101:218–224. https://doi.org/10.1016/j.gexplo.2008.07.002

Mukhopadhyay R, Rosen BP, Phung LT, Silver S (2002) Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol Rev 26:311–325. https://doi.org/10.1111/j.1574-6976.2002.tb00617.x

Nagvenkar GS, Ramaiah N (2010) Arsenite tolerance and biotransformation potential in estuarine bacteria. Ecotoxicology 19:604–613. https://doi.org/10.1007/s10646-009-0429-8

Naik MM, Dubey SK (2013) Lead resistant bacteria: lead resistance mechanisms, their applications in lead bioremediation and biomonitoring. Ecotoxicol Environ Saf 98:1–7. https://doi.org/10.1016/j.ecoenv.2013.09.039

Narendrula-Kotha R, Nkongolo KK (2017) Bacterial and fungal community structure and diversity in a mining region under long-term metal exposure revealed by metagenomics sequencing. Ecol Genet Genom 2:13–24. https://doi.org/10.1016/j.egg.2016.11.001

Narita M, Chiba K, Nishizawa H et al (2003) Diversity of mercury resistance determinants among Bacillus strains isolated from sediment of Minamata Bay. FEMS Microbiol Lett 223:73–82. https://doi.org/10.1016/S0378-1097(03)00325-2

Naylor D, Coleman-Derr D (2017) Drought stress and root-associated bacterial communities. Front Plant Sci 8:2223

Neilson JW, Califf K, Cardona C et al (2017) Significant impacts of increasing aridity on the arid soil microbiome. mSystems 2. https://doi.org/10.1128/mSystems.00195-16

Nguyen LTT, Osanai Y, Anderson IC, Bange MP, Braunack M, Tissue DT, Singh BK (2018) Impacts of waterlogging on soil nitrification and ammonia-oxidizing communities in farming system. Plant Soil. https://doi.org/10.1007/s11104-018-3584-y

Nies DH (1992) Resistance to cadmium, cobalt, zinc, and nickel in microbes. Plasmid 27:17–28. https://doi.org/10.1016/0147-619X(92)90003-S

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27:313–339. https://doi.org/10.1016/S0168-6445(03)00048-2

Nishiuchi S, Yamauchi T, Takahashi H, Kotula L, Nakazono M (2012) Mechanisms for coping with submergence and waterlogging in rice. Plant Rice 52

Niu X, Song L, Xiao Y, Ge W (2018) Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. Front Microbiol 8:2580

Norby RJ, Luo Y (2016) Evaluating ecosystem responses to rising atmospheric CO2 and global warming in a multi-factor world. New Phytol:281–293. https://doi.org/10.1111/j.1469-8137.2004.01047.x@10.1002/(ISSN)1469-8137(CAT)SpecialIssues(VI)Climatechangeandecosystemfunction

Noroozi M, Amoozegar MA, Pourbabaei AA et al (2017) Isolation and characterization of mercuric reductase by newly isolated halophilic bacterium, Bacillus firmus MN8. Glob J Environ Sci Manag 3:427–436

Nwuche CO, Ugoji EO (2008) Effects of heavy metal pollution on the soil microbial activity. Int J Environ Sci Technol 5:409–414. https://doi.org/10.1007/BF03326036

Olmedo MM, Mauricio Ortiz M, Selles G (2015) Effects of transient soil waterlogging and its importance for rootstock selection. Chilean J Agric Res 75:45–56

Oremland RS, Stolz JF (2003) The ecology of arsenic. Science 300:939–944. https://doi.org/10.1126/science.1081903

Pahari A, Pradhan A, Nayak SK, Mishra BB (2017) Bacterial siderophore as a plant growth promoter. 163–180

Pastor JM, Salvador M, Argandona M et al (2010) Ectoines in cell stress protection: uses and biotechnological production. Biotechnol Adv 28(6):782–801

Patel PR, Shaikh SS, Sayyed RZ (2018) Modified chrome azurol S method for detection and estimation of siderophores having affinity for metal ions other than iron. Environ Sustain 1:81–87. https://doi.org/10.1007/s42398-018-0005-3

Pattus F, Abdallah MA (2000) Siderophores and iron-transport in microorganisms. J Chin Chem Soc 47:1–20. https://doi.org/10.1002/jccs.200000001

Paul D, Lade H (2014) Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. Agron Sustain Dev 34(4):737–752

Pedros-Alio C (2012) The rare bacterial biosphere. Annu Rev Mar Sci 4:449–466

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten W (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799. https://doi.org/10.1038/nrmicro3109

Pirrone N, Cinnirella S, Feng X et al (2010) Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmos Chem Phys 10:5951–5964. https://doi.org/10.5194/acp-10-5951-2010

Placella SA, Brodie EL, Firestone MK (2012) Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proc Natl Acad Sci 109:10931–10936

Preece C, Verbruggen E, Liu L, Weedon JT, Penuelas J (2019) Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biol Biochem 131:28–39

Rasooli I, Astaneh SDA, Borna H, Barchini KA (2008) A thermostable Î±-amylase producing natural variant of *Bacillus* spp. isolated from soil in Iran. Am J Agric Biol Sci 3:591–596

Ratkowsky DA, Olley J, McMeekin TA, Ball A (1982) Relationship between temperature and growth rate of bacterial cultures. J Bacteriol 149:1–5

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326:123–125

Reina-Bueno M, Argandoñ M, Nieto JJ et al (2012) Role of trehalose in heat and desiccation tolerance in the soil bacterium *Rhizobium etli*. BMC Microbiol 12:207. https://doi.org/10.1186/1471-2180-12-207

Remonsellez F, Orell A, Jerez CA (2006) Copper tolerance of the thermoacidophilic archaeon *Sulfolobus metallicus*: possible role of polyphosphate metabolism. Microbiology 152:59–66. https://doi.org/10.1099/mic.0.28241-0

Rodríguez-Rojas F, Díaz-Vásquez W, Undabarrena A et al (2016) Mercury-mediated cross-resistance to tellurite in Pseudomonas spp. isolated from the Chilean Antarctic territory. Metallomics 8:108–117. https://doi.org/10.1039/C5MT00256G

Roessler M, Pfluger K, Flach H, Lienard T, Gottschalk G, Muller V (2002) Identification of a salt induced primary transporter for glycine betaine in the methanogen *Methanosarcina mazei* Go¨1. Appl Environ Microbiol 68:2133–2139

Romantsov T, Guan Z, Wood JM (2009) Cardiolipin and the osmotic stress responses of bacteria. Biochim Biophys Acta 1788:2092–2100

Rubiano-Labrador C, Bland C, Miotello G, Armengaud J, Baena S (2015) Salt stress induced changes in the exoproteome of the halotolerant bacterium Tistlia consotensis deciphered by proteogenomics. PLoS One 10(8):e0135065. https://doi.org/10.1371/journal.pone.0135065

Sahrawat KL (2005) Fertility and organic matter in submerged rice soils. Curr Sci 88:735–739

Sairam RK, Tyagi A, Chinnusamy V (2016) Salinity tolerance: cellular mechanisms and gene regulation. Plant-Environ Interactions 137–191

Sandhya V, ASK Z, Grover M, Reddy G, Venkateswarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAPP45. Biol Fertil Soils 46:17–26

Sasidharan R, Bailey-Serres J, Ashikari M, Atwell BJ, Colmer TD, Fagerstedt K et al (2017) Community recommendations on terminology and procedures used in flooding and low oxygen stress research. New Phytol 214:1403–1407

Saum SH, Müller V (2007) Salinity-dependent switching of osmolyte strategies in a moderately halophilic bacterium: glutamate induces proline biosynthesis in *Halobacillus halophilus*. J Bacteriol 189:6968–6975. PMID: 17660292

Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13:2844–2854. https://doi.org/10.1111/j.1462-2920.2011.02556.x

Schiering N, Kabsch W, Moore MJ et al (1991) Structure of the detoxification catalyst mercuric ion reductase from Bacillus sp. strain RC607. Nature 352:168–172. https://doi.org/10.1038/352168a0

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386–1394

Schmidt PA, Schmitt I, Otte J, Bandow C, Rombke J, Balint M, Rolshausen G (2018) Season-long experimental drought alters fungal community composition but not diversity in a grassland soil. Microb Ecol 75(2):468–478

Selin NE (2009) Global biogeochemical cycling of mercury: a review. Annu Rev Environ Resour 34:43–63. https://doi.org/10.1146/annurev.environ.051308.084314

Serrano R, Gaxiola R (1994) Microbial models and salt stress tolerance in plants. Crit Rev Plant Sci 13(2):121–138

Shamseldin A, Nyalwidhe J, Werner D (2006) A proteomic approach towards the analysis of salt tolerance in *Rhizobium etli* and *Sinorhizobium meliloti* strains. Curr Microbiol 52:333–339

Sharma R, Bhardwaj R, Gautam V et al (2018) Microbial siderophores in metal detoxification and therapeutics: recent prospective and applications. In: Egamberdieva D, Ahmad P (eds) Plant microbiome: stress response. Springer, Singapore, pp 337–350

Shaver GR, Canadell J, Chapin FS et al (2000) Global warming and terrestrial ecosystems. Bioscience 50:871–882. https://doi.org/10.1641/0006-3568(2000)050. 0871: GWATEA]2.0.CO;2

Shi Y, Adams JM, Ni Y, Yang T, Jing X, Chen L, He J, Chu H (2016) The biogeography of soil archaeal communities on the eastern Tibetan Plateau. Sci Rep 6:38893

Silver S, Phung LT (2005) A bacterial view of the periodic table: genes and proteins for toxic inorganic ions. J Ind Microbiol Biotechnol 32:587–605. https://doi.org/10.1007/s10295-005-0019-6

Singh P, Cameotra SS (2004) Enhancement of metal bioremediation by use of microbial surfactants. Biochem Biophys Res Commun 319:291–297. https://doi.org/10.1016/j.bbrc.2004.04.155

Smith SR (1997) Rhizobium in soils contaminated with copper and zinc following the long-term application of sewage sludge and other organic wastes. Soil Biol Biochem 29:1475–1489. https://doi.org/10.1016/S0038-0717(97)00036-9

Smith SR, Giller KE (1992) Effective Rhizobium leguminosarum biovar Trifolii present in five soils contaminated with heavy metals from long-term applications of sewage sludge or metal mine spoil. Soil Biol Biochem 24:781–788. https://doi.org/10.1016/0038-0717(92)90253-T

Smith K, Novick RP (1972) Genetic studies on plasmid-linked cadmium resistance in Staphylococcus aureus. J Bacteriol 112:761–772

Smith LT, Smith GM (1989) An osmoregulated dipeptide in stressed *Rhizobium meliloti*. J Bacteriol 171:4714–4717

Sone Y, Nakamura R, Pan-Hou H et al (2013a) Role of MerC, MerE, MerF, MerT, and/or MerP in resistance to mercurials and the transport of mercurials in *Escherichia coli*. Biol Pharm Bull 36:1835–1841. https://doi.org/10.1248/bpb.b13-00554

Sone Y, Nakamura R, Pan-Hou H et al (2013b) Increase methylmercury accumulation in Arabidopsis thaliana expressing bacterial broad-spectrum mercury transporter MerE. AMB Express 3:52. https://doi.org/10.1186/2191-0855-3-52

Sonjak S, Beguiristain T, Leyval C, Regvar M (2009) Temporal temperature gradient gel electrophoresis (TTGE) analysis of arbuscular mycorrhizal fungi associated with selected plants from saline and metal polluted environments. Plant Soil 314:25–34

Sparrow LA, Uren NC (2014) Manganese oxidation and reduction in soils: effects of temperature, water potential, pH and their interactions. Soil Res 52:483–494

Steele RA, Opella SJ (1997) Structures of the reduced and mercury-bound forms of MerP, the periplasmic protein from the bacterial mercury detoxification system. Biochemistry 36:6885–6895. https://doi.org/10.1021/bi9631632

Stieglmeier M, Alves RJE, Schleper C (2014) The prokaryotes: other major lineages of bacteria and the archaea. In: Rosenberg E, Delong EF, Lory S, Stackebrandt E, Thompson F (eds) . Springer, Berlin, pp 347–362

Stomeo F, Makhalanyane TP, Valverde A et al (2012) Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley. FEMS Microbiol Ecol 82(2):326–340

Su C (2014) A review on heavy metal contamination in the soil worldwide: situation, impact and remediation techniques. Environ Skeptics Critics 3:24

Susilowati A, Puspita AA, Yunus A (2018) Drought resistant of bacteria producing exopolysaccharide and IAA in rhizosphere of soybean plant (*Glycine max*) in Wonogiri Regency Central Java Indonesia. IOP Conf Ser Earth Environ Sci 142:012058

Tang Z, Sun X, Luo Z et al (2018) Effects of temperature, soil substrate, and microbial community on carbon mineralization across three climatically contrasting forest sites. Ecol Evol 8:879–891. https://doi.org/10.1002/ece3.3708

Tang J, Zhang J, Ren L et al (2019) Diagnosis of soil contamination using microbiological indices: a review on heavy metal pollution. J Environ Manag 242:121–130. https://doi.org/10.1016/j.jenvman.2019.04.061

TeChien C, Maundu J, Cavaness J, Dandurand LM, Orser CS (1992) Characterization of salt-tolerant and salt-sensitive mutants of Rhizobium leguminosarum biovar viciae strain C1204b. FEMS Microbiol Lett 90:135–140

Tisa LS, Rosen BP (1990) Molecular characterization of an anion pump. The ArsB protein is the membrane anchor for the ArsA protein. J Biol Chem 265:190–194

Todkar S, Todkar R, Kowale L, Karmarkar K, Kulkarni A (2012) Isolation and screening of antibiotic producing halophiles from Ratnagiri coastal area, state of Maharashtra. Int J Sci Res Publ 2(4):2250–3153

Torrens JL, Herman DC, Miller-Maier RM (1998) Biosurfactant (Rhamnolipid) sorption and the impact on rhamnolipid-facilitated removal of cadmium from various soils under saturated flow conditions. Environ Sci Technol 32:776–781. https://doi.org/10.1021/es970285o

Toth Z, Tancsics A, Kriszt B, Kroel-Dulay G, Onodi G, Hornung E (2017) Extreme effects of drought on composition of the soil bacterial community and decomposition of plant tissue. Eur J Soil Sci 68:504–513

Treseder KK, Lennon JT (2015) Fungal traits that drive ecosystem dynamics on land. Microbiol Mol Biol Rev 79:243–262

Tringe SG, von Mering C, Kobayashi A et al (2005) Comparative metagenomics of microbial communities. Science 308:554–557. https://doi.org/10.1126/science.1107851

Tsuzuki M, Moskvin OV, Kuribayashi M, Sato K, Retamal S, Abo M et al (2011) Salt stress-induced changes in the transcriptome, compatible solutes, and membrane lipids in the facultatively phototrophic bacterium *Rhodobacter sphaeroides*. Appl Environ Microbiol 77:7551–7559

United Nations Convention to Combat Desertification (2017). https://www.unccd.int

Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62:504–544

Vera-Gargallo B, Ventosa A (2018) Metagenomic insights into the phylogenetic and metabolic diversity of the prokaryotic community dwelling in hypersaline soils from the Odiel saltmarshes (SW Spain). Genes 9(3):152

Voordouw G (1995) The genus desulfovibrio: the centennial. Appl Environ Microbiol 61:2813–2819

Waditee R, Hibino T, Nakamura T, Incharoensakdi A, Takabe T (2002) Overexpression of a Na/H antiporter confers salt tolerance on a freshwater cyanobacterium, making it capable of growth in sea water. PNAS 99:4109–4114

Wan S, Hui D, Wallace L, Luo Y (2005) Direct and indirect effects of experimental warming on ecosystem carbon processes in a tallgrass prairie. Glob Biogeochem Cycles 19:1–13. https://doi.org/10.1029/2004GB002315

Wan S, Norby RJ, Ledford J, Weltzin JF (2007) Responses of soil respiration to elevated CO2, air warming, and changing soil water availability in a model old-field grassland. Glob Chang Biol 13:2411–2424. https://doi.org/10.1111/j.1365-2486.2007.01433.x

Wang L, Zhuang X, Zhuang G, Jing C (2016) Arsenic resistance strategy in Pantoea sp. IMH: organization, function and evolution of ars genes. Sci Rep 6:39195. https://doi.org/10.1038/srep39195

Waskiewicz A, Muzolf-Panek M, Golinski P (2013) Phenolic content changes in plants under salt stress. In: Ahmad P, Azooz MM, Prasad MNV (eds) Ecophysiology and responses of plants under salt stress. Springer New York, New York, pp 283–314

Wilde P, Manal A, Stodden M, Sieverding E, Hildebrandt U, Bothe H (2009) Biodiversity of arbuscular mycorrhizal fungi in roots and soils of two salt marshes. Environ Microbiol 11:1548–1561

Wilson JR, Leang C, Morby AP et al (2000) MerF is a mercury transport protein: different structures but a common mechanism for mercuric ion transporters? FEBS Lett 472:78–82. https://doi.org/10.1016/S0014-5793(00)01430-7

Wu QS, Zou YN (2017) *Arbuscular mycorrhizal* fungi and tolerance of drought stress in plants. Springer, pp 25–41

Xiao X, Wang M, Zhu H et al (2017) Response of soil microbial activities and microbial community structure to vanadium stress. Ecotoxicol Environ Saf 142:200–206. https://doi.org/10.1016/j.ecoenv.2017.03.047

Yang J, Yang F, Yang Y et al (2016) A proposal of "core enzyme" bioindicator in long-term Pb-Zn ore pollution areas based on topsoil property analysis. Environ Pollut 213:760–769. https://doi.org/10.1016/j.envpol.2016.03.030

Yin K, Zhang W, Chen L (2014) Pyoverdine secreted by Pseudomonas aeruginosa as a biological recognition element for the fluorescent detection of furazolidone. Biosens Bioelectron 51:90–96. https://doi.org/10.1016/j.bios.2013.07.038

Yin K, Wu Y, Wang S, Chen L (2016) A sensitive fluorescent biosensor for the detection of copper ion inspired by biological recognition element pyoverdine. Sensors Actuators B Chem 232:257–263. https://doi.org/10.1016/j.snb.2016.03.128

You Y, Wang J, Huang X et al (2014) Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover. Ecol Evol 4:633–647. https://doi.org/10.1002/ece3.969

Zar MS, Ali S, Shahid AA (2013) The influence of carbon and nitrogen supplementation on alpha amylase productivity of *Bacillusamyloliquefaciens* IIB-14 using fuzzy-logic and two-factorial designs. Afr J Microbiol Res 7(2):120–129

Zhang K, Shi Y, Cui X, Yue P, Li K, Liu X, Tripathi BM, Chu H (2019) Salinity is a key determinant for soil microbial communities in a desert ecosystem. https://doi.org/10.1128/mSystems.00225-18

Functional Diversity in Rhizosphere Microbial Community: Concept to Applications

16

Nafisa Patel, Naresh Butani, and Piyush Desai

Abstract

The microbial diversity attains different level of complexity with microorganisms interacting with the soil as well as with the plants. It is the cumulated interactive multilateral relationship which leads to the establishment of rhizosphere. The soil type or the plant type leads to the formation of different microhabitats varying at micrometer level. Microbial diversity can be studied in terms of species diversity, genetic diversity, and ecological diversity. The microbial activity affects and modifies the exudates and the subsequent rhizodeposition which becomes decisive in establishing the microbial community in the root rhizosphere. The functional behavior of the microbial communities which may include a group of species trait represented as an individual or species leads to the functional diversity in the rhizosphere. Functional diversity influences ecosystem dynamics, stability, productivity, nutrient balance, and other aspects of ecosystem functioning. Rhizospheric microbes are directly or indirectly associated with enhancement of plant growth or plant protection by secreting biomolecules. Thus, functionally these can be categorized as PGPR which directly promotes plant growth or those inhibiting the pathogens which are generally termed as BCA (biological control agents) and indirectly enhancing the plant growth. These functionally diverse microbial types are successfully applied to maintain

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sustainability of the ecosystem as are now available as biofertilizers, bioprotectants, and biostimulants. It is either a co-operative or competitive relation with the other existing microbial populations leading to the establishment of microbial habitat whereupon the functional attributes of theirs which fulfils the ecological need. The ecological dependencies on each other lead to the development of stable microbial communities in the rhizosphere.

Keywords

Niche · Microhabitat · Diversity · Functional diversity · Interactions · PGPR (Plant growth promoting rhizobia) · BCA (Biological control agents)

16.1 Introduction

Microbial community structure is mainly dependent on the complex interaction of microbial population with the type of plant and soil. The determinative force which establishes the community can be either the type of soil or the plant, dominated by either of the one depending on the selective force exhibited or the existing ecosystem sustainability. The formation of soil structure is due to the biochemical activities such as biogeochemical cycling, decomposition, and degradation of organic substances. Soils are source of nutrients and water which enables crop and plant growth. The size, shape, and arrangement of the soil constituents such as gravel, sand, silt, clay, and organic matter makes up the soil texture which has a huge impact on the water holding capacity, water conducting ability, and chemical properties of soil. Soil structure development is influenced by the amount and type of clay, exchangeable ions, water, amount and type of organic matter, cementing agents such as iron, aluminum oxides, polysaccharides, binding between organic and inorganic compounds, plants and the microbial population. All soil components constituting the soil structure differ in the abiotic conditions and the nutrition availability causing the formation of different microhabitats varying at micrometer level. Each microhabitat represents a stable environment where the microbial population has found its niche and established itself. A wide phenotypic and genotypic diversity prevails due to the heterogenous and versatile metabolic capacities of the microorganisms. Moreover, the change in the environmental conditions as well as the man made activities tends to cause a change in the existing conditions and hence it becomes difficult to define the microbial community.

The rhizosphere constitutes the quantity of soil associated with the plant root and root tissue and the surrounding soil around the root which is affected physically, chemically, and biologically due to the varied microbial activities in it. The presence of the endophytes in the internal tissues of the root makes root a part of the rhizosphere while the soil not associated with the root is termed as the bulk soil. Rhizospheric soil is differentiated into three regions, viz. the endorhizospheric zone which includes root tissue, endodermic and cortical layers, rhizoplane zone is the root surface comprising of mucilaginous and epidermic layers and cortex, and the

ectorhizospheric zone which consists of soil adhering to the roots. Apart from these defined zones in a rhizosphere there exist some specialized zones at times like the mycorrhizospheric zone in which the plant root is associated with fungi, the rhizosheath which is a condensed layer made of root hairs, soil particles, mucous substances, and the microorganisms (Lynch and Whipps 1990). The rhizosphere has a very high concentration of easily degradable carbon sources due to rhizodeposition which results into a much higher microbial activity as compared to the bulk soil (Berendsen et al. 2012). It is a myriad of interactions at a different level of complexity between microorganisms and the soil as well as between microorganisms and plants rather it is the multilateral relationships between the plant, soil, and microbial population which makes up the rhizosphere.

16.2 Diversity

Rhizosphere is a hub of a microbial community in terms of quantity as well as quality resulting into a complex network of macro as well as microorganisms which includes versatile range of bacteria, fungi, protozoa, algae as well nematodes and microarthropods which may exist as saprophytes, epiphytes, endophytes, pathogens, and functionally important microorganisms (Jeffery et al. 2010). Thus in terms of microbial diversity, rhizosphere is a richest dwelling microorganism with its ecological niche depending on the soil composition, root exudates, and the environmental conditions. The microbial activity affects and modifies the exudates and the subsequent rhizodeposition which becomes decisive in establishing the microbial community in the root rhizosphere. A triad of microbial community-soil type-plant type determines the microbial diversity (Fig. 16.1). The establishment of microbial community structures is affected by the type of soil and distribution of the soil

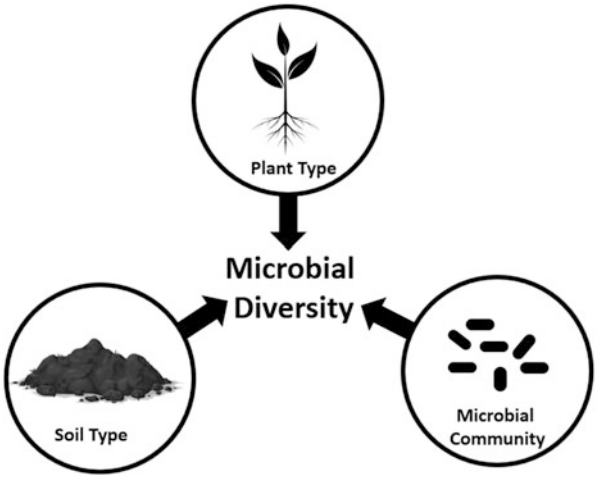

Fig. 16.1 Determinative forces of microbial diversity

particles, the type of plant species and its age, rotation of crops, and the soil management practices.

16.3 Functional Microbial Diversity

Diversity can be at various levels, viz. morphology, phenotypic characteristics, physiology and metabolism, habitat, ecology, genotype. Henceforth, biodiversity is often defined and studied in totality as species diversity, genetic diversity, and ecological diversity. The establishment of microbial community in each ecological niche is attributed to the functional capability and the versatile metabolism of these organisms. The functional behavior of the microbial communities which may include a group of species trait represented as an individual or species leads to the functional diversity in the rhizosphere. The occurrence of such functionally diverse microbial population in the rhizosphere carries out all such functions which directly or indirectly enhances plant growth and increases crop productivity (Tilak et al. 2005). Functional diversity is the measurement of microbial distribution and the varied functions performed by the group of microbial population or individual organism among the existing microbial communities and ecosystem (Díaz and Cabido 2001). Thus, functional diversity involves the study of the existing microbial population at multiple levels exhibiting specific species trait and each trait may represent species or individual manifesting that function which are associated with plant growth and development whereby differentiating the redundant population in the rhizosphere. Functional diversity influences ecosystem dynamics, stability, productivity, nutrient balance, and other aspects of ecosystem functioning (Trivedi et al. 2012). All such functionally important rhizobacteria are termed as the PGPR. These PGPRs are associated with the plant root either extracellular (ePGPR) or intracellular (iPGPR) and many times in the same ecological niche (Martínez-Viveros et al. 2010).

The functional diversity leads to the increase of soil nutritional quality and crop production and hence these can be functionally categorized as those promoting the plant growth (PGPR) or those inhibiting the pathogens (BCA) and indirectly enhancing the plant growth. The PGPR can be further categorized as biofertilizers, bioprotectants, and biostimulants (Martínez-Viveros et al. 2010). Biofertilizers are PGPR strains which improve nutrient uptake and seed germination and hence termed as N_2 fixers, phosphate solubilizers, and organic acid producers. Bioprotectant enhances plant resistance against pathogens and hence may act as antifungal, antibacterial, antiviral as well as renders protection against insects and nematodes. Biostimulants are phytohormone producers and are secondary metabolite producers, viz. auxins, IAA, cytokinins, riboflavin, and vitamins (Fig. 16.2).

The plant rhizosphere is extensively researched to observe diversity of the microbial population in terms of the varied functions exhibited by them. Accordingly, nitrogen fixation in legumes showed by diverse microbial genera, viz. *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium,* and *Rhizobium* (Harman and Uphoff 2019; Kumawat et al. 2019; Wang and Martinez-Romero

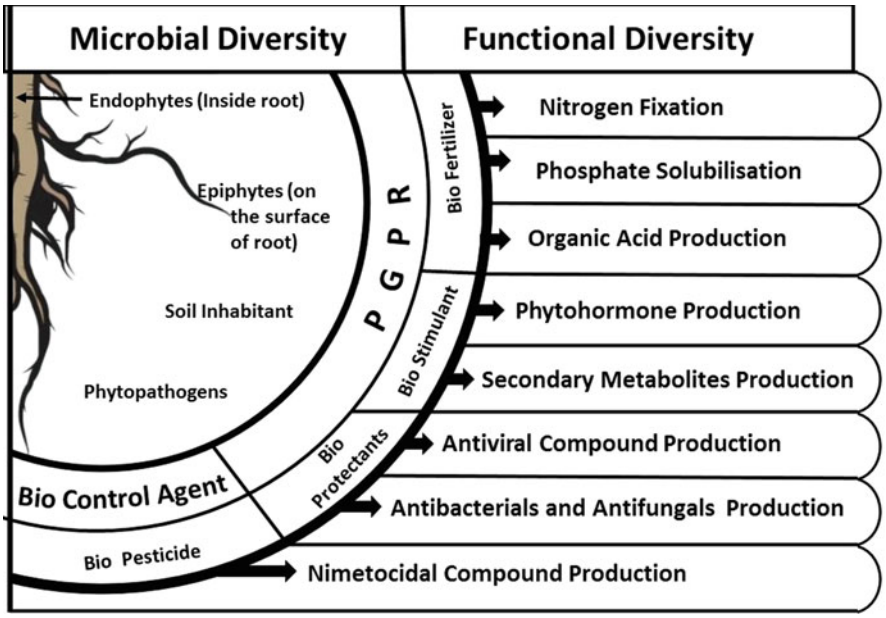

Fig. 16.2 Functional diversity exhibited by rhizospheric microbes. Abbreviation: *PGPR* plant growth promoting rhizobia

2000), while diversity in the PGPR traits was exhibited by *Agrobacterium*, *Flavobacterium*, *Serratia*, *Pseudomonas*, *Providencia, Alcaligenes,* and *Cellulomonas* (Chauhan et al. 2015; Disi et al. 2019; Duy et al. 2016; Gray and Smith 2005; Hossain et al. 2015) while *Pantoea*, *Exiguobacteium*, *Methylobacterium*, *Paenibacillus,* and *Azoarcus* exhibited all the characteristic of PGPR as well as other important metabolites. Diversity in functionally active microorganisms as BCA is a range of *Streptomyces*, *Streptosporangium*, *Thermobifida*, *Micromonospora* (Franco-Correa et al. 2010) while Lee and Hwang (2002) studied diverse genera such as *Actinomadura*, *Streptosporangium*, *Dactylosporangium*, *Micromonospora,* and *Streptomyces* which functioned as anti-fungal agent against fungal plant pathogens.

16.4 Nitrogen Fixers

Nitrogen is an essential element in all the living forms but is extremely unreactive. Plants are incapable to utilize the atmospheric nitrogen in spite of its importance in the metabolism and growth of the plants. Biological nitrogen fixation is an important microbial process producing approximately 200 million tons of nitrogen annually, contributing to almost 50% of the total nitrogen in crop. These microbial populations

which may be symbiotic or free living establish in the vicinity of the roots wherein the exudates released by the plants help in its establishment and henceforth have a higher nitrogen fixation by the resident microbial flora (Egamberdieva and Kucharova 2008). The capacity of nitrogen fixation depends on the moisture, oxygen concentration, and availability of organic C substrates (Church et al. 2008). Rhizobia residing in nodulated legumes constitute 65% of the total nitrogen fixed every year microbiologically. 70% of all nitrogen fixed per year is mediated through the biological nitrogen fixation (Vitousek et al. 2002). Nitrogen fixation is mediated by symbiosis between bacteria and vascular plants, symbiosis between cyanobacteria and fungi, free living heterotrophic or autotrophic bacteria, and abiotic reactions without microbes (Crews 1999). The nitrogen fixers occur in rhizosphere as free living which includes majorly the aerobic *Azotobacter*, anaerobic *Clostridia* or in symbiosis with the higher plants as rhizobia with legumes, Azolla with anabaena, Azollae with azolla. The symbiotic associations may be loosely associated with the plants or may be intercellular symbiosis. *Azospirillum*, *Azotobacter*, *Enterobacter* species are evidenced to occur in rhizosphere of different plants such as sugarcane, maize, rice, grasses, etc. (Affourtit et al. 2001).

Rhizobium-legume association is the most efficient fixing systems fixing approximately 100–300 kg nitrogen per hectare per year (Nghia and Gyurjan 1987). Apart from the N_2 fixing ability, bacteria such as *Azotobacter*, *Azospirillum*, *Rhizobium*, *Mesorhizobium*, *Sinorhizobium* also secrete phytohormones to improve plant development. *Azospirillum* is facultative endophytic diazotroph which increases the root and shoot development by increasing the water and mineral uptake. *Azotobacter* is obligate aerobe, widespread in tropical, subtropical, and temperate regions, these are found in close association with roots of wild and agricultural plants (Doroshenko et al. 2007). *Azolla spp.* are important source of nitrogen for wetland rice, which occurs in symbiosis with blue green algae—nostoc, anabaena. Cyanobacter are global nitrogen fixers which are most widespread and important on earth. They are diverse groups forming complex association with bacteria and green algae in structures called as cyanobacterial mats (Rodrigo and Eberto 2007). *Trichodesmium spp.* is considered as cosmopolitan cyanobacterium found in tropical, subtropical, marine ecosystems. *Gluconacetobacter diazotrophicus* is a nitrogen fixing acetic acid bacterium first isolated form sugarcane. Currently, many are added which include *Acetobacter nitrogenifigens*, *Gluconacetobacter kombuchae*, *Gluconacetobacter johannae*, *Gluconacetobacter azotocaptans*, *Gluconacetobacter diazotrophicus*, *Swaminathania salitolerans*, *Acetobacter peroxydans* (Saravanan et al. 2008).

The functional diversity among the nitrogen fixers has been studied immensely to find variation among the types of microorganisms, type of crop, type of soil, type of climatic habitat. Microorganisms colonize different crops and stimulate plant growth either directly or indirectly as in Table 16.1.

Table 16.1 Microbial diversity colonizing different plant roots to stimulate plant growth

Microbial diversity	Plant type	Reference
Azotobacter vinelandii	Rice	Sahoo et al. (2014)
Bacillus brevis, B. cereus, B. circulans, B. firmus, B. licheniformis, B. megaterium, B. pumilus, and B. subtilis	Rice	Xie et al. (1998)
Azospirillum brasilense, Azospirillum zeae, Pseudomonas stutzeri	Wheat	Venieraki et al. (2011)
Achromobacter insolitus	Wheat	da Silveira et al. (2016)
Bacillus megaterium	Maize, rice	Liu et al. (2006)
Bacillus rhizosphaerae	Sugarcane	Madhaiyan et al. (2011)
Burkholderia tropica	Maize	Reis et al. (2004)
Burkholderia silvatlantica	Sugarcane	Perin et al. (2006)
Delftia tsuruhatensis	Rice	Han et al. (2005)
Enterobacter sacchari	Sugarcane	Zhu et al. (2013)
Gluconacetobacter diazotrophicus	Sugarcane	Vargas et al. (2014)
Stenotrophomonas maltophilia	Sugarcane	Xing et al. (2016)
Pseudomonas koreensis and P. entomophila	Sugarcane	Li et al. (2017)
Acetobacter diazotrophicus	Sugarcane	Boddey et al. (1995)
Burkholderia caballeronis	Tomato	Martínez-Aguilar et al. (2013)
Paenibacillus borealis	Spruce forest	Elo et al. (2001)
Paenibacillus graminis, Paenibacillus odorifer	Plant roots	Berge et al. (2002)
Paenibacillus brasilensis	Maize	von der Weid et al. (2002)
B. amyloliquefaciens, B. aryabhattai, B. safensis, B. aerophilus, B. subtilis	Sugarcane	Kruasuwan and Thamchaipenet (2016)
Lysinibacillus fusiformis	Chickpea	Singh et al. (2013)

16.5 Phosphate Solubilizers

Phosphorus (P) is important in most of the life processes wherein it is directly involved with plant growth and high productivity. Its role is in all the activities in plants like photosynthesis, cell division, and development of healthy root system and utilization of carbohydrate (Kannaiyan et al. 2004). Phosphorous plays a fundamental role in plant metabolism and is important for the function of key enzymes in regulatory metabolic pathways (Theodorou and Plaxton 1993). The presence of phosphorus in the soil humus can be in the form of organic and inorganic P compounds often associated with the cellular components and henceforth the availability of soluble form of phosphorus is very less (Xiao et al. 2011). There exists

microorganism which can solubilize the insoluble phosphates and converts them into soluble and readily usable form in soil and makes it available to the crops (Kang et al. 2017; Pradhan and Sukla 2006). The rhizospheric soil microorganism plays a key role to increase P dynamics and the subsequent availability of phosphate to the plants (Mohammadi and Sohrabi 2012). An extensive variety of rhizospheric microorganisms are enabled to solubilize the insoluble phosphate compounds by either producing organic acids and/or phosphatase enzymes. The conversion of insoluble form of phosphorous to soluble forms by phosphate solubilizing bacteria (PSB) is undertaken by different mechanisms making it available for plants uptake and its growth promotion (Chandler et al. 2008). Gyaneshwar et al. (1999) identified unknown organic acids, viz. oxalic acid, citric acid, lactic acid, etc. from the microbial extracts using TLC and HPLC. Some of the organic acids directly dissolved the mineral phosphate by exchanging it with an acid anion, or aluminum ions or by chelating it with iron. In some cases, phosphate solubilization is induced by phosphate starvation. These organic acids released by the PSBs in the rhizospheric soil solubilized the insoluble phosphate by the lowering of pH, chelation of cations, and competing with phosphate for adsorption sites in soil (Nahas 1996). PSBs release some organic acid which acts as chelators of divalent cations of Ca^{2+} subsequently releasing phosphates from the insoluble phosphatic compounds. Kucey (1988) during their study showed equivalent solubilizing effect by *Penicillium bilaii* as with the addition of 0.05 M EDTA in the medium while Halder and Chakrabartty (1993) in their study confirmed the role of 2-ketogluconic acid in phosphate solubilizing activities of *Rhizobium*. Among the PSBs dwelling in the rhizosphere the most powerful P solubilizers belong to the bacterial genera *Pseudomonas, Bacillus, Enterobacter,* and *Rhizobium* and the fungal strains like *Penicillium* and *Aspergillus*. The common examples belong to species of *Pseudomonas, Bacillus, Micrococcus, Aspergillus, Flavobacterium, Fusarium, Penicillium, Sclerotium,* etc. (Whitelaw 1999). The functional diversity among the phosphate solubilizing bacteria is also attributed to the physical and chemical type of soil (Kim et al. 1997). Phosphate solubilizing bacteria among the PGPRs have capacity to solubilize the inorganic and organic phosphorous in soil. PSBs are found everywhere and it differs in shape and population in the different soil. The genus *Bacillus* and *Pseudomonas* are most powerful and active phosphate solubilizing bacteria (Krishnaraj and Goldstein 2001). The insoluble mineral phosphates are solubilized by different various phosphate solubilizing bacteria such as producing organic acids like fumaric, glycolic, lactic, succinic acids, malonic acid, etc. (Vazquez et al. 2000).

Among the bacterial species are *Oligospora, Alcaligenes sp, Pseudomonas, Bacillus, Burkholderia, and Rhizobium* are most important phosphate solubilizers in soil. Although all fungi are not phosphate solubilizers but some species of *Aspergillus* and *Penicillium* were identified to have more phosphate solubilizing effectiveness (Sagervanshi et al. 2012). The reported fungal strains functioning as phosphate solubilizers are *Aspergillus niger, A. awamori, A. terreus, A. nidulans, A. flavus, A. foetidus, A. wentii* and *P. digitatum, P. lilacinum, P. balaji, P. funicolosum*. Fungal strains solubilize phosphorous to a great level than bacterial

strain. *Aspergillus and Penicillium* are representative genera of phosphate solubilizers among the filamentous fungi. *Rhizocotonia* and *Trichoderma* are also reported as phosphorous solubilizers (Yasser et al. 2014). Fungi are higher producers of organic acids and hence they comparatively show more P-solubilizing activity compared to bacteria (Venkateswarlu et al. 1984).

16.6 Phytohormone Producers

Soil is the habitat of diverse organisms, including bacteria, archaea, many protist, fungi, etc. Plant roots, inhabiting the soil, interact with these microbes and also produce root exudates. The rhizosphere is nutrient rich ecological niche containing various growth enhancing molecules like vitamins, amino acids, carbohydrates (sugars), fatty acids as well as other organic compounds that attracts microbes. The tripartite role among plant root, soil, and microbes enhances the plant growth by various mechanisms which include microbially synthesized biologically active organic molecules, one of them is known as phytohormones. These molecules regulate various plant processes of cell division and differentiation, reproduction, germination and development of seeds, development of leaves and stem, flowering and signaling in plants (Bhatt et al. 2020). Auxins, cytokinins, gibberellins, and ethylene are popularly known as classical plant hormones whereas abscisic acid, brassinosteroids, jasmonic acid, salicylic acid (SA), strigolactones, polyamines, and nitric oxide are recently discovered phytohormones (Spaepen 2015). Diverse range of root associated microbes belonging to different genera and species are reported to producing phytohormones (Sgroy et al. 2009).

A natural auxin, indole acetic acid (IAA), plays key roles in growth regulation in plants, like vascular tissue differentiation and development, root and stem elongation, fruit setting, gravitropism, phototropism, and apical dominance. It is also assumed that over 80% of the rhizospheric bacteria exhibits IAA synthesis capacity (Khalid et al. 2005). A large scale detailed genomic analysis revealed that root associated rhizobacteria possess stronger IAA synthesis abilities as compared to bacteria inhabiting the other environment (Zhang et al. 2019). IAA is mainly synthesized from the aromatic amino acid tryptophan or chemicals similar to it via various pathways by microbes. Phytopathogenic microbes like *Agrobacterium tumefaciens*, *Pseudomonas syringae* as well as symbiotic nitrogen fixers like species belonging to *Rhizobium* and *Bradyrhizobium* are reported to produce IAA via indole-3-acetamide (IAM) pathway. Indole-3-pyruvate (IPA) pathway, the major pathway for IAA production in plant, is also reported in phytopathogens and plant beneficial bacteria. Plant pathogen like *Pantoea agglomerans* and plant growth enhancers such as *Azospirillum brasilense*, *Azospirillum lipoferum*, *Bacillus spp.*, *Bradyrhizobium spp.*, *Enterobacter cloacae*, *Paenibacillus spp.*, *Pseudomonas spp.*, and *Rhizobium spp.* are producing IAA via IPA pathway. The tryptamine (TAM or TRM) pathway, involving the activity of tryptophan decarboxylase, for synthesis of IAA is reported in *Bacillus cereus* and *Burkholderia pyrrocinia* (Liu et al. 2019). Some microbes like *Agrobacterium*, *Rhizobium spp.*, *Variovorax boronicumulans*

produce IAA via the indole-3-acetonitrile (IAN) pathway, the pathway mainly reported in plants (Sun et al. 2018). A unique pathway, tryptophan side-chain oxidase (TSO) pathway, has been only reported in *Pseudomonas fluorescens* CHA0 (Oberhänsli et al. 1991). The tryptophan independent pathway has been also exhibited for synthesis of IAA by *Cyanobacteria* and *Azospirilla* (Prasanna et al. 2010; Prinsen et al. 1993). Furthermore, metagenomic data analysis of rhizobacterial genomes also revealed that the main pathways used for IAA synthesis by rhizobacteria are indole-3-acetamide (IAM) and tryptamine (TMP) pathways (Zhang et al. 2019). The varied amount of IAA production by different strains is owing to different pathway of IAA synthesis, different gene location and gene regulation mechanism, different enzyme actions as well as environmental conditions (Kochar et al. 2013).

Cytokinins are involved in plant developmental and physiological processes like enhancement of cell division, enhancement of root development and root hair formation, chloroplast biogenesis, leaf expansion, stomatal openings, retarding senescence, and enhancement in photosynthesis (Bollag 2017). Various microbes exhibit cytokinins production capacities including *Flavobacterium*, *Citricoccus zhacaiensis, Bacillus amyloliquefaciens, Acinetobacter*, *Arthrobacter*, *Azospirillum*, *Paenibacillus polymyxa, Azospirillum*, *Bradyrhizobium japonicum,* etc. (Kapoor and Kaur 2016; Selvakumar et al. 2018; Sturtevant and Taller 1989; Timmusk et al. 1999). Evidences for role of bacteria in biocontrol of plant diseases or by strengthening the host by production of antimicrobial compounds are well documented, but the novel role of cytokinin has been identified as key determinant for biocontrol of *Pseudomonas syringae* infection in plant model *Arabidopsis*. Cytokinin produced by *Pseudomonas fluorescens* G20-18 form a strong basis of plant strategies to defend disease and also combat abiotic stress (Grobkinsky et al. 2016). Recently, role of cytokinin produced by *Bacillus aryabhattai* strain SRB02 in oxidative and nitrosative stress tolerance has been studied in soybean (Park et al. 2017).

Gibberellins (GAs), a broad group of more than 100 compounds, are crucial phytohormones involved in plant growth and developmental processes like seed dormancy, expansion of leaves and flowering, stem proliferation, and lateral shoot growth (Bottini et al. 2004). GA1, GA3, GA4, and GA7 are usually considered as bioactive GAs which are crucial for growth and development of plants, as well as plant–microbe interaction. Other GAs are involved in species specific bioactivities (Nett et al. 2017). Historically, gibberellins were first reported in culture *Gibberella fujikuroi*. Bacteria and many fungi also produce gibberellins, including *Rhizobium meliloti, Azospirillum lipoferum, Azospirillum brasilense, Bacillus cereus, Herbaspirillum seropedicae, Paecilomyces formosus, Sphingomonas spp., Aspergillus fumigatus, Fusarium proliferatum, Leifsonia xyli,* etc. (Bilal et al. 2018a, 2018b; Desai 2017; Kang et al. 2017; Pandya et al. 2011). Role of microbial gibberellins in plant growth promotion and various biosynthetic pathways has been deeply reviewed by various researchers (Bottini et al. 2004; Rodrigues et al. 2012). Gibberellins are used widely due to its plant promotion activities and its commercial production is mainly carried out by *Fusarium fujikuroi* and other related species.

Annual production of GA3 is estimated around 100 tons but due to lower yield of *Fusarium,* its price is varied between 150 and 500 U.S. dollars per kilogram, so its application is restricted (Camara et al. 2018). Recently, CRISPR/Cas9-based genome editing experiments had been carried out in filamentous fungus *Fusarium fujikuroi* in order to increase the production of GAs (Shi et al. 2019).

Abscisic acid (ABA) is a vital plant hormone which plays important roles in regulation of stomatal closure, leaf senescence, fruit ripening, and morphogenesis of embryo. It also holds a critical role in regulating abiotic environmental stress responses in plants like in drought, salt stress, and metal toxicity (Bhatt et al. 2020). The role of ABA produced by endophytic bacteria (*Bacillus amyloliquefaciens* RWL-1) in salinity stress tolerance in plant (*Oryza sativa*) by establishment of active symbiosis of RWL-1 with plant roots has been deciphered (Shahzad et al. 2017). Various microbes, like *Achromobacter xylosoxidans, Bacillus pumilus, Azospirillum brasilense,* and *Herbaspirillum seropedicae,* have been reported to increase abiotic stress tolerance in plants by producing ABA (Curá et al. 2017; Forchetti et al. 2007). Microbial ABA also helps the plants to fight with toxic metals, like cadmium, by reducing uptake and accumulation of toxic metals. Inoculation of PGPR like *Bacillus subtilis* or *Azospirillum brasilense* to cadmium contaminated soils reduces plant capacity to accumulate cadmium from soil via ABA mediated mechanism (Xu et al. 2018).

Many complex roles are played by phytohormones produced by microbes inside or outside the plant. Researchers are currently focusing on exploration of these microbial phytohormones to increase the plant productivity and enhancement of disease resistance in plant in order to enhance agricultural production.

16.7 VOCs Producers

Rhizosphere, a dynamic region of soil, is governed by complex interaction of plant roots and root associated microbes. Various rhizobacteria can stimulate the growth of plants, directly or indirectly, by secreting various molecules. A wide range of rhizobacteria, ranging from bacteria to fungi, produce volatile metabolites as result of primary or secondary metabolism, popularly known as microbial volatile organic compounds (mVOCs/MVOCs) (Korpi et al. 2009). Its physical and chemical properties make it very suitable as signal molecules to regulate various plant–microbe interactions, like low molecular weight, high vapor pressure, easy evaporation at room temperature, lipophilic moiety, and low boiling point. From its point of origin, MVOCs can travel a far in porous soils as well as in liquids make them ideal info-chemicals. MVOCs play significant ecological and biological roles like mediating intra and interspecies relationships (antagonism, mutualism, etc.), regulating plant growth, stress resistance, and plant defense (Reddy and Hindumathi 2017). The stimulated effect of bacterial volatiles (2,3-butanediol and acetoin) in *Arabidopsis* growth promotion was first reported by Ryu et al. (2003).

Bacterial volatile compounds also help in maintenance of soil health and protection of plants from pathogen attack. Strains of *Pseudomonas fluorescens*, a

well-recognized PGPR, had been reported to improve soil health and plant growth via VOCs mediated mechanisms (Hol et al. 2013; Park et al. 2015). Volatiles from bacteria, viz. *Stenotrophomonas maltophilia*, *Stenotrophomonas rhizophila*, *Serratia plymuthica*, *Serratia odorifera, Pseudomonas trivialis, Pseudomonas fluorescens, Bacillus subtilis,* and *Burkholderia cepacia*, inhibit the mycelial growth of *Rhizoctonia solani* (Kai et al. 2007). Spores or mycelium of *Fusarium oxysporum* are inhibited by volatiles produced by *Bacillus amyloliquefaciens*, *Burkholderia gladioli,* and *Pseudomonas fluorescens* (Elshafie et al. 2012; Guevara-Avendaño et al. 2020; Yuan et al. 2012). Recently, the role of mVOCs, produced by *Bacillus amyloliquefaciens* GB03, in plant salt stress tolerance has been reported (Cappellari and Banchio 2020).

Not only bacteria, fungi also produce wide range of volatile compounds mainly play roles in plant growth promotion and plant pathogen inhibition. A diverse range of volatile compounds are emitted by fungi like alcohols, aldehydes, alkenes, acids, benzenoids, terpenoids, esters and ketones, etc. The first fungal VOC mediated plant growth promotion by plant growth promoting fungi (PGPF), *Talaromyces wortmannii*, was reported by Yamagiwa et al. (2011). Similarly, *Cladosporium cladosporioides* CL-1, PGPF isolated from rhizosphere of red pepper, produced VOCs significantly increase growth of the seedling of tobacco and their root development (Paul and Park 2013). Another important role of fungi VOCs in pathogen inhibition is demonstrated by *Trichoderma,* the well-recognized biocontrol fungus, by Paul and Park (2013). Volatile compounds from *Trichoderma* demonstrated to play an important role in mycoparasitism as well as interaction with plants (Vinale et al. 2008). VOCs from fungi can also be used for mycofumigation, i.e. the use of antimicrobial volatiles produced by fungi for the control of pathogens. For instance, *Oxyporus latemarginatus* EF069 produce a volatile compound, 5-pentyl-2-furaldehyde, applied as a biofumigant for the control of fungal plant diseases (Lee et al. 2009). Pathogenic and non-pathogenic fungal volatiles can modulate plant growth promotion and susceptibility to insect (Moisan et al. 2019).

16.8 Antimicrobial Compounds Producers

Rhizobacteria produce wide array of antimicrobial compounds which play crucial role in antagonism and mediate local population dynamics. These compounds, produced by antagonist microorganisms, include versatile range of antimicrobial secondary metabolites like bacteriocins (ribosomally produced antimicrobial peptides), metabolic by-products (2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, phenazines, cyclic lipopeptides, pyoluteorin), broad-spectrum non-ribosomally synthesized antibiotics (bacillomycin D, fengycin, polyketides, and bacilysin), proteinaceous exotoxins, and lytic enzymes (Hou and Kolodkin-Gal 2020; Kraemer et al. 2017; Nihorimbere et al. 2009; Subramanian and Smith 2015).

Fluorescent pseudomonads, usually known by its notable feature of producing fluorescent pigment, are a large group of species recognized as potential PGPR and

biocontrol agent (BCA) as producing various antimicrobial compounds. These compounds exhibit antibacterial, antitumor, antifungal, antiviral, and anti-nematode properties. *Pseudomonas corrugata* and *P. mediterranea* were reported to produce hydrogen cyanide (HCN) which involved in inhibition of germination of phytopathogenic fungal spores (Strano et al. 2017). HCN production was also reported in various strains like *Pseudomonas spp*. P76 and P124, *Pseudomonas* CF1 and CF5, *P. aeruginosa* P4, *P. putida* R32, and *P. chlororaphis* R47 (Anand et al. 2020; Gupta et al. 2020; Priyanka et al. 2017; Reetha et al. 2014). Pseudomonads, like *P. aeruginosa, P. fluorescens, P. chlororaphis, P. cepacia, P. aurantiaca, P. brassicacearum,* are deeply reviewed for their antimicrobial production by Mishra and Arora (2018).

Bacteriocins, diverse group of ribosomally synthesized bioactive peptides or proteins, are produced by bacteria to inhibit or kill microbial competitors. They exhibit a narrow spectrum activity by inhibiting taxonomically closely related bacteria or a broad spectrum activity by inhibiting a wide range of bacteria (Silva et al. 2018). Various commonly-found Gram positive soil bacteria of genus Bacillus (*Bacillus cereus, B. thuringiensis*, *B. clausii*, *B. subtilis*, *B. amyloliquefaciens, B. stearothermophilus*, *B. megaterium, B. licheniformis,* etc.) are well known for their bacteriocins production. They produce different types of bacteriocins like cerein7, subtilin, Cerein 8A, bac-GM17, amylocyclicin, BL8, thuricin, tochicin, morricin, kenyacin, etc. (Subramanian and Smith 2015). Some other stains like *Lysinibacillus* jx416856, *Erwinia carotovora, Clavibacter michiganensis,* and *Rhizobium lupine* are also reported to produce bacteriocins. Lactic acid bacteria are widely reported for bacteriocins production. 119 different lactic acid bacteria were isolated from the rhizosphere of olive trees and desert truffles were showing good antibacterial and antifungal activity; this activity may be due to bacteriocins production (Fhoula et al. 2013).

Extracellular hydrolytic enzyme production is commonly found in the rhizospheric antagonistic microbes (Adesina et al. 2007). These enzymes play the role in displaying antifungal activity against different plant pathogenic fungi. *Lysobacter enzymogenes* produces extracellular lytic enzyme, β-1,3-glucanases, capable of inhibiting fungal growth by degrading its cell wall (Palumbo et al. 2005). *Serratia liquefaciens, S. plymuthica,* and *S. rubidaea* were isolated from rhizosphere of oilseed rape, found to produce lytic enzymes (like chitinases and β-1,3 glucanases), probably involved in fungal growth inhibition (Kalbe et al. 1996). Chitinase degrades chitin of the fungal cell wall. As a strategy to screen potential PGPR, the trait of chitinase production is widely selected by many researchers. Chitinase producing rhizospheric bacteria*, Pseudomonas putida* B E2, *P. chlororaphis* K15, *Serratia plymuthica* R12*, Pseudomonas spp*. NS-1, and *Bacillus spp*. NS-22, displayed efficient PGPR action in addition to inhibit fungal phytopathogens (Berg et al. 2001; Dukare et al. 2020). Other hydrolytic enzymes like cellulase, protease, polygalacturonase, and glucanase are also involved in lysis of fungal cell wall. *Paenibacillus* spp. 300 and *Streptomyces* spp. 385 were reported to produce chitinase and β-1,3-glucanase and involved in inhibition of Fusarium wilt (Singh et al. 1999). PGPR strain of *Pseudomonas aeruginosa* also produces protease

to exhibit the antifungal activity (Illakkiam et al. 2013). *Bacillus amyloliquefaciens* and *Bacillus subtilis* are expressing glucanolytic enzymes and inhibiting sugarcane fungal pathogens by degrading its cell wall (Zia et al. 2019).

Certain non-ribosomally synthesized antibiotics, bacillomycin D, fengycin, polyketides, and bacilysin, are exhibiting antimicrobial properties (Gu et al. 2017). The aerobic spore forming Gram positive bacterium, *Bacillus amyloliquefaciens* FZB42, is producing these type of antibiotics with other secondary metabolites. Bacillomycin D and fengycin show antifungal activity while polyketides and bacilysin show antibacterial effect. These unique properties make *Bacillus amyloliquefaciens* FZB42, a potential candidate to be used as biocontrol agent. Inhibition mechanism of antibiotics and other secondary metabolites are deeply discussed by Chowdhury et al. (2015). Lantibiotics (lanthionine-containing antibiotics) and non-ribosomally synthesized antibiotics are also produced by *Bacillus subtilis* (Stein 2005).

16.9 Siderophore Producers

The forth most abundant and another important constituent for the plant growth and development is iron which acts as cofactor in several enzymatic reactions as well as in other non-enzymatic reactions. Although of its abundance occurrence the bioavailability is very less due to the formation of insoluble ferric complexes at neutral to alkaline pH. In the rhizospheric soil, a distinct functionally divergent group of organisms plays a role of iron chelation and making it available to the plants by producing low molecular weight secondary metabolites referred as Siderophores. More than 500 such siderophore molecules are identified produced by different rhizospheric microbes (Challis 2005; Visca et al. 2007) whereupon its production is dependent on the iron content of the soil as well as the ecology of the soil with respect to the pH and the presence of other metal ions. A functional diversity exists in the microbial population as per the pH status and the iron deficiency. Fungi and Streptomyces formed a stable population in acidic soils by producing hydroxamate types of siderophores at low pH, while the hydroxamate and catecholate siderophores were seen in neutral to alkaline soils. Hydroxamate siderophores were produced by the fungus Hymenoscyphus ericae a pH range of 3.5 and 5.5 (Federspiel et al. 1991). An interesting phenomenon was exhibited by the *Escherichia coli* strain Nissle 1917 which produced aerobactin at pH 5.6 while salmochelin and yersiniabactin at pH 7.0 or more (Valdebenito et al. 2005). Besides *Vibrio spp.* produced aerobactin and vibrioferrin; enterochelin produced by *E. coli*, *B. cenocepacia* produced *ornibactin* and *pyochelin* (Sathe et al. 2019). Different pH range became an ecological niche for specific organism resulting into a diversed form of microbial community functional in siderophore formation. The occurrence of the functional diversity is further complicated by the co-evolutionary race battling for iron between the siderophore producers and non-producers in the existing diverse microbial community. Therefore, the rhizospheric microbes niches exhibit multiple types of siderophores. *Vibrio anguillarum* produces anguibactin and vanchrobactin

(Lemos et al. 2010), *P. aeruginosa* produces pyoverdine and pyochelin (Dumas et al. 2013), and *B. cenocepacia* produces ornibactin and pyochelin (Tyrrell et al. 2015). The prevailing siderophore producing microbial community may develop either a co-operative or competitive relation with the other microbial populations which results into co-evolutionary race between species. Their ecological dependencies on each other lead to the development of stable microbial communities (Kramer et al. 2020).

16.10 Plant-Parasitic Nematodes (PPN) Controller

A significant damage to several agricultural crops is reported every year by plant-parasitic nematodes (PPN) which are primarily inhabitant of soil. Mainly chemical pesticide, like methyl bromide, based strategies were applied to control PPN but nowadays biocontrol strategies are applied by considering nematocidal potential of various microbes in order to reduce environmental pollution. There are two main groups of rhizobacterial compounds, namely enzymes and secondary metabolites, involved in nematicidal activity which affects the external structural compounds of the nematodes or nematode organs. Lytic enzymes are produced by *Lysobacter capsici*, *Streptomyces cacaoi* GY525, *Bacillus megaterium* PSB2, *Brevibacillus laterosporus*, *Bacillus firmus* DS-1, *Brevibacterium frigoritolerans,* etc. (Aballay et al. 2017; El-Hadad et al. 2010; Lee et al. 2014; Yoon et al. 2012). Other secondary metabolites like, 2,4-diacetylphloroglucinol (DAPG) produced by *P. fluorescens*, H_2S produced by *Tsukamurella paurometabola* C-924 (Marin et al. 2010), HCN produced by *Pseudomonas chlororaphis* PA23 (Nandi et al. 2015), are playing an important role in control of plant-parasitic nematodes.

16.11 Conclusion

The plant microbial interactions in the rhizospheric soil have been studied in different capacities and at different levels ultimately resulting into the plant growth enhancements or plant protection and establishing systemic resistance against pathogens. The functionalities of these diverse heterogenous microbial populations have led to its development and applications as biofertilizers, bioenhancers, bioinoculants, biocontrols, biofilms, or as a bioprocess. The rich functional diversity of the microorganisms is a cumulative effect by the inherent capability of these versatile microorganisms as an individual or as a group, the plant exudates rich in organic substances, and the soil type determined by its physical and chemical characteristics.

References

Aballay E, Prodan S, Zamorano A, Castaneda-Alvarez C (2017) Nematicidal effect of rhizobacteria on plant-parasitic nematodes associated with vineyards. World J Microbiol Biotechnol 33:131

Adesina MF, Lembke A, Costa R, Speksnijder A, Smalla K (2007) Screening of bacterial isolates from various European soils for in vitro antagonistic activity towards Rhizoctonia solani and Fusarium oxysporum: site-dependent composition and diversity revealed. Soil Biol Biochem 39:2818–2828. https://doi.org/10.1016/j.soilbio.2007.06.004

Affourtit J, Zehr JP, Paerl HW (2001) Distribution of nitrogen-fixing microorganisms along the Neuse River Estuary, North Carolina. Microb Ecol 41:114–123

Anand A, Chinchilla D, Tan C, Mène-Saffrané L, L'Haridon F, Weisskopf L (2020) Contribution of hydrogen cyanide to the antagonistic activity of Pseudomonas strains against Phytophthora infestans. Microorganisms 8:1144

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Berg G, Fritze A, Roskot N, Smalla K (2001) Evaluation of potential biocontrol rhizobacteria from different host plants of Verticillium dahliae Kleb. J Appl Microbiol 91:963–971. https://doi.org/10.1046/j.1365-2672.2001.01462.x

Berge O, Guinebretière M-H, Achouak W, Normand P, Heulin T (2002) Paenibacillus graminis sp. nov. and Paenibacillus odorifer sp. nov., isolated from plant roots, soil and food. Int J Syst Evol Microbiol 52:607–616

Bhatt D, Nath M, Sharma M, Bhatt MD, Bisht DS, Butani NV (2020) Role of growth regulators and Phytohormones in overcoming environmental stress, in: protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives. Wiley, Hoboken, p 254

Bilal L, Asaf S, Hamayun M, Gul H, Iqbal A, Ullah I, Lee I-J, Hussain A (2018a) Plant growth promoting endophytic fungi Aspergillus fumigatus TS1 and Fusarium proliferatum BRL1 produce gibberellins and regulates plant endogenous hormones. Symbiosis 76:117–127

Bilal S, Shahzad R, Khan AL, Kang S-M, Imran QM, Al-Harrasi A, Yun B-W, Lee I-J (2018b) Endophytic microbial consortia of phytohormones-producing fungus Paecilomyces formosus LHL10 and bacteria Sphingomonas sp. LK11 to Glycine max L. regulates physio-hormonal changes to attenuate aluminum and zinc stresses. Front Plant Sci 9:1273

Boddey RM, De Oliveira OC, Urquiaga S, Reis VM, De Olivares FL, Baldani VLD, Döbereiner J (1995) Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. In: Management of biological nitrogen fixation for the development of more productive and sustainable agricultural systems. Springer, Berlin, pp 195–209

Bollag JM (2017) Soil biochemistry, vol 6. CRC Press, Boca Raton

Bottini R, Cassán F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503. https://doi.org/10.1007/s00253-004-1696-1

Camara MC, Vandenberghe LPS, Rodrigues C, de Oliveira J, Faulds C, Bertrand E, Soccol CR (2018) Current advances in gibberellic acid (GA 3) production, patented technologies and potential applications. Planta 248:1049–1062

Cappellari LDR, Banchio E (2020) Microbial volatile organic compounds produced by Bacillus amyloliquefaciens GB03 ameliorate the effects of salt stress in Mentha piperita principally through acetoin emission. J Plant Growth Regul 39:764–775. https://doi.org/10.1007/s00344-019-10020-3

Challis GL (2005) A widely distributed bacterial pathway for siderophore biosynthesis independent of nonribosomal peptide synthetases. Chembiochem 6:601–611

Chandler D, Davidson G, Grant WP, Greaves J, Tatchell GM (2008) Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends Food Sci Technol 19:275–283

Chauhan H, Bagyaraj DJ, Selvakumar G, Sundaram SP (2015) Novel plant growth promoting rhizobacteria—prospects and potential. Appl Soil Ecol 95:38–53

Chowdhury SP, Hartmann A, Gao X, Borriss R (2015) Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42 – a review. Front Microbiol 6

Church MJ, Björkman KM, Karl DM, Saito MA, Zehr JP (2008) Regional distributions of nitrogen-fixing bacteria in the Pacific Ocean. Limnol Oceanogr 53:63–77

Crews TE (1999) The presence of nitrogen fixing legumes in terrestrial communities: evolutionary vs ecological considerations. In: New perspectives on nitrogen cycling in the temperate and tropical Americas. Springer, Berlin, pp 233–246

Curá JA, Franz DR, Filosofía JE, Balestrasse KB, Burgueño LE (2017) Inoculation with Azospirillum sp. and Herbaspirillum sp. bacteria increases the tolerance of maize to drought stress. Microorganisms 5:41

da Silveira APD, Sala VMR, Cardoso EJBN, Labanca EG, Cipriano MAP (2016) Nitrogen metabolism and growth of wheat plant under diazotrophic endophytic bacteria inoculation. Appl Soil Ecol 107:313–319

Desai SA (2017) Isolation and characterization of gibberellic acid (GA3) producing rhizobacteria from sugarcane roots. Biosci Discov 8:488–494

Díaz S, Cabido M (2001) Vive la différence: plant functional diversity matters to ecosystem processes. Trends Ecol Evol 16:646–655

Disi JO, Mohammad HK, Lawrence K, Kloepper J, Fadamiro H (2019) A soil bacterium can shape belowground interactions between maize, herbivores and entomopathogenic nematodes. Plant Soil 437:83–92

Doroshenko EV, Boulygina ES, Spiridonova EM, Tourova TP, Kravchenko IK (2007) Isolation and characterization of nitrogen-fixing bacteria of the genus Azospirillum from the soil of a Sphagnum peat bog. Microbiology 76:93–101

Dukare A, Paul S, Arambam A (2020) Isolation and efficacy of native chitinolytic rhizobacteria for biocontrol activities against Fusarium wilt and plant growth promotion in pigeon pea (Cajanus cajan L.). Egypt. J. Biol. Pest Control 30:1–12

Dumas Z, Ross-Gillespie A, Kümmerli R (2013) Switching between apparently redundant iron-uptake mechanisms benefits bacteria in changeable environments. Proc R Soc B Biol Sci 280:20131055

Duy MV, Hoi NT, Ve NB, Thuc LV, Trang NQ (2016) Influence of Cellulomonas flavigena, Azospirillum sp. and Pseudomonas sp. on rice growth and yield grown in submerged soil amended with rice straw. Recent Trends PGPR Res Sustain Crop Prod 238

Egamberdieva D, Kucharova Z (2008) Cropping effects on microbial population and nitrogenase activity in saline arid soil. Turkish J Biol 32:85–90

El-Hadad ME, Mustafa MI, Selim SM, Mahgoob AEA, El-Tayeb TS, Aziz NHA (2010) In vitro evaluation of some bacterial isolates as biofertilizers and biocontrol agents against the second stage juveniles of Meloidogyne incognita. World J Microbiol Biotechnol 26:2249–2256

Elo S, Suominen I, Kämpfer P, Juhanoja J, Salkinoja-Salonen M, Haahtela K (2001) Paenibacillus borealis sp. nov., a nitrogen-fixing species isolated from spruce forest humus in Finland. Int J Syst Evol Microbiol 51:535–545

Elshafie HS, Camele I, Racioppi R, Scrano L, Iacobellis NS, Bufo SA (2012) In vitro antifungal activity of Burkholderia gladioli pv. Agaricicola against some phytopathogenic fungi. Int J Mol Sci 13:16291–16302

Federspiel A, Schuler R, Haselwandter K (1991) Effect of pH, L-ornithine and L-proline on the hydroxamate siderophore production by Hymenoscyphus ericae, a typical ericoid mycorrhizal fungus. Plant Soil 130:259–261

Fhoula I, Najjari A, Turki Y, Jaballah S, Boudabous A, Ouzari H (2013) Diversity and antimicrobial properties of lactic acid bacteria isolated from rhizosphere of olive trees and desert truffles of Tunisia. Biomed Res Int 2013:405708. https://doi.org/10.1155/2013/405708

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G (2007) Endophytic bacteria in sunflower (Helianthus annuus L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 76:1145–1152. https://doi.org/10.1007/s00253-007-1077-7

Franco-Correa M, Quintana A, Duque C, Suarez C, Rodríguez MX, Barea J-M (2010) Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Appl Soil Ecol 45:209–217

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol Biochem 37:395–412

Grobkinsky DK, Tafner R, Moreno MV, Stenglein SA, García de Salamone IE, Nelson LM, Novák O, Strnad M, van der Graaff E, Roitsch T (2016) Cytokinin production by Pseudomonas fluorescens G20-18 determines biocontrol activity against Pseudomonas syringae in Arabidopsis. Sci Rep 6:23310. https://doi.org/10.1038/srep23310

Gu Q, Yang Y, Yuan Q, Shi G, Wu L, Lou Z, Huo R, Wu H, Borriss R, Gao X (2017) Bacillomycin D produced by Bacillus amyloliquefaciens is involved in the antagonistic interaction with the plant-pathogenic fungus Fusarium graminearum. Appl Environ Microbiol 83:e01075–e01017. https://doi.org/10.1128/AEM.01075-17

Guevara-Avendaño E, Bravo-Castillo KR, Monribot-Villanueva JL, Kiel-Martínez AL, Ramírez-Vázquez M, Guerrero-Analco JA, Reverchon F (2020) Diffusible and volatile organic compounds produced by avocado rhizobacteria exhibit antifungal effects against Fusarium kuroshium. Braz J Microbiol 51:861. https://doi.org/10.1007/s42770-020-00249-6

Gupta V, Kumar GN, Buch A (2020) Colonization by multi-potential Pseudomonas aeruginosa P4 stimulates peanut (Arachis hypogaea L.) growth, defence physiology and root system functioning to benefit the root-rhizobacterial interface. J Plant Physiol 248:153144. https://doi.org/10.1016/j.jplph.2020.153144

Gyaneshwar P, Parekh LJ, Archana G, Poole PS, Collins MD, Hutson RA, Kumar GN (1999) Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphate solubilization by Enterobacter asburiae. FEMS Microbiol Lett 171:223–229

Halder AK, Chakrabartty PK (1993) Solubilization of inorganic phosphate byRhizobium. Folia Microbiol (Praha) 38:325–330

Han J, Sun L, Dong X, Cai Z, Sun X, Yang H, Wang Y, Song W (2005) Characterization of a novel plant growth-promoting bacteria strain Delftia tsuruhatensis HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Syst Appl Microbiol 28:66–76

Harman GE, Uphoff N (2019) Symbiotic root-endophytic soil microbes improve crop productivity and provide environmental benefits. Scientifica (Cairo) 2019

Hol WH, Bezemer TM, Biere A (2013) Getting the ecology into interactions between plants and the plant growth-promoting bacterium Pseudomonas fluorescens. Front Plant Sci 4:81

Hossain MJ, Ran C, Liu K, Ryu C-M, Rasmussen-Ivey CR, Williams MA, Hassan MK, Choi S-K, Jeong H, Newman M (2015) Deciphering the conserved genetic loci implicated in plant disease control through comparative genomics of Bacillus amyloliquefaciens subsp. plantarum. Front Plant Sci 6:631

Hou Q, Kolodkin-Gal I (2020) Harvesting the complex pathways of antibiotic production and resistance of soil bacilli for optimizing plant microbiome. FEMS Microbiol Ecol 96. https://doi.org/10.1093/femsec/fiaa142

Illakkiam D, Anuj NL, Ponraj P, Shankar M, Rajendhran J, Gunasekaran P (2013) Proteolytic enzyme mediated antagonistic potential of Pseudomonas aeruginosa against Macrophomina phaseolina. Indian J Exp Biol 51

Jeffery S, Gardi C, Jones A, Montanarella L, Marmo L, Miko L, Ritz K, Peres G, Roombke J, van der Putten W (2010) The soil environment. In: European atlas of soil biodiversity. European Commission, Publications Office of the European Union, pp 17–48

Kai M, Effmert U, Berg G, Piechulla B (2007) Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen Rhizoctonia solani. Arch Microbiol 187:351–360. https://doi.org/10.1007/s00203-006-0199-0

Kalbe C, Marten P, Berg G (1996) Strains of the genus Serratia as beneficial rhizobacteria of oilseed rape with antifungal properties. Microbiol Res 151:433–439. https://doi.org/10.1016/S0944-5013(96)80014-0

Kang S-M, Waqas M, Hamayun M, Asaf S, Khan AL, Kim A-Y, Park Y-G, Lee I-J (2017) Gibberellins and indole-3-acetic acid producing rhizospheric bacterium Leifsonia xyli SE134 mitigates the adverse effects of copper-mediated stress on tomato. J Plant Interact 12:373–380. https://doi.org/10.1080/17429145.2017.1370142

Kannaiyan S, Kumar K, Govindarajan K (2004) Biofertilizers technology for rice based cropping system. Biofertilizers technology for rice based cropping system

Kapoor R, Kaur M (2016) Cytokinins production by fluorescent Pseudomonas isolated from rhizospheric soils of Malus and Pyrus. Afr J Microbiol Res 10:1274–1279

Khalid A, Tahir S, Arshad M, Zahir ZA (2005) Relative efficiency of rhizobacteria for auxin biosynthesis in rhizosphere and non-rhizosphere soils. Soil Res 42:921–926

Kim KY, Jordan D, McDonald GA (1997) Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol Fertil Soils 26:79–87

Kochar M, Vaishnavi A, Upadhyay A, Srivastava S (2013) Bacterial biosynthesis of indole-3-acetic acid: signal messenger service. Mol Microb Ecol Rhizosph 1:309–326

Korpi A, Järnberg J, Pasanen A-L (2009) Microbial volatile organic compounds. Crit Rev Toxicol 39:139–193

Kraemer SA, Soucy J-PR, Kassen R (2017) Antagonistic interactions of soil pseudomonads are structured in time. FEMS Microbiol Ecol 93:93. https://doi.org/10.1093/femsec/fix046

Kramer J, Özkaya Ö, Kümmerli R (2020) Bacterial siderophores in community and host interactions. Nat Rev Microbiol 18:152–163. https://doi.org/10.1038/s41579-019-0284-4

Krishnaraj PU, Goldstein AH (2001) Cloning of a Serratia marcescens DNA fragment that induces quinoprotein glucose dehydrogenase-mediated gluconic acid production in Escherichia coli in the presence of stationary phase Serratia marcescens. FEMS Microbiol Lett 205:215–220

Kruasuwan W, Thamchaipenet A (2016) Diversity of culturable plant growth-promoting bacterial endophytes associated with sugarcane roots and their effect of growth by co-inoculation of diazotrophs and actinomycetes. J Plant Growth Regul 35:1074–1087

Kucey RMN (1988) Effect of Penicillium bilaji on the solubility and uptake of P and micronutrients from soil by wheat. Can J Soil Sci 68:261–270

Kumawat KC, Sharma P, Sirari A, Singh I, Gill BS, Singh U, Saharan K (2019) Synergism of Pseudomonas aeruginosa (LSE-2) nodule endophyte with Bradyrhizobium sp. (LSBR-3) for improving plant growth, nutrient acquisition and soil health in soybean. World J Microbiol Biotechnol 35:47

Lee JY, Hwang BK (2002) Diversity of antifungal actinomycetes in various vegetative soils of Korea. Can J Microbiol 48:407–417. https://doi.org/10.1139/w02-025

Lee SO, Kim HY, Choi GJ, Lee HB, Jang KS, Choi YH, Kim J (2009) Mycofumigation with Oxyporus latemarginatus EF069 for control of postharvest apple decay and Rhizoctonia root rot on moth orchid. J Appl Microbiol 106:1213–1219

Lee YS, Anees M, Park YS, Kim SB, Jung WJ, Kim KY (2014) Purification and properties of a Meloidogyne-antagonistic chitinase from Lysobacter capsici YS1215. Nematology 16:63–72

Lemos ML, Balado M, Osorio CR (2010) Anguibactin-versus vanchrobactin-mediated iron uptake in Vibrio anguillarum: evolution and ecology of a fish pathogen. Environ Microbiol Rep 2:19–26

Li H-B, Singh RK, Singh P, Song Q-Q, Xing Y-X, Yang L-T, Li Y-R (2017) Genetic diversity of nitrogen-fixing and plant growth promoting Pseudomonas species isolated from sugarcane rhizosphere. Front Microbiol 8:1268

Liu X, Zhao H, Chen S (2006) Colonization of maize and rice plants by strain Bacillus megaterium C4. Curr Microbiol 52:186–190

Liu W-H, Chen F-F, Wang C-E, Fu H-H, Fang X-Q, Ye J-R, Shi J-Y (2019) Indole-3-acetic acid in Burkholderia pyrrocinia JK-SH007: enzymatic identification of the Indole-3-Acetamide synthesis pathway. Front Microbiol

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10
Madhaiyan M, Poonguzhali S, Lee J-S, Lee K-C, Hari K (2011) Bacillus rhizosphaerae sp. nov., an novel diazotrophic bacterium isolated from sugarcane rhizosphere soil. Antonie Van Leeuwenhoek 100:437–444
Marin M, Mena J, Franco R, Pimentel E, Sánchez I (2010) Effects of the bacterial-fungal interaction between Tsukamurella paurometabola C 924 and Glomus fasciculatum and Glomus clarum fungi on lettuce microrrizal colonization and foliar weight. Biotecnol Apl 27:48–51
Martínez-Aguilar L, Salazar-Salazar C, Méndez RD, Caballero-Mellado J, Hirsch AM, Vásquez-Murrieta MS, Estrada-De Los Santos P (2013) Burkholderia caballeronis sp. nov., a nitrogen fixing species isolated from tomato (Lycopersicon esculentum) with the ability to effectively nodulate Phaseolus vulgaris. Antonie Van Leeuwenhoek 104:1063–1071
Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10:293–319
Mishra J, Arora NK (2018) Secondary metabolites of fluorescent pseudomonads in biocontrol of phytopathogens for sustainable agriculture. Appl Soil Ecol 125:35–45. https://doi.org/10.1016/j.apsoil.2017.12.004
Mohammadi K, Sohrabi Y (2012) Bacterial biofertilizers for sustainable crop production: a review. ARPN J Agric Biol Sci 7:307–316
Moisan K, Cordovez V, van de Zande EM, Raaijmakers JM, Dicke M, Lucas-Barbosa D (2019) Volatiles of pathogenic and non-pathogenic soil-borne fungi affect plant development and resistance to insects. Oecologia 190:589–604. https://doi.org/10.1007/s00442-019-04433-w
Nahas E (1996) Factors determining rock phosphate solubilization by microorganisms isolated from soil. World J Microbiol Biotechnol 12:567–572
Nandi M, Selin C, Brassinga AKC, Belmonte MF, Fernando WGD, Loewen PC, de Kievit TR (2015) Pyrrolnitrin and hydrogen cyanide production by Pseudomonas chlororaphis strain PA23 exhibits nematicidal and repellent activity against Caenorhabditis elegans. PLoS One 10: e0123184
Nett RS, Montanares M, Marcassa A, Lu X, Nagel R, Charles TC, Hedden P, Rojas MC, Peters RJ (2017) Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution. Nat Chem Biol 13:69–74. https://doi.org/10.1038/nchembio.2232
Nghia NH, Gyurjan I (1987) Problems and perspectives in establishment of nitrogen-fixing symbioses and endosymbioses. Endocytobio Cell Res 4:131–141
Nihorimbere V, Fickers P, Thonart P, Ongena M (2009) Ecological fitness of Bacillus subtilis BGS3 regarding production of the surfactin lipopeptide in the rhizosphere. Environ Microbiol Rep 1:124–130. https://doi.org/10.1111/j.1758-2229.2009.00017.x
Oberhänsli T, Défago G, Haas D (1991) Indole-3-acetic acid (IAA) synthesis in the biocontrol strain CHA0 of Pseudomonas fluorescens: role of tryptophan side chain oxidase. Microbiology 137:2273–2279
Palumbo JD, Yuen GY, Jochum CC, Tatum K, Kobayashi DY (2005) Mutagenesis of β-1, 3-glucanase genes in Lysobacter enzymogenes strain C3 results in reduced biological control activity toward Bipolaris leaf spot of tall fescue and Pythium damping-off of sugar beet. Phytopathology 95:701–707
Pandya ND, Butani NV, Desai PV, Sayyed RZ (2011) Optimization of GA3 biosynthesis by bacteria associated with the rhizosphere of sugarcane. In: Plant growth-promoting rhizobacteria (PGPR) for sustainable agriculture, p 447
Park Y-S, Dutta S, Ann M, Raaijmakers JM, Park K (2015) Promotion of plant growth by Pseudomonas fluorescens strain SS101 via novel volatile organic compounds. Biochem Biophys Res Commun 461:361–365. https://doi.org/10.1016/j.bbrc.2015.04.039
Park Y-G, Mun B-G, Kang S-M, Hussain A, Shahzad R, Seo C-W, Kim A-Y, Lee S-U, Oh KY, Lee DY (2017) Bacillus aryabhattai SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. PLoS One 12: e0173203

Paul D, Park KS (2013) Identification of volatiles produced by Cladosporium cladosporioides CL-1, a fungal biocontrol agent that promotes plant growth. Sensors 13:13969–13977

Perin L, Martínez-Aguilar L, Paredes-Valdez G, Baldani JI, Estrada-De Los Santos P, Reis VM, Caballero-Mellado J (2006) Burkholderia silvatlantica sp. nov., a diazotrophic bacterium associated with sugar cane and maize. Int J Syst Evol Microbiol 56:1931–1937

Pradhan N, Sukla LB (2006) Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr J Biotechnol 5

Prasanna R, Joshi M, Rana A, Nain L (2010) Modulation of IAA production in cyanobacteria by tryptophan and light. Pol J Microbiol 59:99–105

Prinsen E, Costacurta A, Michiels K, Vanderleyden J, Van Onckelen H (1993) Azospirillum brasilense indole-3-acetic acid biosynthesis: evidence for a non-tryptophan dependent pathway. Mol Plant-Microbe Interact 6:609

Priyanka TA, Kotasthane AS, Kosharia A, Kushwah R, Zaidi NW, Singh US (2017) Crop specific plant growth promoting effects of ACCd enzyme and siderophore producing and cynogenic fluorescent Pseudomonas. 3 biotech 7

Reddy BN, Hindumathi A (2017) Potential of microbial volatile organic compounds for crop protection against Phytopathogenic Fungi BT - volatiles and food security: role of volatiles in agro-ecosystems. In: Choudhary DK, Sharma AK, Agarwal P, Varma A, Tuteja N (eds) Volatiles and food security. Springer, Singapore, pp 271–284. https://doi.org/10.1007/978-981-10-5553-9_14

Reetha AK, Pavani SL, Mohan S (2014) Hydrogen cyanide production ability by bacterial antagonist and their antibiotics inhibition potential on Macrophomina phaseolina (Tassi.) Goid. Int J Curr Microbiol Appl Sci 3:172–178

Reis VM, Estrada-De Los Santos P, Tenorio-Salgado S, Vogel J, Stoffels M, Guyon S, Mavingui P, Baldani VLD, Schmid M, Baldani JI (2004) Burkholderia tropica sp. nov., a novel nitrogen-fixing, plant-associated bacterium. Int J Syst Evol Microbiol 54:2155–2162

Rodrigo V, Eberto N (2007) Seasonal changes in periphyton nitrogen fixation in a protected tropical wetland. Biol Fertil Soils 43:367–372

Rodrigues C, de Vandenberghe LPS, de Oliveira J, Soccol CR (2012) New perspectives of gibberellic acid production: a review. Crit Rev Biotechnol 32:263–273

Ryu C-M, Farag MA, Hu C-H, Reddy MS, Wei H-X, Paré PW, Kloepper JW (2003) Bacterial volatiles promote growth in Arabidopsis. Proc Natl Acad Sci 100:4927–4932

Sagervanshi A, Kumari P, Negi A, Kumar A (2012) Isolation and characterization of phosphate solubilizing bacteria from Anand agriculture soil. Adv Res Pharm Biol 2

Sahoo RK, Ansari MW, Dangar TK, Mohanty S, Tuteja N (2014) Phenotypic and molecular characterisation of efficient nitrogen-fixing Azotobacter strains from rice fields for crop improvement. Protoplasma 251:511–523

Saravanan VS, Madhaiyan M, Osborne J, Thangaraju M, Sa TM (2008) Ecological occurrence of Gluconacetobacter diazotrophicus and nitrogen-fixing Acetobacteraceae members: their possible role in plant growth promotion. Microb Ecol 55:130–140

Sathe S, Mathew A, Agnoli K, Eberl L, Kümmerli R (2019) Genetic architecture constrains exploitation of siderophore cooperation in the bacterium Burkholderia cenocepacia. Evol Lett 3:610–622

Selvakumar G, Bindu GH, Bhatt RM, Upreti KK, Paul AM, Asha A, Shweta K, Sharma M (2018) Osmotolerant Cytokinin producing microbes enhance tomato growth in deficit irrigation conditions. Proc Natl Acad Sci India Sect B Biol Sci 88:459–465. https://doi.org/10.1007/s40011-016-0766-3

Sgroy V, Cassán F, Masciarelli O, del Papa MF, Lagares A, Luna V (2009) Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera. Appl Microbiol Biotechnol 85:371–381

Shahzad R, Khan AL, Bilal S, Waqas M, Kang S-M, Lee I-J (2017) Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in Oryza sativa. Environ Exp Bot 136:68–77. https://doi.org/10.1016/j.envexpbot.2017.01.010

Shi T-Q, Gao J, Wang W-J, Wang K-F, Xu G-Q, Huang H, Ji X-J (2019) CRISPR/Cas9-based genome editing in the filamentous fungus Fusarium fujikuroi and its application in strain engineering for gibberellic acid production. ACS Synth Biol 8:445–454. https://doi.org/10.1021/acssynbio.8b00478

Silva CCG, Silva SPM, Ribeiro SC (2018) Application of Bacteriocins and protective cultures in dairy food preservation. Front Microbiol 9:594. https://doi.org/10.3389/fmicb.2018.00594

Singh PP, Shin YC, Park CS, Chung YR (1999) Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. Phytopathology 89:92–99

Singh RK, Kumar DP, Solanki MK, Singh P, Srivastva AK, Kumar S, Kashyap PL, Saxena AK, Singhal PK, Arora DK (2013) Optimization of media components for chitinase production by chickpea rhizosphere associated Lysinibacillus fusiformis B-CM18. J Basic Microbiol 53:451–460

Spaepen S (2015) Plant hormones produced by microbes. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer, Cham, pp 247–256. https://doi.org/10.1007/978-3-319-08575-3_26

Stein T (2005) Bacillus subtilis antibiotics: structures, syntheses and specific functions. Mol Microbiol 56:845–857. https://doi.org/10.1111/j.1365-2958.2005.04587.x

Strano CP, Bella P, Licciardello G, Caruso A, Catara V (2017) Role of secondary metabolites in the biocontrol activity of Pseudomonas corrugata and Pseudomonas mediterranea. Eur J Plant Pathol 149:103–115

Sturtevant DB, Taller BJ (1989) Cytokinin production by Bradyrhizobium japonicum. Plant Physiol 89:1247–1252. https://doi.org/10.1104/pp.89.4.1247

Subramanian S, Smith DL (2015) Bacteriocins from the rhizosphere microbiome – from an agriculture perspective. Front Plant Sci 6

Sun S-L, Yang W-L, Fang W-W, Zhao Y-X, Guo L, Dai Y-J (2018) The plant growth-promoting Rhizobacterium Variovorax boronicumulans CGMCC 4969 regulates the level of Indole-3-acetic acid synthesized from Indole-3-acetonitrile. Appl Environ Microbiol 84. https://doi.org/10.1128/AEM.00298-18

Theodorou ME, Plaxton WC (1993) Metabolic adaptations of plant respiration to nutritional phosphate deprivation. Plant Physiol 101:339–344

Tilak K, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK, Johri BN (2005) Diversity of plant growth and soil health supporting bacteria. Curr Sci:136–150

Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by Paenibacillus polymyxa. Soil Biol Biochem 31:1847–1852. https://doi.org/10.1016/S0038-0717(99)00113-3

Trivedi P, He Z, van Nostrand JD, Albrigo G, Zhou J, Wang N (2012) Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere. ISME J 6:363–383

Tyrrell J, Whelan N, Wright C, Sá-Correia I, McClean S, Thomas M, Callaghan M (2015) Investigation of the multifaceted iron acquisition strategies of Burkholderia cenocepacia. Biometals 28:367–380

Valdebenito M, Bister B, Reissbrodt R, Hantke K, Winkelmann G (2005) The detection of salmochelin and yersiniabactin in uropathogenic Escherichia coli strains by a novel hydrolysis-fluorescence-detection (HFD) method. Int J Med Microbiol 295:99–107

Vargas L, Santa Brigida AB, Mota Filho JP, De Carvalho TG, Rojas CA, Vaneechoutte D, Van Bel M, Farrinelli L, Ferreira PCG, Vandepoele K (2014) Drought tolerance conferred to sugarcane by association with Gluconacetobacter diazotrophicus: a transcriptomic view of hormone pathways. PLoS One 9:e114744

Vazquez P, Holguin G, Puente ME, Lopez-Cortes A, Bashan Y (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol Fertil Soils 30:460–468

Venieraki A, Dimou M, Pergalis P, Kefalogianni I, Chatzipavlidis I, Katinakis P (2011) The genetic diversity of culturable nitrogen-fixing bacteria in the rhizosphere of wheat. Microb Ecol 61:277–285

Venkateswarlu B, Rao AV, Raina P (1984) Evaluation of phosphorus solubilisation by microorganisms isolated from Aridisols. J Indian Soc Soil Sci 32:273–277

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, Woo SL, Lorito M (2008) A novel role for Trichoderma secondary metabolites in the interactions with plants. Physiol Mol Plant Pathol 72:80–86. https://doi.org/10.1016/j.pmpp.2008.05.005

Visca P, Imperi F, Lamont IL (2007) Pyoverdine siderophores: from biogenesis to biosignificance. Trends Microbiol 15:22–30

Vitousek PM, Cassman KEN, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB (2002) Towards an ecological understanding of biological nitrogen fixation. In: The nitrogen cycle at regional to global scales. Springer, Berlin, pp 1–45

von der Weid I, Duarte GF, van Elsas JD, Seldin L (2002) Paenibacillus brasilensis sp. nov., a novel nitrogen-fixing species isolated from the maize rhizosphere in Brazil. Int J Syst Evol Microbiol 52:2147–2153

Wang ET, Martinez-Romero E (2000) Sesbania herbacea–rhizobium huautlense nodulation in flooded soils and comparative characterization of S. herbacea-nodulating rhizobia in different environments. Microb Ecol 40:25–32

Whitelaw MA (1999) Growth promotion of plants inoculated with phosphate-solubilizing fungi. In: Advances in agronomy. Elsevier, Amsterdam, pp 99–151

Xiao C, Chi R, Li X, Xia M, Xia Z (2011) Biosolubilization of rock phosphate by three stress-tolerant fungal strains. Appl Biochem Biotechnol 165:719–727

Xie G, Su B, Cui Z (1998) Isolation and identification of N2-fixing strains of Bacillus in rice rhizosphere of the Yangtze River valley. Wei sheng wu xue bao= Acta Microbiol Sin 38:480–483

Xing Y-X, Wei C-Y, Mo Y, Yang L-T, Huang S-L, Li Y-R (2016) Nitrogen-fixing and plant growth-promoting ability of two endophytic bacterial strains isolated from sugarcane stalks. Sugar Tech 18:373–379

Xu Q, Pan W, Zhang R, Lu Q, Xue W, Wu C, Song B, Du S (2018) Inoculation with Bacillus subtilis and Azospirillum brasilense produces abscisic acid that reduces Irt1-mediated cadmium uptake of roots. J Agric Food Chem 66:5229–5236. https://doi.org/10.1021/acs.jafc.8b00598

Yamagiwa Y, Inagaki Y, Ichinose Y, Toyoda K, Hyakumachi M, Shiraishi T (2011) Talaromyces wortmannii FS2 emits β-caryphyllene, which promotes plant growth and induces resistance. J Gen Plant Pathol 77:336–341

Yasser MM, Mousa ASM, Massoud ON, Nasr SH (2014) Solubilization of inorganic phosphate by phosphate solubilizing fungi isolated from Egyptian soils. J Biol Earth Sci 4

Yoon GY, Lee YS, Lee SY, Park RD, Hyun HN, Nam Y, Kim KY (2012) Effects on Meloidogyne incognita of chitinase, glucanase and a secondary metabolite from Streptomyces cacaoi GY525. Nematology 14:175–184

Yuan J, Raza W, Shen Q, Huang Q (2012) Antifungal activity of Bacillus amyloliquefaciens NJN-6 volatile compounds against Fusarium oxysporum f. sp. cubense. Appl Environ Microbiol 78:5942–5944

Zhang P, Jin T, Kumar Sahu S, Xu J, Shi Q, Liu H, Wang Y (2019) The distribution of tryptophan-dependent indole-3-acetic acid synthesis pathways in bacteria unraveled by large-scale genomic analysis. Molecules 24:1411

Zhu B, Zhou Q, Lin L, Hu C, Shen P, Yang L, An Q, Xie G, Li Y (2013) Enterobacter sacchari sp. nov., a nitrogen-fixing bacterium associated with sugar cane (Saccharum officinarum L.). Int J Syst Evol Microbiol 63:2577–2582

Zia MA, Yasmin H, Shair F, Jabeen Z, Mumtaz S, Hayat Z, Shah SZH, Afghan S, Hafeez FY, Hassan MN (2019) Glucanolytic Rhizobacteria produce antifungal metabolites and elicit ROS scavenging system in sugarcane. Sugar Tech 21:244–255. https://doi.org/10.1007/s12355-018-0654-7

Epiphytic Microbes of Roots: Diversity and Significance

17

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Abstract

Microbes residing on the surface of roots are termed as root epiphytic microbes. They contribute majorly in biogeochemical mechanisms of soil. There are various approaches for deciphering root epiphytes which are either culture dependent or culture independent methods. Plants play a decisive role in selecting root epiphytic community which is driven by factors such as nature of soil, host plant, and root exudates. Root surface harbors both prokaryotic and eukaryotic microbes. *Proteobacteria* and *Actinobacteria* are major phyla present as root epiphytes. *Alphaproteobacteria* and *Gammaproteobacteria* predominate among bacteria. *Pseudomonas*, *Burkholderia*, *Erwinia*, *Acinetobacter*, and *Sphingomonas* are the major genera isolated from different plant root surfaces. Few other genera such as *Bacillus*, *Agrobacterium*, *Streptomyces*, *Klebsiella*, *Nocardia*, etc. are also found on some of the root surfaces. Fungi of class *Ascomycota* represents eukaryotic root epiphytic community. Epiphytes play a significant role in plant metabolism. Many epiphytic microbes produce phytohormones such as auxin, gibberellins as well as other growth promoting substances that contribute in plant growth. Root epiphytes involve in root defense via mechanisms such as biofilm formation, releasing antimicrobial substances, or competing for space and nutrients with pathogens. Epiphytic microbes play a role in framing root morphology and root structure thereby molding shape of roots.

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Rhizosphere · Root epiphytes · Diversity · Plant defense · Phytohormones

17.1 Introduction

Soil popularly known as BLACK BOX among the science fraternity is known to be the most significantly diverse uppermost layer of the earth's crust in the lithosphere ecosystem. Because of its composition consisting of organic matter, minerals, water, and gases, this layer is the home for vast diversity of microorganisms. As a matter of fact, soil acts as the greatest reservoir of biodiversity of microorganisms in the world. Soil is also a layer that has roots of vegetation embedded in it. Rhizoplane is a part of the plant that is shielded from sunlight and open air by the bulky layer of soil; however, it shows lesser variations in other conditions, namely humidity and temperature. It is a home to the diverse form of microorganisms. Studies suggest that about 10^{11} microbial cells involving more than 30,000 species of prokaryotes resides per gram of roots and rhizosphere soil (Berendsen et al. 2012). To be more precise, below ground ecological niches can be categorized as the bulky layer of soil, rhizosphere, rhizoplane, epiphytes, and finally endophytes (Rout 2014). However, the microflora of epiphytes and endophytes differs greatly from that of the bulk soil suggesting that plants have evolved in selecting the microbial community in their environment (Zamioudis et al. 2013). Among this vast world of microbes, a small but significant portion involves members of rhizoplane of the plants better known as **Root epiphytes**. They are the organisms that adhere to and reside on the surface of roots of the plant. However, as there is a significant amount of soil particles which adheres to the root surfaces, the confusion and dispute regarding the precise definition largely prevail among the researchers. Furthermore, root adhering microbes are those genera that are preferentially stimulated from the members present in the surrounding rhizosphere. Because of this, most of the studies related with root associated microbes extends its lay out to rhizosphere. Reinhold-Hurek et al. (2015) have given a more confined term of the original rhizosphere. They classified the rhizosphere into ectorhizosphere and endorhizosphere. Ectorhizosphere comprises of the soil attached to and on the root surface where the bacteria reside, while endorhizosphere covers the free spaces present in inner root tissues such as cortex and endodermis in which microbes can reside. Because of their existence at the interface of vegetation and external environment, the members of ectorhizosphere have a very little but significant share in the ecological niche. These minute residents of roots are teaming with the plants causing significant impact on the plant growth and metabolism. However, the architecture of the diversity of microbes is highly sensitive towards the continuously altering biogeochemical conditions on and around the root surface. As a result, they may vary in numbers and types based on the availability of nutrients as well as other environmental conditions. Microbes of these microecological niches show varying degree of beneficial, neutral, or damaging interactions with their host plants. As a result, they

have a role to play in the dynamics of root ecosystem and plant trait expression (Rout 2014). Even though some of the plant–microbiome interactions are studied largely, mostly they are focused on rhizobium species or mycorrhizal interactions. There is not much information available regarding their diversity and significance of root epiphytic members in the literature in spite of their contributions in biogeochemical mechanisms of rhizoplane. Henceforth, we have made an attempt to provide an insight into the diverse and compact world of root surface associated microbes. However, due to dispute and confusion regarding the precise boundary of the rhizoplane, looking to the broader outline, we have integrated reports of rhizosphere in some of the discussion in this chapter.

17.2 Diversity of Root Epiphytes

17.2.1 Approaches for Studying Root Epiphytic Microbiome

Complexity of the environment has a deep correlation with the biodiversity of that particular habitat. As a storehouse of minerals and organic materials, plant rhizosphere is likely to provide accommodation and nutrition to the members of various domains of life. There are number of methods available to study the microbial members present in rhizoplane (Mendes et al. 2013; Turner et al. 2013). Most of the methods with simpler approach involve culturing and studying the microorganisms in the laboratory (Tsavkelova et al. 2007; Castillo et al. 2015). However, it fails to provide the total picture of members of those genera which are unculturable but still the residents of the rhizoplane. Recently, techniques involving genome-based analysis such as PCR amplification, rRNA genome sequencing, next generation sequencing, metagenomics, metabolomics, metatranscriptomics, metaproteomics, etc. have been largely employed in biodiversity studies (Turner et al. 2013). Culture independent techniques have uncovered the fact that the microorganisms detected via culturing constitute merely 5% of the total microbial community and a significant proportion of the phyla detected using high end culture independent technologies does not have a single culturable member (Mendes et al. 2013). These methods have unearthed the secrets of underground microbial world along with root harboring microbes, not obtained through culture techniques. **Metagenomics** is the study of metagenome, referred as a complete set of total genomic DNA in any particular sample. The samples used for such studies mostly comprise the environmental samples. Metagenomics involves the isolation of total DNA of the samples, its amplification followed by its sequencing. The sequence is shot against the pool of enormous database and the members of various genera are designated. Similarly, **metatranscriptomics** study is performed by isolation of total RNA and its sequencing. Successively, data analysis via comparison of DNA/RNA sequences, with the vast array of databases is performed as per the requirement and taxonomic ranks of the isolates are designated. Recently, science of **metabolomics** has been employed for diversity studies of plants. Metabolomics involves overall study of small molecules of either substrates or products of metabolism, better

known as metabolites. This approach can provide a deep insight towards the microbial diversity as a function of biochemical changes related with various developmental alterations during plant growth. The area of **metaproteomics** deals with the protein content present in the environmental samples. As proteins are the direct products of gene expression, in addition to the diversity studies, they can also reflect the genetic regulation of the total microbiome communities of rhizoplane under specific environmental conditions. These methodologies have been frequently employed in the diversity studies of root epiphytic microflora. War Nongkhlaw and Joshi (2014) have isolated biofilm forming epiphytic microbes from various ethnomedicinal plants of North East India and identified them using the concept of PCR amplification and 16 s rRNA sequencing. Flores-Núñez et al. (2020) have performed diversity analysis of epiphytic prokaryotic microbes on agave and cacti species through metagenomic studies. Turner et al. (2013) have followed the RNA based metatranscriptomics approach for determining total community structure and diversity of microorganisms residing in pea, oats, and wheat rhizosphere. They isolated total RNA from the bulk soil and rhizosphere and roots of the sample plants and performed multiplex and sequencing analysis. From 19 independent samples, 1,674,231 reads were generated and were analyzed further using UNSEARCH with SSU rRNA database. Full length rRNA transcripts were used to derive rRNA sequences to which the taxonomic ranks were assigned using the Lowest Common Ancestor Algorithm (by MEGAN package). This conservative algorithm removed the possibility of false assignment of conservative sequences to low taxonomic ranks as a result, most of the reads were assigned high taxonomic ranks mainly, prokaryotes, eukaryotes, phylum, and genus level. However, few reads were also analyzed up to species and strain level. The sample collections from rhizoplane for all the genomic studies mostly involves a common protocol of initial steps that comprised of washing the roots, separation of rhizoplane microbes using mechanical methods such as aberration with glass beads or pebbles or through ultrasonication, followed by centrifugation and collection of supernatants for further processing. However, it has been found that mechanical separation does not provide complete detachment of rhizoplane microbes from the root surface and nearly equal amount of the microbes still remains attach to the root surface after the procedure (Reinhold-Hurek et al. 2015).

Along with genome-based studies, rhizoplane microbes are also observed microscopically to get an idea of their morphological characteristics. However, this do not have direct contribution to the diversity studies.

17.2.2 Factors Affecting Diversity of Root Epiphytes

In the rhizosphere, plant roots have to compete with the nearby roots and microorganisms for space, water, and nutrients (Phillips et al. 2004). Root community structure largely depends on number of factors that directly or indirectly affects the microbial load as well as diversity. This majorly includes the nature of soil

surrounding the roots (rhizosphere), environmental factors, type of vegetation, and root exudates.

17.2.2.1 Type of Soil

Types of microbes residing in particular rhizosphere are largely dependent on the soil covering the root surfaces. Soil composition and structure take shape based on the edaphic and environmental factors as well as the history of vegetation in that soil. As root epiphytes are to be recruited from the microbes present in this rhizosphere soil, it has a significant effect on the root epiphytes. Besides, rhizosphere zones are considered as "hot spots" for the microbial diversity. The microflora of naïve soil differs from that of the processed soil. Agricultural soil communities have great impact of pesticides, fertilizers, growth enhancers, and soil sterilizing methods, while naïve soil communities do not have such impact. Roots of healthy plants are inhabited by soil-derived fungi, bacteria, oomycetes, and other microorganisms that have evolved independently in distinct kingdoms of life (Durán et al. 2018). Along with that there is also a significant difference in the community of bulk and soil and rhizosphere soil. As we travel from rhizosphere towards the inner core, i.e. rhizoplane, microbial diversity decreases while specificity, affinity, and avidity of microbes towards the plant roots increase (Sare et al. 2020). Lundberg et al. (2012) have claimed that soil type has a strong effect on bacterial community of bulk soil, rhizosphere as well as endophytic compartments of the plant. Reinhold-Hurek et al. (2015) stated the microbial diversity of the bulk soil compared with the rhizosphere soil of oats, pea, and wheat. They compared the studies performed by various researchers using two approaches that involved 16s rRNA gene amplification and deep sequencing as well as metatranscriptome studies for determining the diversity of bulk soil and rhizosphere but surprisingly deciphered difference in the results of both studies. Their analysis suggested no significant difference in diversity of bulk soil and rhizosphere soil when determined through 16 s rRNA gene amplification, on the contrary, metatranscriptome analysis revealed differences in the bulk soil and rhizosphere soil. The difference may be because the rRNA transcripts represent the active members in contrast to just the mere all the members represented by DNA sequencing.

Nowadays, as the concern for environmental sustainability has observed a sharp rise, agricultural communities have also conducted multiple attempts on the way of finding alternatives of chemical fertilizers, pesticides, and other growth promoting chemical substances. Recently, microbial amendments that contain plant growth promoting microorganisms along with the metabolically fermented products of that microorganisms are supplied to the agricultural crops. One of the types of such commercial microbial amendment is VESTA. It is a fermented liquid that consists of fermented products, organic acids, and broad range of microorganisms that are involved either directly or indirectly in plant growth promotion. This product is commercially produced and supplied by SOBEC Corp. Fowler, CA. Deng et al. (2019) have investigated the effect of soil amendment VESTA on the soil properties, bacterial communities, diversity and composition, and growth of strawberry plant. They employed 16srRNA gene amplicon-based illumina sequencing analysis for

depicting the bacterial community structure of the naïve soil (without amendment) and that supplied with amendment in strawberry rhizosphere. Their study revealed that there was not only the difference in the physicochemical properties of the soil and root growth but also substantial changes in microbial flora in the areas that were under the effect of amendment; however, it was found that microbes present in the amendment did not replace the rhizosphere community, instead they played a significant role in modulation of bacterial community of strawberry rhizosphere. Also, the investigations indicated that diversity of control soil was greater than bacterial diversity of amendment supplied soil. When the treatment was correlated with change in different taxonomic level, they found significant rise in the number of Gram negative, aerobic or facultative aerobic *Betaproteobacteria* with wide range of metabolic capabilities along with the nitrogen fixing capacity. There was a parallel decrease in *Actinobacteria* in the treated soil. When the soil undergoes such treatments, it demonstrates subsequent alterations in water stress on the plant roots, microbial exudates, and biogeochemical cycles. This has a great impact on the naïve communities which may also produce antagonistic exudates for other microbes.

Soil properties also differ with the geographical locations. Soil in higher altitudes is found to bare more harsh conditions with coarser texture, low nutrient availability, and less water holding capacity. The root microflora composition tends to be affected by such environmental stress factors (Castillo et al. 2015). Additionally, the changes in temperature and pH of the soil are also reflected on the diversity of microbial flora in the soil as well as the rhizosphere. It is evident that pH and temperature affect the enzyme activity, membrane functioning, and ionic compositions of the microbes hence their growth and metabolism. Also, at higher altitudes, temperature drop downs below sub-zero level at which water is hardly in its liquid state making its availability difficult for the microbial cells. Similarly, roots facing anoxic conditions, for e.g. lake sediments, will be colonized with facultative or obligate anaerobes. Praeg et al. (2019) have analyzed the microbial diversity in the rhizosphere of *Ranunculus gracilis* along the high alpine altitudinal gradient located on Mt. Schrankogel, Central Alps, Austria. Their study revealed the fact that 47% of prokaryotic diversity and 37.4% fungal variations depend on pH and temperature of particular soil.

17.2.2.2 Type of Host Plant and its Roots

For the terrestrial plants, root surface is the major site of interaction with soil microorganisms and even if soil type plays a key role in designing root community structure, plant genotype is the driving force of root microbiome diversity for identical soil types. Nevertheless, plant genome acts as a filter for selection of root epiphytic microbes. There are studies claiming that plants modulate their rhizosphere community by selectively benefiting the proliferation of microorganisms with positive traits for plant growth (Mendes et al. 2013). It can be assumed that different plant species release different root exudates which attract specific microbial species according to their metabolic framework. Microbes apply mechanisms such as chemotaxis, motility, adhesins, etc. and adhere to the root surface leading to biofilm

formation. The nature of root surface sets the stage for the establishment of the microbial community. The root exudates excreted by the plants act as nutrients for the microorganisms, hence there is more amount of microbial activity around the roots compared to bulk soil. Furthermore, these microbes release biomolecules in their vicinity based on which the plant immune system shows tendency to differentiate between pathogenic and non-pathogenic microbes and acts accordingly. This phenomenon affects the colonization of microbes on root surface thereby avoiding the pathogenic microorganisms. Studies indicate that when grown under the same soil types, different plant species showed difference in root community (Hashidoko 2005; Reinhold-Hurek et al. 2015). Hashidoko (2005) investigated rhizoplane microflora of a chlorogenic acid rich plant, *Aegopodium podagraria* of the family *Umbelliferae* and a hydrolysable, tannin rich plant of *Geranaceae*, *Geranium robertianum* and concluded that the bacterial communities of both the rhizoplane were totally different even after sharing the same bulk soil. Other factors such as root age, root length, and diameter, etc. are related with the microbial load present on any of the root surface. Castillo et al. (2015) suggested that there is difference in the epiphytic bacterial load in the roots of paddy with respect to the age of plant. It is obvious that aging process decreases the functional capacity of the roots (Liu et al. 2019). Younger roots are metabolically active and can produce more amount of root exudates attracting higher numbers of microbes in contrast to the aged roots which shows lesser nutrient uptake and sugar release capacity that reflects not only on the total microbes present on its surface but also on the load and diversity of the root residential microbes. Statistical dimensions of root decide its surface area that is available to provide "housing" to the epiphytic microbes. More the surface area, higher the harboring of microbes.

In addition to the terrestrial land plants, a major portion of the vegetation also falls under the aquatic category which makes it necessary to consider the roots of aquatic plants for its effect of epiphytic community. It has been observed that aquatic roots, even though submerged in water, interact with microbes related to the mechanisms of plant growth promotion, biogeochemical cycles such as nitrification, denitrification, and also for bioremediation of water contaminants (Tanaka et al. 2009). However, compared to the terrestrial roots, aquatic roots tend to have lesser microbial load as they are under the effect of water currents. Furthermore, the water immersed roots also face variety of surrounding conditions based on the aquatic body in which it is immersed which also acts as one of the contributing factors in the diversity of the root epiphytes. For example, freshwater roots have microbes which are either non-halophilic or maximally, halotolerant, on the contrary, most of the fresh water microbes cannot survive on the marine root surface. Marine roots are the home to mostly halophilic or halotolerant microbes. In fresh water bodies, root community depends on the properties of water such as pH, oxygen availability, electrical conductivity, salt concentration, presence of organic matter, and toxicants (Srivastava et al. 2017).

Tanaka et al. (2009) have analyzed bacterial community harbored by aquatic roots of reed (*Phragmites australis*) and Japanese loosestrife (*Lythrum anceps*) from a floating treatment wetland pond. They found taxonomical difference in the

bacterial communities of both the roots suggesting plant species specificity with respect to its epiphytic community. Reed roots were predominated by *Betaproteobacteria,* while Japanese loosestrife harbored *Alphaproteobacteria.* Also compared to pond water, plant roots microorganisms were more diverse. This may be because of the nutrients supplied by the roots in form of root exudates. Their studies claimed the presence of higher number of novel phylogenetic species on the roots of these plants. Another major study was carried out by Crump and Koch (2008), on four species of aquatic angiosperms, namely *Vallisneria americana* (Wild celery), *Potamogeton perfoliatus* (Red head grass), *Stuckenia pectinata* (Saga pond weed), and *Zostera marina* (Eel grass), respectively from fresh water, brackish water, and marine water of Chesapeake Bay. They analyzed that all the four plant roots had difference in diversity of microorganisms on their surface supporting the idea of species specificity in terms of root–microbe interrelations.

As this section discusses diversity of root epiphytes based on the type of roots, another root type which can be considered for mention in this section is aerial roots. They are the roots present above ground and are mostly adventitious in nature. They are present on the epiphytic plants such as orchids. Studies (Tsavkelova et al. 2007) revealed that in epiphytic orchid which consist of aerial roots, microbial community not only differed than those present on the roots of terrestrial orchid but also was more abundant compared to its terrestrial counterpart. It has been stated that the presence of velamen which is a spongy hygroscopic tissue on the aerial roots may be responsible for the presence of higher number of microbes on the aerial roots. Furthermore, epiphytic aerial roots were allowed the harboring of gram-negative *Pseudomonas* and *Flavobacterium*, while gram positive strains of *Streptomyces* and *Bacillus* dominated the rhizoplane of terrestrial roots. These studies also claimed that orchid bacterial communities depend on the species and root types mainly depending on the composition of root exudates.

17.2.2.3 Root Exudates

Root exudates are defined as smaller, low molecular weight compounds comprising of sugars, amino acids, organic acids, and secondary metabolites (Phillips et al. 2004). Damaged root surface cells, secretions, lysates, and mucilage are other organic materials that can be utilized by rhizosphere flora (Andrews and Harris 2000). There are number of studies that claim dependence of root microflora communities on the type of exudates (Tsavkelova et al. 2007; Srivastava et al. 2017). There are strong evidences demonstrating the interdependence of microbial diversity and the root exudates (Mendes et al. 2013; Canarini et al. 2019). But the knowledge of mechanisms and level of such interactions are still in its infancy. Roots are known to release variety of exudates depending on the plant species and the factors discussed in the above two sections. Those compounds, mainly amino acids, phytoalexins, phenolics, etc., act as a sort of "biochemical magnets" to attract specific groups of microbes. However, their profile seems to be interdependent in a way that microbes surrounding the roots release certain metabolites that stimulate secretion of primary root metabolites such as amino acids (Phillips et al. 2004) and there happens to be a cross talk between plant roots and the surrounding microbial

members through these chemotactic molecules that plays a crucial role in deciding the root epiphytic community. Additionally, microbes utilize those molecules there by affecting the concentration gradient of those metabolites. Roots tend to release primary metabolites as diffusion process which acts along the concentration gradient releasing more exudates subsequently. Generally due to the presence of these root exudates, that comprises of both primary and secondary metabolites, rhizoplane appears to be a nutrient rich niche which is directly responsible for higher diversity compared to the bulk soil in which the microbes face oligotrophic conditions most of the times. However, reports also claim that nutrient poor rhizosphere leads to increase in biodiversity because of the synergistic interactions among various species as an approach for increasing the bioavailability of required elements. Additionally, metabolic properties of root surface microbes largely depend on the polyphenolic profile of the plant species (Hashidoko 2005). These secondary metabolites also play a role in the phytopathogenic mechanisms of the plant as well as in framing the root architecture (Canarini et al. 2019).

The relationship of root exudates with the root community has been studied using the mutant version of the plant blocking the expression of specific secondary metabolite, sequentially analyzing its effect on the root community structure. It has been stated that under or over expression of certain biometabolites like indole glucosinolates, ABC transporter, certain transcription factors, tyrosine derived metabolites, etc. reflected the changes in the bacterial communities as well as its abundance in the vicinity of roots of *A. thaliana* (Reinhold-Hurek et al. 2015). There are recent studies (Mendes et al. 2013) on genomic profiling to analyze release of root exudates on gene expression of community microbial strains using number of methods such as "one gene at a time" approach, whole genome transcriptome profiling, stable isotope probing (SIP), and other "Omics" related approaches. Mark et al. (2005) have evaluated the effect of sugar beet root exudates on the gene expression of *Pseudomonas aeruginosa* using whole genome transcriptome profiling approach. They deciphered alterations in expression level of around 104 gene in the bacteria with response to the exudate released from the roots. Orchids release various phenolics, phytoalexins, and tryptophan. Phytoalexins and phenolics have a tendency to suppress various microorganisms, while tryptophan that acts as a precursor for Indol Acetic Acid (IAA) attracts the strains such as *Bacillus sp*., that are capable of converting IAA into Auxin (Tsavkelova et al. 2007). Significant amount of isoflavones and saponins are released as secondary metabolites from soybean (Glycine max) roots for biochemical interaction with rhizosphere bacteria (Sugiyama 2019). The story of root exudates does not hold much significance when we talk about aquatic roots. Terrestrial plant roots attract microorganisms by releasing array of root exudates while aquatic roots release organic nutrients mainly carbon and oxygen and invite microbes by providing these so-called offerings to them (Srivastava et al. 2017).

17.2.3 Prokaryotic Microbes

A major portion of rhizoplane community consists of prokaryotic microbes, members of certain bacterial genera to be present as highest in number against other domains. Furthermore, as root exudates contributes about 25% of the organic matter, the bacterial load is about 10-fold to 100-fold more in the rhizosphere area compared to the bulk soil (Andrews and Harris 2000). Microbial communities in the root vicinity can be classified as either PGPB (Plant growth promoting bacteria) or ISR (Induce systemic resistance) (Rout 2014).

Bacteria are the most abundant microbial candidates present in rhizosphere, rhizoplane as well as on the root surfaces. They cover the root surface through biofilm formation. Most of the studies suggest that *Proteobacteria* is the predominating phyla in the root community along with few members of *Actinobacteria* (Bodenhausen et al. 2013; Reinhold-Hurek et al. 2015; Deng et al. 2019; Dong et al. 2019). The two classes of *Proteobacteria*, *Gammaproteobacteria* (25%), and *Alphaproteobacteria* (38%) dominated the root surfaces (Reinhold-Hurek et al. 2015). However, strawberry roots treated with microbial amendments showed increased number of *Betaproteobacteria* and decrease in *Actinobacteria* demonstrating that treatments performed in soil rhizosphere can likely change the root community structure (Deng et al. 2019). Root microbiota are enriched with the members of *Acidobacteria*, *Verrucomicrobia*, *Bacteroidetes,* and *Planctomycetes* in addition to *Proteobacteria* and *Actinobacteria*, derived from surrounding soil and can be transferred horizontally (Compant et al. 2019). Different plant roots have certain common bacterial inhabitants while many of the genera are also plant specific (Table 17.1).

The quantitative load of the root associated bacteria also differs slightly in different plant species. The number of bacteria were found to be around 10^6 to 10^7 CFU/g in paddy (Castillo et al. 2015) and between the range of 2.5 to 4.5×10^5 CFU/g in lettuce roots depending upon the type of protocol and number of washing steps applied for the sample processing (Sare et al. 2020).

Bodenhausen et al. (2013) have compared the root epiphytic community of *Arabidopsis thaliana* with its endophytic counterpart. Their studies suggested the presence of higher number of bacteria on root surface compared to the endophytic portion. Proteobacteria, Actinobacteria, and Bacteroides were the predominant phyla present along with small portion of Firmicutes. They deciphered more than 4000 sequences and found high abundance of certain OTUs (Operational Taxonomic Units) denoting the presence of *Pseudomonas* representing the class *Gammaproteobacteria*, *Actinomycetales,* and *Actinoplanes* as members of *Actinobacteria* and genus *Chitinophagaceae* representing *Bacteroides*. High number of *Arthrobacter* sp., *Flavobacterium* sp., and *Sphingomonas* sp. were also associated with the *A. thaliana* roots. Two non-corelated free-living nitrogen fixing members of α proteobacteria, facultative anaerobic bacterial strain of *Klebsiella pneumoniae* IFO3318 and a strain of aerobic nitrogen fixing free-living bacteria *Beijeirinkia indica* subsp. *Indica* IFO3744, were isolated from the rhizoplane of stressed soil tolerant plants (Hashidoko 2005). High phosphate solubilizing bacterial strain of

Table 17.1 Root epiphytic bacterial members

No.	Plant	Root epiphytic bacteria	Reference
1	Tomato	*Pseudomonas, Acinetobacter*	Dong et al. (2019)
2	Paddy	*Erwinia, Pantoea, Klebsiella, Enterobacter, Aeromonas, Acinetobacter, Pseudomonas.*	Castillo et al. (2015)
3	Terrestrial orchid (*Paphiopedilum appletonianum*)	*Streptomyces, Bacillus, Pseudomonas, Erwinia, Burkholderia, Nocardia*	Tsavkelova et al. (2007)
4	Epiphytic orchid (*Pholidota articulata*)	*Pseudomonas, Flavobacterium, Stenotrophomonas, Pantoea, Chryseobacterium, Bacillus, Agrobacterium, Erwinia, Burkholderia, Paracoccus*	Tsavkelova et al. (2007)
5	Lettuce	*Burkholderia, Sphingobium, Blastopirellula, Luteolibacter, Methylophilus, Nitrospira, Hydrogenophaga,* etc.	Sare et al. (2020)
6	*Arabidopsis thaliana*	*Proteobacteria, Actinobacteria, Bacteroides*	Bodenhausen et al. (2013)
7	Rice from acid sulfate soil paddocks	*Sphingomonas sp.*	Hashidoko (2005)
8	*Xyris complanata*	*Fracteuria sp.*	Hashidoko (2005)
9	Strawberry	*Betaproteobacteria*	Deng et al. (2019)
10	Wheat	*Burkholderiales, Sphingobacteriales, Xanthomonadales*	Kawasaki et al. (2016)

Pantoea eucalypti and IAA (Indol Acetic Acid) producers *Raoultella ornithinolytica* are epiphytes of ethanomedicinal plants (War Nongkhlaw and Joshi 2014).

Bacterial community differs in the rhizoplane of *Brachypodium distachyon* (wheat) in nodal roots and seminal roots along their root tips and root bases. *Oxalobacteraceae* of *Betaproteobacteria* dominated in seminal root tips while *Comamonadaceae* (*Betaproteobacteria*) was strongly associated with nodal roots (Kawasaki et al. 2016).

In addition to bacteria, there are certain members of archaea that colonizes specific microbial niche in the root communities. This may be due to the ubiquitous presence of archaea. Archaea interact with plants as well as members of microbial world present in the rhizosphere. Methanogenic archaea are closely associated with rice roots and contribute to the release of large portion of methane from the crop (Deng et al. 2019). In arugula plants that are widely used in salads, archaeal phylum Thaumarchaeota (73.4%) dominated the rhizosphere followed by Euryarchaeota (20.9%), Woesearchaeota (3%), and Bathyarchaeota (0.2%)(Taffner et al. 2019). Agave and cacti episphere consist of archaeal members of classes *Nitrosospira*, *Halobacteria,* and *Methanomicrobia* (Flores-Núñez et al. 2020).

17.2.4 Eukaryotic Microbes

As the rhizosphere consists of the members from all the domains of life, fungi represent the eukaryotic category in majority of the epiphytic community. They co-exist with the prokaryotic microflora in the rhizoplane and perform interkingdom interactions. However, the number of eukaryotic members is very less compared to the prokaryotes.

Fungi mainly of phylum Ascomycota dominate the root communities of various plants (Kawasaki et al. 2016; Sugiyama 2019). In wheat plants, Kawasaki et al. (2016) studied and compared the microbial communities of tightly bound soil portion of the roots, loosely bound fractions as well as bulk soil and depicted that dominant fungal communities remain nearly same in the tightly attached fraction (which majorly consist of root epiphytes) and loosely attached soil in the rhizosphere. *Emericellopsis mirabilis* of *Ascomycota* was dominant OTU found in the tightly bound soil samples attached on the root surfaces.

Salazar-Cerezo et al. (2018) performed study of culturable fungal diversity on *Stanhopea tigrina* (Mexican orchid) and isolated nearly 140 fungi from the roots. The epiphytic fungi consisted majority of *Trichoderma* sp. and *Penicillium* sp. of Ascomycota, followed by *Fusarium*, *Scedosporium*, etc.

17.3 Significance

17.3.1 Growth of Plants

Epiphytic microbes play an important ecological role in the growth of plants by producing various molecules like phytohormones and bacterial volatile organic compounds (VOCs). These microbes are inhabiting in the surroundings of the roots so these molecules serve as signaling molecules between plant and microbes. These plant–microbe interactions have significant effect on the growth of the plants, nutrient uptake by the plant, soil health, and soil fertility.

Physiological process of plants is influenced by small organic molecules or substances at very low concentration known as phytohormones or plant hormones. These chemical messengers are able to coordinate cellular activities of plants. Total nine categories are reported, out of which auxins, cytokinins (CKs), and gibberellins (GAs), a gaseous hormone ethylene (ET) are known as classical plant hormones and whereas abscisic acid (ABA), brassinosteroids (BRs), jasmonic acid (JA), salicylic acid (SA), and strigolactones (SLs) are categorized as class of new phytohormones. Classical hormones play major role in plant growth and development, whereas others are having prominent role in plant defense as well as in mitigation of biotic and abiotic stress (Bhatt et al. 2020).

Apart from plants, phytohormone production capacity has been also detected in many plants associated bacteria and fungi. Microbial production of phytohormones plays a role in plant growth promotion and root architecture but the degree of proof for their contribution is varying a lot depending as per the phytohormone producing

microbial strain. Only presence of phytohormone in the supernatant of microbial culture is insufficient to ascertain their role in plant growth but experiments with mutant, incapable to produce phytohormone, can directly demonstrate their function role in plant growth (Spaepen 2015). The postulation that hormones induce plant growth is not always correct, because some hormones also show opposite effect.

Auxins are involved in several plant growth and developmental events like cell division, cell elongation, and differentiation. Indole-3-acetic acid (IAA) is synthesized from its chemically similar compound tryptophan and its production is reported in microbes. As per a rough estimation, over 80% of the rhizospheric bacteria are assumed to capable of producing IAA (Khalid et al. 2005). IAA production by microbes has been linked with promotion of plant growth because when these microbes were inoculated experimentally, it resulted in increase in root and shoot biomass (Spaepen 2015). Based on several genetic and biochemical methods, at least five different pathways for synthesis of IAA have been described, which include the indole-3-acetamide (IAM), the indole-3-acetonitrile (IAN), the indole-3-pyruvate (IPyA), the tryptophan side-chain oxidase (TSO), and the tryptamine (TAM) pathways (Kunkel and Harper 2018). Certain recent evidences suggest that, apart from plant promotion, elevated IAA levels or enhanced auxin signaling involved in disease development in some plant pathogen interaction. The role of pathogen producing IAA has been investigated on *Arabidopsis thaliana*. The IAA produced by plant pathogen *Pseudomonas syringae* strain DC3000 contributes to its virulence and also suppresses salicylic acid (SA) mediated defense mechanism in *Arabidopsis thaliana* (McClerklin et al. 2018).

Cytokines (CKs) are involved in cell division and differentiation of root and shoot meristematic tissues, organ formation, root and root hair development, as well as prevention of senescence. Relatively less species and strains are reported, may be due to problems associated with cytokinin assay, as compared to that of auxins. Increased root and shoot biomass has been resulted when plant rhizosphere is inoculated with cytokine producing bacteria (Arkhipova et al. 2006). In rhizosphere, microbially produced CKs are interacting with other plant hormone signaling pathways as similar as to plant derived CKs. Thus, microbially produced CKs play similar role in protection against pathogens by exogenously providing CKs or overexpressing the CK biosynthetic genes. Its role can be expanded in abiotic and biotic stress resiliency. Epiphytes, capable of producing CKs can be effectively applied for biocontrol agent as a part of integrated crop management (Akhtar et al. 2020).

The class of gibberellins (GAs), tetracyclic diterpenoid acids, is a broad group comprising more than 100 compounds. Gibberellins are involved in plant developmental and physiological processes like cell division, leaf and stem growth, seed germination, seedling emergence, floral induction, and flower and fruit growth. GAs are also playing the role in promotion of root growth, root hair abundance, inhibition of floral bud differentiation (Bottini et al. 2004). Endophytic fungi are major producers of GAs, but in free-living microbes GAs production has been widely reported and studied. Gibberellins produced by free living rhizospheric bacteria play various roles like it can increase the ^{15}N uptake in wheat roots, promote the root

growth in maize (Fulchieri et al. 1993), promote growth of both roots and shoots under drought (Cohen et al. 2001), help plants to mitigate metal induced stress (Kang et al. 2017), increase soil salinity resistance (Kim et al. 2017). Nowadays, interest in searching the free-living microbes with GAs production capacity has been observed as these organisms can be effectively used as PGPR.

Abscisic acid holds a critical role in stomatal closure, morphogenesis of embryo, fruit ripening, leaf senescence, and inhibition of seed germination. It is also involved in synthesis of stored proteins and lipids, as well as shows protective response against abiotic stress like drought, low temperature, metal toxicity, and salinity (Bhatt et al. 2020). ABA is well recognized as a central regulator in adaptation of plants to abiotic stress and a key molecule in plant response to microbes. However, some plant pathogens produce ABA and induce ABA accumulation in the infected plant, resulting in progression of infection. *Bacillus megaterium, B. cereus*, *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, P. vulgaris* are reported to produce ABA (Karadeniz et al. 2006).

Ethylene, the simplest unsaturated hydrocarbon, is gaseous phytohormone, also produced by rhizospheric microbes. It is also known as fruit ripening hormone and involved in plant senescence, seed germination, cell expansion. Ethylene plays a defensive role against plant pathogen by showing synergistic effect with jasmonate dependent defense response. Under abiotic stress, elevated ethylene level may lead to inhibitory effects on the plants. Various microbes including bacteria, actinomycetes, yeast, molds, and algae are deeply discussed in the review by Fukuda et al. (1993). On the other hand, microbes can also reduce the level of ethylene by producing enzyme ACC deaminase. This enzyme degrades ACC (1-aminocyclopropane-1-carboxylic acid), an important intermediate of ethylene production pathway, in order to reduce the effect of ethylene (Ravanbakhsh et al. 2018). Recently, an interesting trait of some angiosperm plants is reported as an array to combat with drought, called formation of rhizosheath. Rhizosheath, the soil which remains strongly attached to root upon excavation, is a protective layer that enhance nutrient and water uptake by maintenance of direct contact between soil and the root. Formation of rhizosheath is affected by various factors like formation of root hairs, root and microbial mucilage, and the surrounding microflora (Hartman 2020). By an unclear mechanism, under draught conditions, rhizosheath is produced (Brown et al. 2017). Ethylene reduces the growth of the primary root but promotes root hair promotion, necessary to form rhizosheath under moderate draught stress. The evidences from the transcriptomics experiments on rhizosheath formation rice (*Oryza sativa*) by Zhang et al. (2020) also support these phenomena and also unravel ethylene signaling. Plant growth promoting *Enterobacteriaceae* rhizobacteria was found upon bacterial community analysis of rhizosheath soil, many of which showed high ACC deaminase activity, as a result plant growth is promoted.

Many soil epiphytes produce VOCs, though these VOCs are produced from a distance but have potential role to improve plant growth. The first report of VOCs in plant growth promotion was the production of VOCs (2,3- butanediol and acetoin) by *Bacillus subtilis* GB03 (Ryu et al. 2003). After that, many reporters have documented various VOCs for plant growth promotion like VOCs from

Pseudomonas fluorescens SS101 enhance the growth of *Nicotiana tabacum* (Park et al. 2015), VOCs from *Pseudomonas simiae* AU increased growth of soybean seedlings (Vaishnav et al. 2015), soil bacterium *Streptomyces coelicolor* promote growth of *Arabidopsis thaliana* (Dotson et al. 2020). Interaction between VOCs and plants is deeply discussed by Raza and Shen (2020).

17.3.2 Plant Defense

Plant, as a rich source of nutrients, eventually attacked by many plant pathogens like bacteria, fungi, protists, insects, and vertebrates. During the process of evolution, plant has developed many successful defense mechanisms against plant pathogens. Some of these mechanisms involved role of microbiome near to roots. Epiphytes, as an integrated part of root rhizosphere, interact with roots to provide protection against pathogen to the plants. One of the mechanisms involved formation of biofilms surrounds the roots for protection from plant pathogens. The production of antimicrobial compounds and VOCs also protects plants from pathogens. Competition between pathogen and non-pathogenic organism can also help plants by inhibiting colonization of pathogenic organisms.

A biofilm is defined as an assemblage of microorganisms that are irreversibly associated (cannot be removed by gentle rinsing) with a surface and encased in a matrix of primarily polysaccharide material. Usually non-cellular materials like mineral crystals, blood components, corrosion particles, clay or silt particles, depending on the surrounding environment in/on which the biofilm has developed, may also be found in the biofilm matrix. Biofilms may form on various surfaces, including living tissues, ship hull, indwelling medical devices, industrial settings, water system piping, or natural aquatic systems (Donlan 2002).

Biofilms on plant surfaces have been described as surface attached, structured microbial communities, normally observed as various assemblages like aggregates, microcolonies, and symplasmata (Morris and Monier 2003). They form microniches of conditions which are markedly different from its ambient environment.

A protective and antibacterial biofilm of *B. subtilis*, on root surfaces, was evidently reported for biocontrol of *P. syringae* root infection in model plant *Arabidopsis*. Interaction between a root pathogenic *P. syringae* and *Arabidopsis* root was studied to elucidate the mechanism of infection inhibition. Wild type *B. subtilis* 6051 was used in the experiment because of its biocontrol activity in a variety of plants, seed protectant, and antifungal activity as well as its biofilm forming capacity on root surfaces in vitro (Kinsinger et al. 2003; Bais et al. 2004). The control of root infection by *B. subtilis* 6051 was directly proportional to its ability to colonize and formation of biofilms on plant root surfaces and mediated by the secretion of antimicrobial a lipopeptide antibiotic, surfactin at the root surface. *B. subtilis* is known to produce surfactin and iturin in vitro (Peypoux et al. 1999), but surfactin is found stable for prolong period in rhizosphere (Asaka and Shoda 1996). The exact role of surfactin in biocontrol was proved by using a surfactin-minus mutant of *B. subtilis* which did not effectively control *P. syringae* pathogenicity and

exhibited poor biofilm formation on plant roots (Bais et al. 2004). Surfactin damage membrane barrier properties cause structural fluctuations in membrane and act rapidly on membrane integrity rather than other cellular vital process (Carrillo et al. 2003). This study suggests the good evidence of role of epiphytic bacterial biofilm in control of plant pathogen infection on root.

Gram positive, endospore former, aerobic rhizobacteria *Bacillus amyloliquefaciens* subsp. *plantarum* are known for their capacity to enhance yield of crop plants and their efficient role in biocontrol of bacterial and fungal plant pathogens. FZB42, the type strain of this subspecies is commercially used as biofertilizer and biocontrol agent in agriculture. The epiphytic *Bacillus amyloliquefaciens* FZB42 has been reported to form biofilm on the root surface *of Zea mays, Arabidopsis thaliana*, *Lemna minor,* tomato, and lettuce. Their ability to form biofilm was confirmed by green fluorescent protein expression based assay and confocal laser scanning microscopy by using genetically engineered *Bacillus amyloliquefaciens* FZB42 (Fan et al. 2011).

The soil-borne fungus *Rhizoctonia solani* is necrotrophic plant pathogen of economically important crops such as rice, maize, eggplant, sugar beet, soybean, potato, pepper, cabbage, lettuce, cauliflower, and tomato. 14 distinct anastomosis groups (AGs) of *R. solani* species have been described, some of them are subdivided into additional subgroups which can be differentiated based on genotypic characteristics and ecological criteria, such as specific host range. Bottom rot of lettuce is caused by members of the subgroup AG1-IB, very difficult to control, occurs wherever lettuce grows and resulted in high economical losses (Verwaaijen et al. 2017). The initial establishment of infection by *R. solani* can be delayed by addition of FZB42 to the soil. FZB42 produces non-ribosomally synthesized secondary metabolites like surfactin, fengycin, and bacillomycin D in the lettuce rhizosphere which exhibit direct antagonism and at the same time also enhancing the plant defense responses by mediating plant defense gene expression towards fungal pathogen (Chowdhury et al. 2015b).

Significant yield losses have been observed due to root-knot nematode (RKN), *Meloidogyne incognita*. It attacks on the roots of various trees, shrubs, and herbaceous plants including tomatoes and cotton. The infected roots develop rounded or irregular galls of 1 to 20 mm in size and become distorted leads to stunted growth and poor crop yield. The nematodes also exasperate the deleterious effects of pathogenic bacteria and fungi. Application of FZB42 has been significantly reduce nematode eggs in roots, juvenile worms in soil, and plant galls on tomato. Moderate nematicidal activity is exhibited by FZB42 due to the production of plantazolicin by a gene within the *pzn* gene cluster (Liu et al. 2013). Various mechanism of diseases suppression by FZB42 is deeply reviewed by Chowdhury et al. 2015a, including the stimulation of plant ISR by various bacterial secondary metabolites like surfactin and volatile organic compounds (VOCs).

Every year reports of life-threatening outbreaks due to food borne pathogen, *Escherichia coli* O157: H7, contamination to various plant parts indicates compulsion to search new control strategies. *Escherichia coli* O157: H7 can cause hemorrhagic colitis and, in very severe cases, hemolytic uremic syndrome. Two of the

widespread *Arabidopsis* epiphytes, *Enterobacter asburiae,* and *Wautersia paucula* studied for demonstration of competition with *Escherichia coli* O157: H7 in order to suppress contamination on lettuce. When applied in experimental soils, *Enterobacter asburiae* effectively compete and control *Escherichia coli* O157:H7 contamination (Cooley et al. 2006).

Microbial volatile organic compounds (VOCs) exhibit their potential in inhibiting the growth of phytopathogens. Small VOCs produced by bacterial antagonist reported to inhibit the mycelial growth of soil-borne phytopathogen *Rhizoctonia solani* Kühn (Kai et al. 2007). *Bacillus weihenstephanensis, B. simplex, B. subtilis, Serratia marcescens* demonstrate nematocidal activities by producing VOCs against *Panagrellus redivivus*—a free-living nematode (Gu et al. 2007). Many bacterial and fungal species producing VOCs are summarized and discussed by Campos et al. 2010 and de Boer et al. 2019.

17.3.3 Root Morphology and Architecture

Root morphology simply denotes the surface features of the root and all the subparts like root hairs, root diameter, root patterns, etc. Root architecture refers to the temporal and spatial configuration of entire root system in the heterogeneous matrix of the soil which determines the ability of plant roots to obtain mobile and immobile nutrients (Lynch 1995). Apart from nutrients uptakes, roots are essential anchoring and mechanical support to the plants. In order to improve crop yield and growth, knowledge of root system architecture is an essential part.

By the experiments involving gnotobiotic condition, the role of epiphytes has been elucidated in root development. In a model dicot *Arabidopsis thaliana*, root development was monitored in the terms of hair elongation, length of the primary root, branching patterns, and light dark cycle in the presence or absence (gnotobiotic) of epiphytic microbial strains and found that root development was governed by the inoculated epiphytic microbes (Klikno and Kutschera 2017).

Regulation of root system architecture involves endogenous signals from plant itself and some outer stimuli like signals generated by microbes like plant growth promoting rhizobacteria (PGPR) and environmental stimuli, like the availability of water and nutrients. Involvement of PGPR in root system architecture was evidently studied in rice seedling and PGPR strain, *Bacillus altitudinis* (strain FD48) by comparative experiments involving gnotobiotic conditions. Role of PGPR in phytohormone modulation was proved by studying expression of auxin responsive genes (Ambreetha et al. 2018). *Bacillus megaterium*, a rhizospheric bacteria and PGPR, can effectively modulate root architecture by involving auxin- and-ethylene independent mechanisms.

Bacterial cross talk (quorum sensing) signals, N-acyl-homoserine lactones (AHLs), are also responded by plants and it alter post-embryonic root development. AHLs able to regulate primary root growth, lateral root formation, and root hair development (Ortíz-castro et al. 2008). Root colonization by the *Pseudomonas putida* and *P. fluorescens* can modulate root architecture by modulating auxin-

responsive gene expression with the involvement of bacterial cyclodipeptides (Ortiz-Castro et al. 2020). These evidences strongly suggest the role of bacterial molecules in the root system architecture which involves the role of epiphytes in ecology of root system.

17.4 Future Aspects

Diversity and functional properties of epiphytic microbes can be exploited in order to enhance the plant growth by production of phyto-stimulating molecules. Epiphytic diversity can be used in production of commercial biocontrol agents for many plant diseases. The role of microbes in root development is established. Scientific communities are nowadays focusing on developing the crop varieties which are more efficient in acquisition of nutrients and water under limiting conditions by understanding root system architecture and its remodeling. Still, there is a great need to understand interactions of plant behavior modifying compounds with plants, in order to understand their role in root system architecture.

References

Akhtar SS et al (2020) Role of Cytokinins for interactions of plants with microbial pathogens and Pest insects. Front Plant Sci 10:1777. https://doi.org/10.3389/fpls.2019.01777

Ambreetha S et al (2018) Plant-associated Bacillus modulates the expression of auxin-responsive genes of rice and modifies the root architecture. Rhizosphere 5:57–66. https://doi.org/10.1016/j.rhisph.2017.12.001

Andrews JH, Harris RF (2000) The ecology and biogeography of microorganisms on plant surfaces. Annu Rev Phytopathol 38:145–180

Arkhipova TN et al (2006) Comparison of effects of bacterial strains differing in their ability to synthesize cytokinins on growth and cytokinin content in wheat plants. Russ J Plant Physiol 53 (4):507–513

Asaka O, Shoda M (1996) Biocontrol of Rhizoctonia solani damping-off of tomato with Bacillus subtilis RB14. Appl Environ Microbiol 62(11):4081–4085

Bais HP, Fall R, Vivanco JM (2004) Biocontrol of Bacillus subtilis against infection of Arabidopsis roots by Pseudomonas syringae is facilitated by biofilm formation and Surfactin production. Plant Physiol 134(1):307–319. https://doi.org/10.1104/pp.103.028712

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Bhatt D et al (2020) Role of growth regulators and Phytohormones in overcoming environmental stress. In: Protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives. Wiley, p 254

Bodenhausen N, Horton MW, Bergelson J (2013) Bacterial communities associated with the leaves and the roots of Arabidopsis thaliana. PLoS One 8(2). https://doi.org/10.1371/journal.pone.0056329

Bottini R, Cassán F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65(5):497–503. https://doi.org/10.1007/s00253-004-1696-1

Brown LK et al (2017) The rhizosheath – a potential trait for future agricultural sustainability occurs in orders throughout the angiosperms. Plant Soil 418(1):115–128. https://doi.org/10.1007/s11104-017-3220-2

Campos VP, de Pinho RSC, Freire ES (2010) Volatiles produced by interacting microorganisms potentially useful for the control of plant pathogens. Ciência e Agrotecnol 34(3):525–535

Canarini A et al (2019) Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front Plant Sci 10:157

Carrillo C et al (2003) Molecular mechanism of membrane permeabilization by the peptide antibiotic surfactin. Biochim Biophys Acta Biomembr 1611(1):91–97. https://doi.org/10.1016/S0005-2736(03)00029-4

Castillo I et al (2015) Isolation of endophytic, epiphytic and rhizosphere plant growth-promoting bacteria from cultivated rice paddy soils of the Guadalquivir river marshes. Global Adv Res J Agric Sci 4(2):127–136

Chowdhury SP, Hartmann A et al (2015a) Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42 – a review. Front Microbiol:780. https://www.frontiersin.org/article/10.3389/fmicb.2015.00780

Chowdhury SP, Uhl J et al (2015b) Cyclic lipopeptides of Bacillus amyloliquefaciens subsp. plantarum colonizing the lettuce rhizosphere enhance plant defense responses toward the bottom rot pathogen Rhizoctonia solani. Mol Plant Microbe Interact 28(9):984–995

Cohen, A. C. *et al.* (2001) Azospirillum inoculation and inhibition of gibberellin and ABA synthesis in maize seedling under drought. In: Proceedings-plant growth regulation society of America-annual meeting, pp 88–93

Compant S et al (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. J Adv Res 19:29–37. https://doi.org/10.1016/j.jare.2019.03.004

Cooley MB, Chao D, Mandrell RE (2006) Escherichia coli O157:H7 survival and growth on lettuce is altered by the presence of epiphytic Bacteria. J Food Prot 69(10):2329–2335. https://doi.org/10.4315/0362-028X-69.10.2329

Crump BC, Koch EW (2008) Attached bacterial populations shared by four species of aquatic angiosperms. Appl Environ Microbiol 74(19):5948–5957

de Boer W et al (2019) Pathogen suppression by microbial volatile organic compounds in soils. FEMS Microbiol Ecol 95(8). https://doi.org/10.1093/femsec/fiz105

Deng S et al (2019) A plant growth-promoting microbial soil amendment dynamically alters the strawberry root bacterial microbiome. Sci Rep 9(1):1–15

Dong CJ et al (2019) Bacterial communities in the rhizosphere, phyllosphere and endosphere of tomato plants. PLoS One 14(11):1–17. https://doi.org/10.1371/journal.pone.0223847

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890. https://doi.org/10.3201/eid0809.020063

Dotson BR, et al. (2020) The Streptomyces volatile 3-octanone alters auxin/cytokinin and growth in Arabidopsis thaliana via the gene family KISS ME DEADLY. bioRxiv. Cold Spring Harbor Laboratory. https://doi.org/10.1101/2020.02.15.949685

Durán P et al (2018) Microbial Interkingdom interactions in roots promote Arabidopsis survival. Cell 175(4):973–983.e14. https://doi.org/10.1016/j.cell.2018.10.020

Fan B et al (2011) Efficient colonization of plant roots by the plant growth promoting bacterium Bacillus amyloliquefaciens FZB42, engineered to express green fluorescent protein. J Biotechnol 151(4):303–311. https://doi.org/10.1016/j.jbiotec.2010.12.022

Flores-Núñez VM et al (2020) Functional signatures of the epiphytic prokaryotic microbiome of agaves and cacti. Front Microbiol 10(January):1–13. https://doi.org/10.3389/fmicb.2019.03044

Fukuda H, Ogawa T, Tanase S (1993) Ethylene production by micro-organisms. In: Advances in microbial physiology. Academic Press, pp 275–306. https://doi.org/10.1016/S0065-2911(08)60101-0

Fulchieri M, Lucangeli C, Bottini R (1993) Inoculation with Azospirillum lipoferum affects growth and gibberellin status of corn seedling roots. Plant Cell Physiol 34(8):1305–1309

Gu Y-Q et al (2007) Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biol Biochem 39(10):2567–2575
Hartman S (2020) Trapped in the Rhizosheath: root-bacterial interactions modulate ethylene signaling. Plant Physiol 183(2):443–444. https://doi.org/10.1104/pp.20.00379
Hashidoko Y (2005) Ecochemical studies of interrelationships between epiphytic bacteria and host plants via secondary metabolites. Biosci Biotechnol Biochem 69(8):1427–1441. https://doi.org/10.1271/bbb.69.1427
Kai M et al (2007) Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen Rhizoctonia solani. Arch Microbiol 187(5):351–360. https://doi.org/10.1007/s00203-006-0199-0
Kang S-M et al (2017) Gibberellins and indole-3-acetic acid producing rhizospheric bacterium Leifsonia xyli SE134 mitigates the adverse effects of copper-mediated stress on tomato. J Plant Interact 12(1):373–380. https://doi.org/10.1080/17429145.2017.1370142
Karadeniz A, Topcuoğlu ŞF, Inan S (2006) Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. World J Microbiol Biotechnol 22(10):1061–1064. https://doi.org/10.1007/s11274-005-4561-1
Kawasaki A et al (2016) Microbiome and exudates of the root and rhizosphere of Brachypodium distachyon, a model for wheat. PloS One 11(10):e0164533
Khalid A et al (2005) Relative efficiency of rhizobacteria for auxin biosynthesis in rhizosphere and non-rhizosphere soils. Soil Res 42(8):921–926
Kim M-J et al (2017) Plant growth promoting effect of Bacillus amyloliquefaciens H-2-5 on crop plants and influence on physiological changes in soybean under soil salinity. Physiol Mol Biol Plants 23(3):571–580. https://doi.org/10.1007/s12298-017-0449-4
Kinsinger RF, Shirk MC, Fall R (2003) Rapid surface motility in Bacillus subtilis is dependent on extracellular surfactin and potassium ion. J Bacteriol 185(18):5627–5631
Klikno J, Kutschera U (2017) Regulation of root development in Arabidopsis thaliana by phytohormone-secreting epiphytic methylobacteria. Protoplasma 254(5):1867–1877. https://doi.org/10.1007/s00709-016-1067-7
Kunkel BN, Harper CP (2018) The roles of auxin during interactions between bacterial plant pathogens and their hosts. J Exp Bot 69(2):245–254
Liu Z et al (2013) The highly modified microcin peptide plantazolicin is associated with nematicidal activity of Bacillus amyloliquefaciens FZB42. Appl Microbiol Biotechnol 97(23):10081–10090
Liu Z et al (2019) An age-dependent sequence of physiological processes defines developmental root senescence. Plant Physiol 181(3):993–1007
Lundberg DS et al (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488 (7409):86–90
Lynch J (1995) Root architecture and plant productivity. Plant Physiol 109(1):7
Mark GL et al (2005) Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. Proc Natl Acad Sci 102(48):17454–17459. https://doi.org/10.1073/pnas.0506407102
McClerklin SA et al (2018) Indole-3-acetaldehyde dehydrogenase-dependent auxin synthesis contributes to virulence of Pseudomonas syringae strain DC3000. PLoS Pathogens 14(1): e1006811
Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37 (5):634–663. https://doi.org/10.1111/1574-6976.12028
Morris CE, Monier J-M (2003) The ecological significance of biofilm formation by plant-associated bacteria. Ann Rev Phytopathol 41(1):429–453
Ortíz-castro R, Martínez-trujillo M, López-bucio J (2008) N-acyl-L-homoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in Arabidopsis thaliana. Plant Cell Environ 31(10):1497–1509
Ortiz-Castro R, Campos-García J, López-Bucio J (2020) Pseudomonas putida and Pseudomonas fluorescens influence Arabidopsis root system architecture through an Auxin response mediated

by bioactive Cyclodipeptides. J Plant Growth Regul 39(1):254–265. https://doi.org/10.1007/s00344-019-09979-w

Park Y-S et al (2015) Promotion of plant growth by Pseudomonas fluorescens strain SS101 via novel volatile organic compounds. Biochem Biophys Res Commun 461(2):361–365. https://doi.org/10.1016/j.bbrc.2015.04.039

Peypoux F, Bonmatin JM, Wallach J (1999) Recent trends in the biochemistry of surfactin. Appl Microbiol Biotechnol 51(5):553–563

Phillips DA et al (2004) Microbial products trigger amino acid exudation from plant roots. Plant Physiol 136(1):2887–2894

Praeg N, Pauli H, Illmer P (2019) Microbial diversity in bulk and rhizosphere soil of Ranunculus glacialis along a high-alpine altitudinal gradient. Front Microbiol 10:1429

Ravanbakhsh M et al (2018) Microbial modulation of plant ethylene signaling: ecological and evolutionary consequences. Microbiome 6(1):52. https://doi.org/10.1186/s40168-018-0436-1

Raza W, Shen Q (2020) Volatile organic compounds mediated plant-microbe interactions in soil. In: Sharma, V., Salwan, R., and Al-Ani, L. (eds) Molecular aspects of plant beneficial microbes in agriculture. Academic Press, pp. 209–219. doi: https://doi.org/10.1016/B978-0-12-818469-1.00018-3

Reinhold-Hurek B et al (2015) Roots shaping their microbiome: global hotspots for microbial activity. Annu Rev Phytopathol 53(1):403–424. https://doi.org/10.1146/annurev-phyto-082712-102342

Rout ME (2014) The plant microbiome. In: Advances in botanical research, 1st edn. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-12-417163-3.00011-1

Ryu C-M et al (2003) Bacterial volatiles promote growth in Arabidopsis. Proc Natl Acad Sci 100 (8):4927–4932

Salazar-Cerezo S et al (2018) Fungal diversity and community composition of culturable fungi in Stanhopea tigrina cast gibberellin producers. Front Microbiol 9:612

Sare AR et al (2020) Standardization of plant microbiome studies: which proportion of the microbiota is really harvested? Microorganisms 8(3). https://doi.org/10.3390/microorganisms8030342

Spaepen S (2015) Plant hormones produced by microbes. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer, Cham, pp 247–256. https://doi.org/10.1007/978-3-319-08575-3_26

Srivastava JK et al (2017) Plant–microbe interaction in aquatic system and their role in the management of water quality: a review. Appl Water Sci 7(3):1079–1090

Sugiyama A (2019) The soybean rhizosphere: metabolites, microbes, and beyond—a review. J Adv Res 19:67–73. https://doi.org/10.1016/j.jare.2019.03.005

Taffner J et al (2019) Novel insights into plant-associated archaea and their functioning in arugula (Eruca sativa mill.). J Adv Res 19:39–48. https://doi.org/10.1016/j.jare.2019.04.008

Tanaka Y et al (2009) Microbial community analysis in the roots of aquatic plants and isolation of novel microbes including an organism of the candidate phylum OP10. Microbes Environ:1111290336

Tsavkelova EA et al (2007) Bacteria associated with orchid roots and microbial production of auxin. Microbiol Res 162(1):69–76. https://doi.org/10.1016/j.micres.2006.07.014

Turner TR et al (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME J 7(12):2248–2258. https://doi.org/10.1038/ismej.2013.119

Vaishnav A et al (2015) Putative bacterial volatile-mediated growth in soybean (Glycine max L. Merrill) and expression of induced proteins under salt stress. J Appl Microbiol 119 (2):539–551. https://doi.org/10.1111/jam.12866

Verwaaijen B et al (2017) The Rhizoctonia solani AG1-IB (isolate 7/3/14) transcriptome during interaction with the host plant lettuce (Lactuca sativa L.). Plos One 12(5):e0177278. https://doi.org/10.1371/journal.pone.0177278

War Nongkhlaw FM, Joshi SR (2014) Epiphytic and endophytic bacteria that promote growth of ethnomedicinal plants in the subtropical forests of Meghalaya, India. Rev Biol Trop. http://creativecommons.org/licenses/by/3.0 62(4):1295–1308

Zamioudis C et al (2013) Unraveling root developmental programs initiated by beneficial Pseudomonas spp. Bacteria. Plant Physiol 162(1):304–318. https://doi.org/10.1104/pp.112.212597

Zhang Y et al (2020) Root-Bacteria associations boost Rhizosheath formation in moderately dry soil through ethylene responses. Plant Physiol 183(2):780–792

Evaluation of Dynamic Microbiome Ecology Within the Plant Roots

18

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Abstract

Belowground plant root–soil interface is a dynamic region, where numerous biogeochemical changes take place and are determined by the physical and chemical activities. A vast number of microbes including bacteria and fungi are associated with soil and plants. Roots carry out several functions like attachment and absorbance of nutrients, water and microorganisms necessary for plant growth. Plants have evolved with a wide range of microorganisms within (endophytes) or surrounding (epiphytes) and play a significant role in plant growth and health. Environmental factors, soil condition, impact of plant genotype, rhizosphere and root exudates have an impact on plant endophytes and their mechanism. Therefore, this chapter reviews the plant root associated endophytes, factors affecting, functionalities and understanding the interaction between microbiome associated within plant root.

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Keywords

Root endophytes · Microbial community · Plant–microbe interactions · Cell signalling · Biocontrol agent

18.1 Introduction

A large number of beneficial microorganisms are associated with surrounding or within plants and play a major role in enhancing plant health and development. Those microbial communities associated with plant cells are known as endophytes. The endophytes belong to a vast group of microorganisms that have their life cycle partly or entirely inside the plant and are located in intra- and inter-cellular spaces or in the vascular tissue. It can be found in aerial or root parts of plant. The entire or partial life cycle of these microorganisms takes place within the different parts of the host without triggering any disease. They are omnipresent in nature and show multifaceted interactions with hosts like antagonism, mutualism and sometimes parasitism (Nair and Padmavathy 2014). It acts as reservoirs for microbial secondary metabolites such as phenolic acids, terpenoids, alkaloids, quinones, tannins, steroids and saponins and has potential properties like anti-insect, antimicrobial and anticancer (Gouda et al. 2016). Due to this reason endophytic microbes also scrutinize for novel drug discovery. Furthermore, various endophytic microorganisms that have been characterized as plant growth-promoting bacteria (PGPB) improve the plant capacity to withstand various environmental stresses.

The plant roots endophytes can differ significantly as compared to rhizosphere microbiome due to plant inner environment (Germida et al. 1998; Gottel et al. 2011). Diverse bioproducts such as biofertilizers and biofungicides or to modify and/or introduce beneficial bacteria into the phytomicrobiome for agricultural purposes are being developed (Souza et al. 2015; Mitter et al. 2017).

For better understanding, we must see insight on how these microorganisms live in soil and plants. Plant endophytes interactions are extremely multi-layered involving community assembly and its functioning. Therefore, this chapter aims to comprehend the plant root associated endophytes, interaction between plant root endophytes–soil microbiome and cross-talk between different endophytes (Fig. 18.1).

18.2 Plant Root Associated Endophytes

Bary (1866) introduced the term endophyte, well-defined microorganisms (fungal or bacterial) that raises within plant tissues and relationships with the host plant is obligate or facultative type (Petrini 1991; Cabral et al. 1993; Hallmann and Berg 2006; Rosenblueth and Martínez-Romero 2006).

Obligate endophytes are those which depend on the metabolism of plants for survival, being spread among plants by the activity of different types of vectors or by

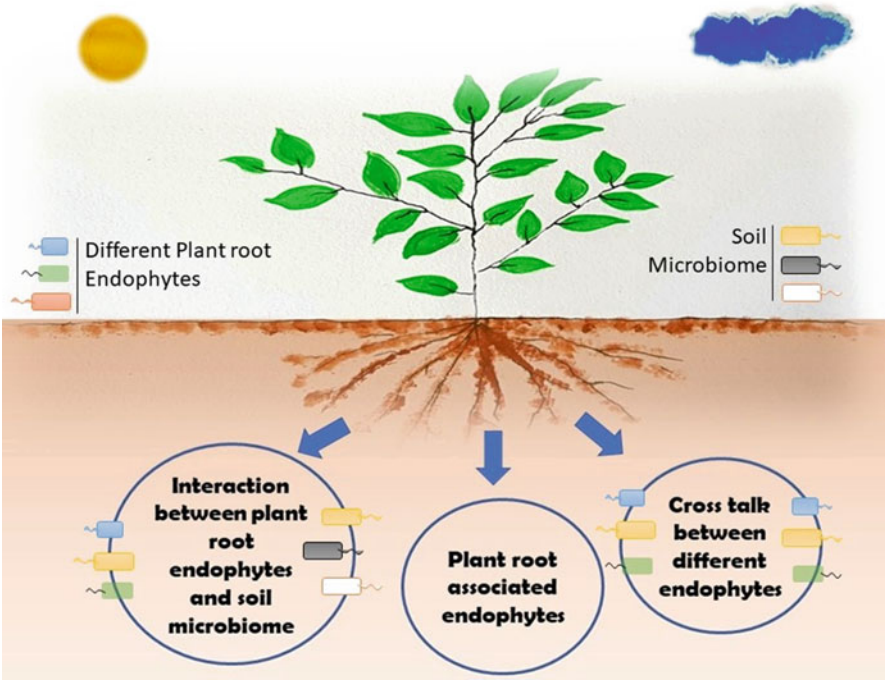

Fig. 18.1 Overview of dynamic microbiome interactions within plant roots

vertical transmission (Hardoim et al. 2008). Whereas, the facultative endophytes are residing on the exterior of the plant body up to a certain lifespan and association depend upon soil and atmospheric environment (Abreu-Tarazi et al. 2010). A large number of plant species provide shelter to a diverse range of endophytes (Mundt and Hinkle 1976; Hallmann and Berg 2006). The microbial community of endophyte within plants depends on two factors: (1) plant resources (biotic and abiotic) and (2) ability to colonize. Endophytes especially one living inside roots often colonize and penetrate the epidermis from different sites such as root hair zone, root emergence and root cracks (Dong et al. 2003; Compant et al. 2005; Zakria et al. 2007). Successfully endophytes are colonized in plant both intra- and intercellularly (Zakria et al. 2007). Several microbial endophytes can locomote to peripheral regions of plant through vascular tissues and spreading systemically after initial colonization (Compant et al. 2005; Zakria et al. 2007). This movement was observed by Johnston-Monje and Raizada (2011). They observed that the transport of endophytes (labeled with a green-fluorescent-protein) moves from seeds into plant roots and tissues, while endophytes injected into stems moved into the roots and rhizosphere, and continuing movement of organisms throughout the root microbiome.

18.2.1 Biotic and Abiotic Factors Influencing Plant Microbiota

Microbial composition in any portion of plant is influenced by a variety of abiotic/biotic factors. Various factors below- and aboveground affect plant microbiome such as soil properties including soil type, salinity, pH, structure, moisture, organic matter, soil–plant exudates (Fierer 2017) and environmental conditions like agricultural practices, pathogen presence and climate (Hardoim et al. 2015). Apart from soil environment recruitment of plant microbiota depends upon plant species, genotype, age, developmental stage, health, root morphology, exudates and rhizodepositions (Hartmann et al. 2009; Ladygina and Hedlund 2010; Reinhold-Hurek et al. 2015). Even, it was found that plant species growing in same soil conditions have significantly different microbial communities in root compartments and rhizosphere (Reinhold-Hurek et al. 2015; Hacquard 2016; Samad et al. 2017). Plant roots excrete several organic compounds that promote microbial growth that affects the overall structure of microbial rhizosphere community (Grayston et al. 1998; Miethling et al. 2000). Nowadays researchers' efforts have been directed towards the understanding of the composition of rhizosphere microbiome, its signalling and determining the impact on plant growth and health (Mendes et al. 2011; Straub et al. 2013; Berg et al. 2016).

18.2.2 Types of Microorganism Associated Within Plant Root

Plant root endophytes in form of bacteria or fungi are associated with plant or colonized inside plant tissues. From 16 phyla, more than 200 genera of bacterial species have been reported as endophytes and most of them found to be species belonging to the phyla firmicutes proteobacteria and actinobacteria (Golinska et al. 2015).

The diverse range of gram-positive to gram-negative endophytic bacterial species include *Bacillus*, *Agrobacterium*, *Microbacterium. Achromobacter*, *Xanthomonas*, *Brevibacterium*, *Pseudomonas*, *Acinetobacter*, etc. (Sun et al. 2013). The endophyte community structure of a particular plant depends on several factors such as host progression stage, inoculum density, host species and environmental factors (Dudeja and Giri 2014; Khare et al. 2018). A list of some plant specific root endophytes is shown in Table 18.1.

18.2.3 Function and Application of Root Endophytes

Endophytic microbes are important components of plants, and they function in the following ways:

1. nutrient uptake by plants (White et al. 2012; Prieto et al. 2017),
2. protect plants from pathogens and insects (Soares et al. 2016; Verma et al. 2018a, b),

Table 18.1 Several plant specific root inhabiting microorganisms

Sr. No.	Root endophytes	Plant	References
1	*Rhizoscyphus ericae*	*Calluna vulgaris* *Erica andevalensis* *Cephaloziella varians* *Vaccinium macrocarpon* *Colobanthus quitensis*	Vrålstad et al. (2002) Upson et al. (2007) Turnau et al. (2007) Upson et al. (2009)
2	*Chloridium paucisporum*	*Pinus resinosa* *Picea rubens* *Betula alleghaniensis*	Wang and Wilcox (1985)
3	*Acephala applanata*	*P. sylvestris* *Picea abies*	Grunig and Sieber (2005)
4	*Phialophora finlandia*	*P. resinosa* *P. silvestres* *Betula alleghaniensis*	Wang and Wilcox (1985)
5	*Phialocephala fortinii*	*Salix glauca* *Abies alba* *Rhododendron* sp. *P. resinosa* *Alnus rubra*	Wang and Wilcox (1985)
6	*Mollisia* sp.	*P. abies* *Deschampsia antarctica*	Menkis et al. (2005) Upson et al. (2009)
7	*Leptodontidium orchidicola*	*Platanthera hyperborean*	Grunig and Sieber (2005)
8	*Bionectria rossmaniae*	*S. lycopersicum* L.	Andrade-Linares et al. (2011)
9	*Doratomyces* sp.	*Arabidopsis thaliana*	Junker et al. (2012)
10	*Trichoderma* sp.	*S. lycopersicum* L.	Andrade-Linares et al. (2011)
11	*Periconia macrospinosa*	*Andropogon gerardii*	Mandyam et al. (2010)
12	*Phoma* sp.	*A. thaliana*	Junker et al. (2012)
13	*Rhizopycnis vagum*	*Pinus halepensis* *Rosmarinus officinalis* *Dioscorea zingiberensis* *S. lycopersicum* L.	Girlanda et al. (2002) Xu et al. (2008) Andrade-Linares et al. (2011)
14	*Plectosphaerella cucumerina*	*A. thaliana* *S. lycopersicum* L.	Junker et al. (2012) Andrade-Linares et al. (2011)
15	*Microdochium* sp.	*Andropogon gerardii*	Mandyam et al. (2010)
16	*Piriformospora indica*	*Glomus mosseae*	Verma et al. (1998)
17	*Piriformospora williamsii* sp. nov.	*Glomus fasciculatum*	Williams (1985) Basiewicz et al. (2012)
18	*Sebacina vermifera*	*Terrestrial orchids* *Acianthus reniformis* *Caladenia* sp. *Eriochilus* sp.	Warcup (1988)
19	*Xylaria* sp.	*Dendrobium* sp.	Chen et al. (2013)

3. increase stress tolerance in plants (Redman et al. 2002; Irizarry and White 2018),
4. modulate plant development (Irizarry and White 2018; Verma et al. 2018a, b) and
5. suppress weed growth (White et al. 2018).

Function varied depending upon the type of microorganisms and plant species. As we know endophytes are beneficial to their host cells and so its application is observed in every aspect of life. The agricultural domain chiefly depends on stable climate conditions and fertile soil. Increasing environmental pollution has a major impact on the quality of water, soil, and ecological balance and preservation of biological diversity. Moreover, it also affects directly or indirectly economic framework conditions in the agricultural sector (Sturz et al. 2000; Yadav 2017). We have briefly reviewed the potential application of root endophytes in various sectors such as plant growth-promoting endophytes, biocontrol agent, beneficial to their host by producing a range of natural products and its potential use in the field of medicine, agriculture or industry and alternative to conventional methods. Furthermore, it has been reported that application of endophytes on pollutants sites eliminate contaminants from soil using phytoremediation technique by enhancing nitrogen fixation and phosphate solubilization leading to soil fertility improvement. Recently research is more focusing towards the potential biotechnological applications of endophytes for improving phytoremediation and the sustainable biofuel and biomass production from non-food crops (Ryan et al. 2008).

18.3 Cross-Talk Between Different Endophytes

Significant information is available on cross-talk between plant–microbes. The interesting aspect of dynamic microbiome ecology is to understand how microbe–microbe plays a role within plant root for successive establishment. Generally, root colonization is the prime requirement for potent endophytes to reach the root surface via the chemotactic mechanism. In the process, endophytes have to outcompete other microbial species for successful insertion on to plant root surface. For invasion in root tissue, endophytes stimulate specific plant gene expression in a very well-coordinated way to their successful accommodation with resisting plant immune responses (Bais et al. 2006; Rosenblueth and Martínez-Romero 2006; Compant et al. 2010).

18.3.1 Microbe–Microbe Signalling in Plant Root

Synchronized incursion by microorganisms on root surface comprises numerous signalling pathways and shared signalling between plants and endophytes (Morris and Monier 2003; Rudrappa et al. 2008). Quorum sensing (QS) is a well-understood microbe–microbe signalling mechanism. QS decides the fate of microbial behaviour through well-coordinated cell density-dependent regulator (Teplitski et al. 2000). Quorum sensing system operates through low molecular weight autoinducers, which

influx and outflux between communicating bacterial cells (Chernin 2011). The quorum sensing system allows each microorganism to involve in harmonization to achieve signal threshold and endurance of the microbial community because all involved microbes express genes together to achieve colonization potential (Elasri et al. 2001). N-acyl homoserine lactones (AHLs) have been found to be the most common QS signals in gram-negative bacteria. Bacterial community uses diverse biomolecules to communicate/interact between and within species (Steidle et al. 2001; Chen et al. 2002). It is most appropriate for the microbial species sharing the same niche.

18.3.2 Microbial Shifts Within the Root Microbiome

The microbial communities inhabiting in plant roots were reported to be showing spatiotemporal shifts in a range of plant species. Even the root endophyte microbiome structure changes with the ageing of plant (Monteiro et al. 2011). Researchers have observed declination in the abundance of *actinobacteria* and *Pseudomonas* microbial communities in potato roots upon ageing (van Overbeek and van Elsas 2008). In *Chrysopogon zizanioides*, endophyte community structure more noticeably changes during initial growth stages (Monteiro et al. 2011). With change in endophyte community structure could directly affect hormonal changes and physiology in plant system with ageing (Taiz and Zeiger 1998). Some random probability patterns of endophyte colonization have been observed in terms of influencing the plant system for allowing endophytes for pre- and post-root infiltration (Hardoim et al. 2008). These consequences lead to diverse plant species growing on the same soil to have distinct structure of endophytes community (Weber et al. 1999). Endophytes community structure can establish in soil over the course of time, which is remarkable in agriculture systems as to the establishment of endophytes from one crop to the following crop due to rotation of crop as a common agricultural practice (Sturz et al. 1998).

18.3.3 Role of Technological Advancement in Understanding Microbial Community

Research in the field of endophyte interaction has reached to new heights due to continued efforts and technological advancement. An array of next generation tools are leveraged to understand very complex microbial dynamics inhabiting plant root system. Exploration of Meta-genomics, proteomics, and transcriptomics in the field of plant endophytes and their interaction is a recent trend. This multifaceted approach has made discovery and characterization of many very low molecular weight autoinducers achievable. Recent advancement in microarray multiplex technology can elucidate microbial diversity and gene expression patterns from very multi-layered microenvironments (Gao and Tao 2012). Various tools such as high throughput sequencing, metagenomic analysis, phylogenetic characterization,

Table 18.2 Next generation technologies and their outcome to probe complex endophytes interaction and community structure

Technology platform used	Outcome of research	References
High throughput sequencing (454-pyrosequencing)	Extracts from potato root examined in which 5 out of the 10 genera had not been reported previously as potato root endophytes	Manter et al. (2010)
Metagenomic analysis	Well-characterized, adaptations, metabolic processes and PGPR characteristics in rice	Sessitsch et al. (2012)
	Reported broad range of novel endophyte phylogenetic lineages	Sun et al. (2013)
	Study on potential protein having colonization ability and plant growth promotion was characterized	Barret et al. (2011) Sessitsch et al. (2012)
	Gene expression related to protein secretion systems, motility and detoxification of reactive oxygen species was demonstrated	Hérouart et al. (2002) Cheng et al. (2010)
Phylogenetic characterization	Understanding of the community composition	Korf (2004)
Proteomic analysis	Changes in plant protein expression pattern under influence of endophyte and changes in endophyte protein expression due to the influence of plant	Cheng et al. (2010)
2D gel electrophoresis clubbed with mass spectrometry	Plant protein expression related to defence response and hormone production	Pradet-Balade et al. (2001); Cheng et al. (2010)
	Identified plant defence-related proteins in rice treated with *Sinorhizobium meliloti* using mass spectrometry-based technique	Chi et al. (2010)
Transcriptome analysis	Identification of gene expression in endophyte *Azoarcus* sp. BH72, mandatory for effective establishment of plant surfaces and within roots	Hauberg-Lotte et al. (2012)
Nucleic acid-based isotope probing (SIP-DNA and SIP-rRNA methods)	Determined nitrogen fixation by *Klebsiella pneumoniae* in wheat plant by using isotopes	Radajewski et al. (2000)
	Explored both rhizosphere and stem endophytes	Rasche et al. (2009)

proteomic analysis, 2D gel electrophoresis clubbed with mass spectrometry, transcriptome analysis and nucleic acid-based stable isotope probing (SIP-DNA and SIP-rRNA methods) have been summarized with its significant outcome in the field of endophyte dynamics in Table 18.2.

18.4 Interaction Between Plant Root Endophytes and Soil Microbiome

Soil microbiome can greatly influence endophytes community structure apart from various physical conditions such as latitude, elevation, temperature and precipitation. Increasing literature emphasize on the regulation of bacterial community structure within the plant roots, called as the root microbiome or root-endophytic microbial community. Despite the strong curiosity in the finding of origins and significances of plant–soil feedbacks, there is a significant gap in understanding the mechanism of root-endophytic bacterial communities influence on microbial communities residing in the rhizosphere of soil (Wagg et al. 2014).

18.4.1 Interspecies and Intergenus Interactions

In complex ecosystems, microorganisms simultaneously cross-talk with their own species as well as other genus and even with candidates of another kingdom. QS signal molecules are well discussed in terms of the communications within members of single species. N-acyl-homoserine lactones (AHLs) produced among bacteria of the same type were also observed in diverse genera (Arora et al. 2010). Soil microbiome candidates such as *Aeromonas hydrophila*, *Serratia liquefaciens* and *Pseudomonas aeruginosa* were reported to produce analogous N-butanoyl homoserine lactone (C4-HSL) (Eberl 1999; Swift et al. 1997). Different rhizobia involve in legume nodulation produced diverse signal molecules of different categories. Among rhizobial species *Rhizobium leguminosarum* bv. *viciae*, bv. *Phaseoli*, *Rhizobium fredii*, and *Trifolii*, *Sinorhizobium meliloti* communicate through 3-oxo-C8-HSL similar to AHL. This communication within the microbiome community is either competitive or synergistic nature and plays a typical function in dynamics (Marketon and González 2002).

Both gram-positive and gram-negative bacteria harbour genes for autoinducer type 2 (AI-2) signalling biomolecules. Interestingly, certain zoosporic pathogens were found to produce autoinducer type 2, which acts as a mutual bridge of interaction with other nearby microorganisms (Kong et al. 2010). This makes pathogen able to exist in soil along with a wide range of microorganisms.

Diffusible signal factors (DSF) were reported as interspecies communicating signals. cis-2-dodecenoic acid (BDSF) produced by *Burkholderia cenocepacia*, categorized as DSF, is a structural analog of cis-11-methyl2-dodecenoic acid found in *Xanthomonas campestris*. The biofilm production was found to be restored by BDSF in *X. campestris* DSF-deficient mutants (Boon et al. 2008). BDSF was also observed to restrict the growth of *Candida albicans* (Boon et al. 2008). Shank and Kolter (2009) have reported the presence of cis-2-decenoic acid in *Pseudomonas aeruginosa* which is chemically same as BDSF and DSF (Shank and Kolter 2009). The cis-2-decenoic acid was found to have an inhibitory effect of biofilm formation in various types of microorganisms including *Bacillus subtilis*, *Candida albicans*,

P. aeruginosa, *Escherichia coli* and *Streptococcus pyogenes* (Shank and Kolter 2009; Dow 2017).

18.4.2 Root Endophytes Inhibiting Phytopathogens

The plant growth promotion through protection against disease caused by phytopathogens is known as biocontrol mechanism. Several phytopathogen species habituating in soil adversely affect overall plant health. Certain endophytes synthesize antibiotics and siderophores which counteract against such pathogens. Siderophores competing with phytopathogens for obtaining trace metals for essential growth. Some siderophores such as salicylic acid and pyochelin act as iron chelator inhibiting the disease-causing microbial communities. Certain antimicrobial metabolites such as 2,4-diacetylphloroglucinol (DAPG) produced by endophytic isolates were found to reduce 70% of wilt in eggplant (Ramesh et al. 2009).

18.5 Conclusion

The role of omnipresence beneficial microbial endophytes in plant root system is undeniable. The complexity of plant physiological factors, cross communications between plant–microbe, microbe–microbe interaction shapes dynamic ecology. Endophytes definitely express different strategies for plant growth promotion in both single species and multiplex microbial community scenario. In a similar context, external factors may also affect strategies of endophytes such as microbial community interaction with the host plant, type of soil, and phytopathogen management. These affect altogether structure and function of the root endophytes. The endophytic relationship is multiplex and affected by several biotic and abiotic factors interaction that materializes at many temporal and spatial ranges. The dynamics of endophyte interaction endure as a significant research domain. Investigators now have technologically advanced tools to decode the very much complex interactions affected by abiotic and biotic aspects that have a great influence on microbial communities followed by plant health.

References

Abreu-Tarazi MF, Navarrete AA, Andreote FD, Almeida CV, Tsai SM, Almeida M (2010) Endophytic bacteria in long-term in vitro cultivated "axenic" pineapple microplants revealed by PCR–DGGE. World J Microbiol Biotechnol 26(3):555–560

Andrade-Linares DR, Grosch R, Franken P, Karl HR, Kost G, Restrepo S et al (2011) Colonization of roots of cultivated Solanum lycopersicum by dark septate and other ascomycetous endophytes. Mycologia 103:710–721

Arora NK, Khare E, Maheshwari DK (2010) Plant growth promoting rhizobacteria: constraints in bioformulation, commercialization, and future strategies. In: Plant growth and health promoting bacteria. Springer, Berlin, pp 97–116

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Barret M, Morrissey JP, O'Gara F (2011) Functional genomics analysis of plant growth-promoting rhizobacterial traits involved in rhizosphere competence. Biol Fertil Soils 47(7):729

Bary A (1866) Morphologie und physiologie der pilze, flechten und myxomyceten. W. Engelmann, Leipzig

Basiewicz M, Weiss M, Kogel K-H, Langen G et al (2012) Molecular and phenotypic characterization of *Sebacina vermifera* strains associated with orchids, and the description of Piriformospora williamsii sp. nov. Fungal Biol 116:204–213

Berg G, Rybakova D, Grube M, Köberl M (2016) The plant microbiome explored: implications for experimental botany. J Exp Bot 67(4):995–1002

Boon C, Deng Y, Wang LH, He Y, Xu JL, Fan Y, Pan SQ, Zhang LH (2008) A novel DSF-like signal from *Burkholderia cenocepacia* interferes with Candida albicans morphological transition. ISME J 2(1):27–36

Cabral D, Stone JK, Carroll GC (1993) The internal mycobiota of Juncus spp.: microscopic and cultural observations of infection patterns. Mycol Res 97(3):367–376

Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM (2002) Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415 (6871):545–549

Chen J, Zhang L-C, Xing Y-M, Wang Y-Q, Xing X-K et al (2013) Diversity and taxonomy of endophytic xylariaceous fungi from medicinal plants of Dendrobium (Orchidaceae). PLoS One 8(3):e58268

Cheng Z, McConkey BJ, Glick BR (2010) Proteomic studies of plant–bacterial interactions. Soil Biol Biochem 42(10):1673–1684

Chernin LS (2011) Quorum-sensing signals as mediators of PGPRs' beneficial traits. In: Bacteria in agrobiology: plant nutrient management. Springer, Berlin, pp 209–236

Chi F, Yang P, Han F, Jing Y, Shen S (2010) Proteomic analysis of rice seedlings infected by *Sinorhizobium meliloti* 1021. Proteomics 10(9):1861–1874

Compant S, Reiter B, Sessitsch A, Nowak J, Clément C, Barka EA (2005) Endophytic colonization of Vitis vinifera L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71(4):1685–1693

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42(5):669–678

Dong Y, Iniguez AL, Triplett EW (2003) Quantitative assessments of the host range and strain specificity of endophytic colonization by Klebsiella pneumoniae 342. Plant Soil 257(1):49–59

Dow JM (2017) Diffusible signal factor-dependent quorum sensing in pathogenic bacteria and its exploitation for disease control. J Appl Microbiol 122(1):2–11

Dudeja SS, Giri R (2014) Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria in legumes and non-legumes. Afr J Microbiol Res 8:1562–1572

Eberl L (1999) N-acyl homoserine lactone-mediated gene regulation in gram-negative bacteria. Syst Appl Microbiol 22(4):493–506

Elasri M, Delorme S, Lemanceau P, Stewart G, Laue B, Glickmann E, Oger PM, Dessaux Y (2001) Acyl-homoserine lactone production is more common among plant-associated Pseudomonas spp. than among soilborne Pseudomonas spp. Appl Environ Microbiol 67(3):1198–1209

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15(10):579–590

Gao D, Tao Y (2012) Current molecular biologic techniques for characterizing environmental microbial community. Front Environ Sci Eng 6(1):82–97

Germida JJ, Siciliano SD, Renato de Freitas J, Seib AM (1998) Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiol Ecol 26(1):43–50

Girlanda M, Ghignone S, Luppi AM (2002) Diversity of root associated fungi of two Mediterranean plants. New Phytol 155:481–498
Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M (2015) Endophytic actinobacteria of medicinal plants: diversity and bioactivity. Antonie Van Leeuwenhoek 108(2):267–289
Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher ED, Tuskan GA, Vilgalys R, Doktycz MJ (2011) Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. Appl Environ Microbiol 77(17):5934–5944
Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1538
Grayston SJ, Wang S, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30(3):369–378
Grunig CR, Sieber TN (2005) Molecular and phenotypic description of the widespread root symbiont *Acephala applanata* gen. et sp. nov., formerly known as dark-septate endophyte type 1. Mycologia 97(3):628–640
Hacquard S (2016) Disentangling the factors shaping microbiota composition across the plant holobiont. New Phytol 209(2):454–457
Hallmann J, Berg G (2006) Spectrum and population dynamics of bacterial root endophytes. In: Microbial root endophytes. Springer, Berlin, pp 15–31
Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16(10):463–471
Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320
Hartmann A, Schmid M, Van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321(1-2):235–257
Hauberg-Lotte L, Klingenberg H, Scharf C, Böhm M, Plessl J, Friedrich F, Völker U, Becker A, Reinhold-Hurek B (2012) Environmental factors affecting the expression of pilAB as well as the proteome and transcriptome of the grass endophyte Azoarcus sp. strain BH72. PLoS One 7(1): e30421
Hérouart D, Baudouin E, Frendo P, Harrison J, Santos R, Jamet A, Van de Sype G, Touati D, Puppo A (2002) Reactive oxygen species, nitric oxide and glutathione: a key role in the establishment of the legume–Rhizobium symbiosis? Plant Physiol Biochem 40(6-8):619–624
Irizarry I, White JF (2018) *Bacillus amyloliquefaciens* alters gene expression, ROS production and lignin synthesis in cotton seedling roots. J Appl Microbiol 124(6):1589–1603
Johnston-Monje D, Raizada MN (2011) Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. PLoS One 6(6):e20396
Junker C, Draeger S, Schulz B (2012) A fine line e endophytes or pathogens in Arabidopsis thaliana. Fungal Ecol 5(3):657–662
Khare E, Mishra J, Arora NK (2018) Multifaceted interactions between endophytes and plant: developments and prospects. Front Microbiol 9:2732
Kong P, Lee BW, Zhou ZS, Hong C (2010) Zoosporic plant pathogens produce bacterial autoinducer-2 that affects Vibrio harveyi quorum sensing. FEMS Microbiol Lett 303(1):55–60
Korf I (2004) Gene finding in novel genomes. BMC Bioinf 5(1):59
Ladygina N, Hedlund K (2010) Plant species influence microbial diversity and carbon allocation in the rhizosphere. Soil Biol Biochem 42(2):162–168
Mandyam K, Loughin T, Jumpponen A (2010) Isolation and morphological and metabolic characterization of common endophytes in annually burned tallgrass prairie. Mycologia 102 (4):813–821
Manter DK, Delgado JA, Holm DG, Stong RA (2010) Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. Microb Ecol 60(1):157–166
Marketon MM, González JE (2002) Identification of two quorum-sensing systems in *Sinorhizobium meliloti*. J Bacteriol 184(13):3466–3475

Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PA, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332(6033):1097–1100

Menkis A, Vasiliauskas R, Taylor AF, Stenlid J, Finlay R (2005) Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. Mycorrhiza 16:33–41

Miethling R, Wieland G, Backhaus H, Tebbe CC (2000) Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. Microb Ecol 40(1):43–56

Mitter B, Pfaffenbichler N, Flavell R, Compant S, Antonielli L, Petric A, Berninger T, Naveed M, Sheibani-Tezerji R, von Maltzahn G, Sessitsch A (2017) A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. Front Microbiol 8:11

Monteiro JM, Vollú RE, Coelho MR, Fonseca A, Neto SC, Seldin L (2011) Bacterial communities within the rhizosphere and roots of vetiver (*Chrysopogon zizanioides* (L.) Roberty) sampled at different growth stages. Eur J Soil Biol 47(4):236–242

Morris CE, Monier JM (2003) The ecological significance of biofilm formation by plant-associated bacteria. Annu Rev Phytopathol 41(1):429–453

Mundt JO, Hinkle NF (1976) Bacteria within ovules and seeds. Appl Environ Microbiol 32 (5):694–698

Nair DN, Padmavathy S (2014) Impact of endophytic microorganisms on plants, environment and humans. Sci World J 2014:250693

Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 179–197

Pradet-Balade B, Boulmé F, Beug H, Müllner EW, Garcia-Sanz JA (2001) Translation control: bridging the gap between genomics and proteomics? Trends Biochem Sci 26(4):225–229

Prieto KR, Echaide-Aquino F, Huerta-Robles A, Valério HP, Macedo-Raygoza G, Prado FM, Medeiros MH, Brito HF, da Silva IG, Felinto MC, White JF Jr (2017) Endophytic bacteria and rare earth elements; promising candidates for nutrient use efficiency in plants. In: Plant macronutrient use efficiency. Academic, New York, pp 285–306

Radajewski S, Ineson P, Parekh NR, Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. Nature 403(6770):646–649

Ramesh R, Joshi AA, Ghanekar MP (2009) Pseudomonads: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). World J Microbiol Biotechnol 25(1):47–55

Rasche F, Lueders T, Schloter M, Schaefer S, Buegger F, Gattinger A, Hood-Nowotny RC, Sessitsch A (2009) DNA-based stable isotope probing enables the identification of active bacterial endophytes in potatoes. New Phytol 181(4):802–807

Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermotolerance generated by plant/fungal symbiosis. Science 298(5598):1581

Reinhold-Hurek B, Bünger W, Burbano CS, Sabale M, Hurek T (2015) Roots shaping their microbiome: global hotspots for microbial activity. Annu Rev Phytopathol 53:403–424

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact 19(8):827–837

Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148(3):1547–1556

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278(1):1–9

Samad A, Trognitz F, Compant S, Antonielli L, Sessitsch A (2017) Shared and host-specific microbiome diversity and functioning of grapevine and accompanying weed plants. Environ Microbiol 19(4):1407–1424

Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B, Hauberg-Lotte L, Friedrich F, Rahalkar M, Hurek T (2012) Functional characteristics of an endophyte community

colonizing rice roots as revealed by metagenomic analysis. Mol Plant-Microbe Interact 25 (1):28–36

Shank EA, Kolter R (2009) New developments in microbial interspecies signaling. Curr Opin Microbiol 12(2):205–214

Soares MA, Li HY, Bergen M, Da Silva JM, Kowalski KP, White JF (2016) Functional role of an endophytic *Bacillus amyloliquefaciens* in enhancing growth and disease protection of invasive English ivy (Hedera helix L.). Plant Soil 405(1-2):107–123

Souza RD, Ambrosini A, Passaglia LM (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38(4):401–419

Steidle A, Sigl K, Schuhegger R, Ihring A, Schmid M, Gantner S, Stoffels M, Riedel K, Givskov M, Hartmann A, Langebartels C (2001) Visualization of N-acylhomoserine lactone-mediated cell-cell communication between bacteria colonizing the tomato rhizosphere. Appl Environ Microbiol 67(12):5761–5770

Straub D, Yang H, Liu Y, Tsap T, Ludewig U (2013) Root ethylene signalling is involved in *Miscanthus sinensis* growth promotion by the bacterial endophyte *Herbaspirillum frisingense* GSF30T. J Exp Bot 64(14):4603–4615

Sturz AV, Christie BR, Matheson BG (1998) Associations of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. Can J Microbiol 44 (2):162–167

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Rev Plant Sci 19(1):1–30

Sun H, He Y, Xiao Q, Ye R, Tian Y (2013) Isolation, characterization, and antimicrobial activity of endophytic bacteria from *Polygonum cuspidatum*. Afr J Microbiol Res 7(16):1496–1504

Swift S, Karlyshev AV, Fish L, Durant EL, Winson MK, Chhabra SR, Williams P, Macintyre S, Stewart GS (1997) Quorum sensing in Aeromonas hydrophila and Aeromonas salmonicida: identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J Bacteriol 179(17):5271–5281

Taiz L, Zeiger E (1998) Plant physiology, 2nd edn. Sinauer Associates, Inc., Sunderland

Teplitski M, Robinson JB, Bauer WD (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol Plant-Microbe Interact 13(6):637–648

Turnau K, Henriques FS, Anielska T, Renker C, Buscot F (2007) Metal uptake and detoxification mechanisms in *Erica andevalensis* growing in a pyrite mine tailing. Environ Exp Bot 61:117–123

Upson R, Read DJ, Newsham KK (2007) Widespread association between the ericoid mycorrhizal fungus *Rhizoscyphus ericae* and a leafy liverwort in the sub- and maritime Antarctic. New Phytol 176:460–471

Upson R, Newsham K, Bridge PD, Pearce DA, Read DJ (2009) Taxonomic affinities of dark septate root endophytes of *Colobanthus quitensis* and *Deschampsia antarctica*, the two native Antarctic vascular plant species. Fungal Ecol 2:184–196

Van Overbeek L, Van Elsas JD (2008) Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (*Solanum tuberosum* L.). FEMS Microbiol Ecol 64(2):283–296

Verma S, Varma A, Rexer KH, Hassel A, Kost G, Sarbhoy A, Bisen P, Bütehorn B, Franken P (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia 90 (5):896–903

Verma SK, Kingsley K, Bergen M, English C, Elmore M, Kharwar RN, White JF (2018a) Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. Plant Soil 422 (1-2):223–238

Verma SK, Kingsley KL, Bergen MS, Kowalski KP, White JF (2018b) Fungal disease protection in rice (Oryza sativa) seedlings by growth promoting seed-associated endophytic bacteria from invasive *Phragmites australis*. Microorganisms 6:21

Vrålstad T, Myhre E, Schumacher T (2002) Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the Helotiales in burnt and metal polluted habitats. New Phytol 155(1):131–148

Wagg C, Bender SF, Widmer F, van der Heijden MG (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc Natl Acad Sci 111(14):5266–5270

Wang CJ, Wilcox HE (1985) New species of ectendomycorrhizal and pseudomycorrhizal fungi: *Phialophora finlandia*, *Chloridium paucisporum*, and *Phialocephala fortinii*. Mycologia 77 (6):951–958

Warcup JH (1988) Mycorrhizal associations of isolates of *Sebacina vermifera*. New Phytol 110 (2):227–231

Weber OB, Baldani VL, Teixeira KD, Kirchhof G, Baldani JI, Dobereiner J (1999) Isolation and characterization of diazotrophic bacteria from banana and pineapple plants. Plant Soil 210 (1):103–113

White JF, Crawford H, Torres MS, Mattera R, Irizarry I, Bergen M (2012) A proposed mechanism for nitrogen acquisition by grass seedlings through oxidation of symbiotic bacteria. Symbiosis 57(3):161–171

White JF, Kingsley KI, Kowalski KP, Irizarry I, Micci A, Soares MA, Bergen MS (2018) Disease protection and allelopathic interactions of seed-transmitted endophytic pseudomonads of invasive reed grass (*Phragmites australis*). Plant Soil 422(1-2):195–208

Williams PG (1985) *Orchidaceous rhizoctonias* in pot cultures of vesicular–arbuscular mycorrhizal fungi. Can J Bot 63(7):1329–1333

Xu L, Zhou L, Zhao J, Li J, Li X, Wang J (2008) Fungal endophytes from *Dioscorea zingiberensis* rhizomes and their antibacterial activity. Lett Appl Microbiol 46(1):68–72

Yadav AN (2017) Agriculturally important microbiomes: biodiversity and multifarious PGP attributes for amelioration of diverse abiotic stresses in crops for sustainable agriculture. Biomed J Sci Technol Res 1:1–4

Zakria M, Njoloma J, Saeki Y, Akao S (2007) Colonization and nitrogen-fixing ability of Herbaspirillum sp. strain B501 gfp1 and assessment of its growth-promoting ability in cultivated rice. Microbes Environ 22(3):197–206

Manoeuvring Soil Microbiome and Their Interactions: A Resilient Technology for Conserving Soil and Plant Health

19

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Abstract

The soil microbial community hugely affects the growth and development of the plants through direct or indirect interactions. The rhizospheric microbial community dwelling in the soil are major drivers of this phenomenon. Manipulation of soil microbial population and community through various treatments of an array of beneficial microbes such as plant growth-promoting rhizobacteria, plant growth-promoting fungi, endophytic bacteria, biocontrol agents, etc. helps in alleviating various abiotic and biotic stresses of the plants. This, in turn, leads to the achievement of the yield which is close to the potential yield of the crop. Apart from increasing the yield of the crop, some of the beneficial microbes also enhance the nutrient content in the soil and availability of certain minerals to the plants eventually leading to conservation of soil health. Thus, manipulation of plant–soil microbiome paves the way for sustainable and green agriculture without imparting excessive monetary expenses, thereby creating increased crop production and embellishment of soil health. This chapter will so focus on the strategies and methods that are adopted to manipulate the plant–soil microbiome interactions, various mechanisms that are involved in the interactions, and the impact of this technology on the plant and soil.

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Keyword

PGPR · Soil health · Soil microbiome

19.1 Introduction

The agricultural ecosystems are experiencing an enormous pressure of providing the food to the growing population along with maintaining environmental sustainability. The agricultural lands are constantly degrading because of the faulty farming packages, changing climate, invasion of foreign species, accumulation of pollutants and chemicals, and many other reasons. Since the soil is at the receiving end of this cycle, the organisms which thrive on it are also very gravely affected as it is the most complex and diverse habitat. The crops permanently require a soil system to grow as it provides the base and required nutrients to them, with exception to hydroponics and aeroponics. The microorganisms which dwell in the soil are also at risk which is also a reason to worry as they are the critical players of various functions and services provided by the agricultural ecosystem (Jiao et al. 2019). Soil microbiome can be defined as the total count of microorganisms inhabiting the soil which co-exist together in the rhizospheric as well as the non-rhizospheric zones and are able to perform various functions either individually or together that ultimately changes the properties of soil and health of plants grown on them. These microbes include bacteria, fungi, protozoa, algae, and actinomycetes. These microbes are diverse in nature and range from beneficial ones to harmful ones. The beneficial ones support plant growth and development either directly and/or indirectly by providing the nutrients, stimulating plant growth, and acting as antagonists to phytopathogenic microbes. The major component of soil microbiome is fungi, bacteria, and archaea groups, which altogether makes more than 99% of soil microbial biomass (Fierer 2017). Additionally, various saprophytic, mutualistic, and phytopathogenic microbes also constitute the soil microbiome which also has important roles to play (Peay et al. 2016). The effects of soil microbiome on plants and soil are now very much evident and it is now a proven fact that a right microbial composition is essential for the betterment of both.

The relationship between soil, plants, and soil microbiome is now deciphered day-by-day, and now a new tier of connection is also added to it which is animals (Attwood et al. 2019). This new connection is very well evident from the proof that different ecto-endophytic plant microbes which pass onto plants from soil microbiome also enter the rumen of animals and aid in digestion (Kingston-Smith et al. 2008). The interactions between plants and the soil microbiome are also highly coordinated and dependent on various factors such as biotic or abiotic stresses, microbial population, climatic condition, soil, host plant, root exudates, and microbial secretions (Bais et al. 2006; Lakshmanan et al. 2014). This along with various other functions is carried out by the soil microbiome that is essential for the vitality of earth. It is so grievous that the soil microbiome is under threat due to urbanization, industralization, climate change, land degradation, changing rainfall pattern,

malformed agricultural practices, and poor land management practices (Amundson et al. 2015). Additionally, the pressure of feeding the population has adjured farmers to increase the agricultural production by adopting intensive agriculture which in turn has led to detrimental effects on soil physical and chemical properties and also loss of soil microbial diversity. The consequence of all these factors and their impact on soil microbiome is still poorly understood and needs more focus in order to make a sustainable agricultural blueprint (Köhl et al. 2014; Kumar et al. 2015). Hence, in recent times the scientific farming community is having enhanced interest and attention manoeuvring soil microbiome as a means for increasing crop production and/or productivity, soil restoration, and ecological balance (Calderón et al. 2017).

The habitat of soil-derived plant microbiome ranges from the whole plant to specific organs to the zone of interactions between plant and soil, i.e. rhizosphere and plant and atmosphere, i.e. phyllosphere (Rout and Southworth 2013). The rhizospheric region of the soil is dynamic in nature and is constantly remodelled by the influences of growing plants through exudation/secretion/deposition of various molecules and compounds (Bais et al. 2006; Badri and Vivanco 2009; Hinsinger et al. 2009; Shi et al. 2011; Moe 2013). The plants thereby through these influences bring changes in inhabiting microbiome. Reciprocally, the microbiome also brings changes in plants through production of different regulatory compounds which can have a positive or negative impact on the growth and fitness of the former (Carney et al. 2007; Mendes et al. 2011; Lebeis 2015). The soil microbiome thus behaves as an immensely evolved exterior force which possesses excellent potential of making changes in the cultivating crop plants (Philippot et al. 2013; Turner et al. 2013; Spence et al. 2014; Vaishnav et al. 2019); therefore, it is also aptly called as plant's second genome (Berendsen et al. 2012). Higher buffering capacity and reproduction potential of microbes have led to advanced genetic evolution in them which enables them to adapt to different environmental conditions. The stability of microbes under wavering soil conditions is also because of their abundance, physiological tolerance, molecular flexibility, widespread dispersal, and horizontal gene transfer (Allison and Martiny 2008; Fuhrman et al. 2015). The three main mechanisms which function behind their stability are resilience, resistance, and functional redundancy (Allison and Martiny 2008). The mechanism of resilience can be described as the ability of microbes to recover very readily to its stable state after the changes brought by any of the disturbances (Griffiths and Philippot 2013; Hodgson et al. 2015). Resistance in microbes is the ability to exhibit a significant magnitude of tolerance against any disturbances. The microbial functional redundancy is described as the phenomenon where the disturbed microbial ecosystem possesses the same traits to that of original one even though the community is significantly modified without recover (Allison and Martiny 2008).

The manoeuvring of plant's soil microbiome is one of the best alternatives which can ease of the dual pressure of increasing the agricultural production but with eco-friendly and sustainable agriculture, without imparting excessive monetary expenses. A particular strain of microbe or a consortium of many compatible microbe scans be thereby used for increasing agricultural production and enrichment of soil health (Yadav et al. 2019; Mukherjee et al. 2020; Patel et al. 2020). There are

many commercial microbial formulations present in the market which are even utilized by the farmers successfully as biofertilizers and seed inoculants (Patel et al. 2019; Prabha et al. 2019). Since both soil and plants are meta-organism, our knowledge and understanding about the precise mechanisms and processes which are involved during their interactions with the microbial community and their outcome are still insufficient. In this chapter, we will mainly focus on knowledge of the importance of soil microbiome, its composition, their interactions with host plant and their outcome, their effects on soil health, and the role of soil microbiome in achieving sustainable agriculture along with increased production and productivity.

19.2 Composition of Soil Microbial Community and Population

Soil and the microbes inhabiting in them together make the base of food webs carrying out functions like maintenance of terrestrial life, nutrients recycling, and elemental cycling pathways of production and degradation (Wilpiszeski et al. 2019). Microbes are known to form communities that are complex in nature, having varying structure, interactions, and functions. They are the most diversified form of life and have an indispensable role to play in different ecological functions and biogeochemical cycles. The soil microbial community lives in close association with the soil particles in the form of a single cell or as matrix embedded biofilms (Maier et al. 2009; Kamal et al. 2010). The diversity of soil microbes and their composition in different communities are the major indicators of soil fertility and productivity (Wang et al. 2019). The soil microbial community constitutes of bacterial species, archaeal species, and species from eukaryotic taxa (Curtis et al. 2002). The bacterial and archaeal species are the most ancient microbial life-forms and are thus found in more diverse environmental conditions. Among the eukaryotic taxa, fungal species are more prominent ones which are comparatively more modern microbial life-form in their appearance and are evolved in close association with the plants (Maier et al. 2009; Kamal et al. 2010). The total life from an estimate in the soil varies diversely, among which the bacterial species solely range from over thousands to millions in one gram of soil (Curtis et al. 2002; Torsvik and Øvreås 2002; Schloss and Handelsman 2006). These soil microbes are either the supporter or inhibitor of the plant's growth and development through direct and indirect means. The supporters are known as the beneficial microbes which function as symbionts, mutualists, or endophytes. The inhibitors are known as the phytopathogens and negatively affect plants through tissue damage and the production of toxins (Roper and Gupta 1995; Mukherjee et al. 2020). In addition to bacteria and fungi, the soil microbial population also has viruses and protozoan species (Jansson and Hofmockel 2019). The abundance level of soil microbial population is so much so that it constitutes about 60% of the earth's biomass (Bar-On et al. 2018).

The microbial population in the soil is majorly categorized into three groups. The first group comprises beneficial microorganisms like nitrogen-fixing bacteria, plant growth-promoting rhizobacteria, mineral solubilizing bacteria, mycoparasitic fungi,

mycorrhizal fungi, biocontrol agents, etc. which is the most studied one. The second group comprises phytopathogenic microorganisms which are deleterious to plant and the third group comprises human-pathogenic microorganisms which are deleterious to human health (Teplitski et al. 2011; Kaestli et al. 2012). The plant growth-promoting rhizobacteria (PGPR) are free-living bacterial species which are found in the rhizospheric region of the soil and expend beneficial effects on plants through direct and indirect means. They provide nutrients to plants by nutrient acquisition, help in signal transduction and growth by phytohormones production, and form a channel of cross-talk with other microbes in soil and plants (Backer et al. 2018; Patel et al. 2020). The nitrogen-fixing bacteria are the symbiotic bacterial species which fixes atmospheric nitrogen in the soil and is majorly found associated with legume crops with some exceptions to non-leguminous crops (Mahmud et al. 2020). The mineral solubilizing bacteria are yet another group of bacterial species which aids in mineralization of nutrients that are present in fixed states in the soil (Mukherjee et al. 2019). The mycoparasitic fungi and the biocontrol agents are the species of microbes which are parasitic to pathogenic fungi and microbes, respectively. The mycorrhizal fungi is another group of fungi that lives in a symbiotic association with plants performing the functions of nutrients mineralization and activation of defence genes in plants against phytopathogens (Maharshi et al. 2019).

There are now many modern and efficient methods of estimating the soil microbial population but the classical ones are still more reliable. The classical method of estimation of soil microbial population is serial dilution along with isolation and culturing on different mediums (Lakshmanan et al. 2014). The population is calculated by counting the number of colonies of microbes formed at a specific dilution level of the soil. The only drawback of this method is that we do not get the exact population level as the unculturable microbes are not counted. Once the microbes are in pure culture form, then they can be identified through PCR methods and also evaluated for their potentiality as plant growth promotion microbes, biocontrol agent, and others (Forchetti et al. 2007; Beneduzi et al. 2008; Taulé et al. 2012). The exact estimation of soil microbial population is also dependent on sampling and sequencing techniques. In modern times, the soil microbiome population and diversity are calculated through the high-throughput sequencing methods, DNA/RNA SIP method, and DNA arrays (Mendes et al. 2011; Uhlik et al. 2013; Nkongolo and Narendrula-Kotha 2020). The sequencing methods also provide only a partial coverage as it is estimated that one gram of soil contains up to 1000 Gbps of metagenomic DNA (Frisli et al. 2013). The structure and function of soil microbial population are controlled by different factors which are host-dependent and host-independent. The host-dependent factors include host-plant species, host-plant genotype, host-plant signalling pathways, secretions from host-plants root, etc. The host-independent factors include soil type, temperature, soil pH, moisture, soil porosity, etc. (Lakshmanan et al. 2014). The taxonomic diversity of microbes found in the rhizosphere of different plants is given in Table 19.1.

Table 19.1 Composition of soil microbes in rhizosphere of different plants

Host	Main rhizospheric microbiome	References
Erica andevalensis	Actinobacteria, followed by the acidobacteria, and proteobacteria. Archaea: community was composed of crenarchaeota	Mirete et al. (2007)
Zea mays	Azospirillum, bradyrhizobium, and ideonella	Roesch et al. (2007)
Avena sativa	Proteobacteria, firmicutes, actinobacteria, verrucomicrobia, and nitrospira	De Angelis et al. (2009)
Deschampsia antarctica and *Colobanthus quitensis*	Firmicutes, few acidobacteria Bifidobacterium (phylum actinobacteria), Arcobacter (phylum proteobacteria), and Faecalibacterium (phylum firmicutes)	Teixeira et al. (2010)
Oak	Proteobacteria, acidobacteria, and actinobacteria.	Uroz et al. (2010)
Beta vulgaris	Proteobacteria, firmicutes, and actinobacteria. Gamma- and betaproteobacteria and firmicutes	Mendes et al. (2011)
Solanum tuberosum	Proteobacteria, firmicutes, actinobacteria, bacteroidetes, and acidobacteria. Bacterial families streptomycetaceae, micromonosporaceae, and pseudomonadaceae	Weinert et al. (2011)
Rhizophora mangle and *Laguncularia racemosa*	Halobacteria, methanobacteria, methanomicrobia, and thermoprotei	Pires et al. (2012)
Mammillaria carnea	Acidobacteria, actinobacteria, proteobacteria, and bacteroidetes	Torres-Cortés et al. (2012)
Arabidopsis thaliana	Acidobacteria, proteobacteria, planctomycetes, and actinobacteria	Bulgarelli et al. (2012)
Glycine max	Proteobacteria	Vaishnav et al. (2018)
Vitis vinifera	Proteobacteria, actinobacteria, acidobacteria, bacteroidetes, ascomycota, basidiomycota, and zygomycota	Berlanas et al. (2019)
Glycine max	Proteobacteria, acidobacteria, actinobacteria, bacteroidetes, firmicutes, verrucomicrobia, and planctomycetes	Liu et al. (2019)
Adenium obesum, Aloe dhufarensis, and *Cleome austroarabica*	Actinobacteria, proteobacteria, bacteroidetes, planctomycetes, acidobacteria, verrucomicrobia, ascomycota, basidiomycota, and mucoromycota (only in *A. obesum* and *A. dhufarensis*)	Khan et al. (2020)
Panax ginseng	Proteobacteria, actinobacteria, acidobacteria, bacteroidetes, chloroflexi, firmicutes, gemmatimonadetes, plactomycetes, nitrospirae, latescibacteria, mucoromycota, ascomycota, and basidiomycota	Wei et al. (2020)

19.3 Effects of Soil Microbiome on Plants

As stated earlier, there is a massive count of microbes which colonize plant roots and offer distinct valuable assistance to them. These microbes can work independently or in interaction with each other also. In a recent study, it was shown that the arbuscular-mycorrhizal parasites and the plant growth-promoting rhizobacteria could live as symbionts in a model grassland system and supplement each other for better acquirement of nutrients. Thus, consequently, it was deduced that symbionts possessing diverse functions could supplement the root microbiome which would help in mitigation of nutrients constraint (Van Der Heijden et al. 2015; Vyas et al. 2018). From the evolution of life-forms from amphibian to terrestrial habitat, plants got exposed to a considerable array of microbes constituting of bacteria, fungi, and protists (Kenrick and Crane 1997). This exposition of land plants to microbes led to the establishment of different interactions between them and making up of a flawless soil microbiome which became a trademark characteristic of plants in adapting to a new habitat. Evidences from the fossil remains showed that the plants which were engaged in advantageous interactions with the arbuscular-mycorrhizal fungi and other microbes, were well equipped with improved nutrient uptake from the soil and came into being 400 million years ago (Lambers et al. 2009). The phyletic examinations also support the hypothesis that developmental advancement of land plants in solitary mode happened only 100 million years ago which is later than mutualistic mode as mentioned. This was concluded by studying the ability of angiospermic plants to create a specific positive association with the nitrogen fixers (Werner et al. 2014). The modern researches aided with computational techniques have enabled scientist to declassify microbial diversity and their possible interactions with plants (Lebeis et al. 2012). The researches have shown that plants have dynamic and conglomerated microbial communities consisting of fungal, bacterial species working in a consortium as mutualist, commensals, and parasites (Schlaeppi and Bulgarelli 2015).

Advanced disclosures from the experiments have proved that the immune system of a plant is an outcome of its interactions with the soil microbial community. As, for example, in *Arabidopsis thaliana*, a complete defence system is established by a consortium of non-pathogenic endophytes, and root microbes (Lebeis et al. 2015; Hiruma et al. 2016). This perception is also supported with the revelations that soil microbial partners are also equipped with different machineries which tune the defence system of plants, for example, the T3SS (type III secretion system) in bacterial species (Guttman et al. 2014). The soil microbes which can have beneficial effects on the plants essentially have two kinds of mechanisms. First category is made of the mechanisms which have a direct effect on plants growth and development. It includes protections from phytopathogens (Lugtenberg and Kamilova 2009), improved nutrient acquizition (Pii et al. 2015; Terrazas et al. 2016), and regulation of phytohormones (Glick 2012). The second category is made of the mechanisms which have indirect effects on plants growth and development. These include activation of induced systemic response (ISR), inducing the production of stress-related molecules (Parray et al. 2016; Vaishnav et al. 2014), and all the

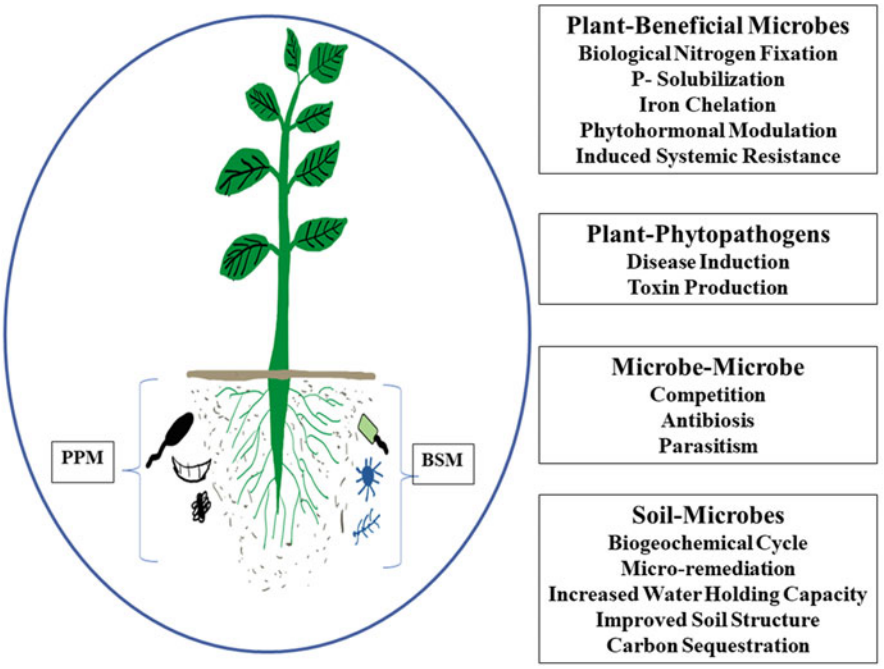

Fig. 19.1 The effects which come out after interactions between plant–soil microbes. *PPM* phytopathogenic microbes, *BSM* beneficial soil microbes

different activities which indirectly shield plants from phytopathogens (Lugtenberg and Kamilova 2009) as given in Fig. 19.1.

1. *Direct effects*: The major mechanisms of soil microbiome which have a direct effect on the plants are improvement in the availability of nutrients, production of phytohormones, and inciting of plant diseases. Nutrients bioavailability in the rhizospheric region is most important for the plants as its development and profitability firmly rely on them. Some of the primary mechanisms involved in minerals bioavailability for plants are nitrogen fixation, phosphorus solubilization, and siderophore production (Pii et al. 2015; Terrazas et al. 2016). The phytopathogenic microbes also have a direct impact on the plant through the production of phytotoxins and incitation of plant diseases. Additionally, the beneficial soil microbes also help plants to survive under different abiotic stresses such as drought conditions through direct mechanisms, i.e. through the production of extracellular polysaccharides (EPS) (Lakshmanan et al. 2017; Naylor and Coleman-Derr 2018). There is also a massive surge in the researches where the soil microbes can be used as inoculants to support plants against the changing environment (Compant et al. 2010). The osmotic stress in plants is also alleviated by rhizospheric microbes through the production of specific metabolites and

inducing aquaporins in plants (Casanovas et al. 2002; Pereyra et al. 2012; Quiroga et al. 2017; Kapilan et al. 2018).

a. *Biological nitrogen fixation (BNF) and nitrate availability*: This is one of the most studied direct mechanisms of beneficial microbes on plants as well as soil. Many of the commercial formulations of microbes are available in the market, which is used for nitrogen fixation in crop plants (Yadav et al. 2019). It is also one of the functions of soil microbes which is applied to a large scale in the agricultural sector. Biological nitrogen fixation is the process in which atmospheric nitrogen (N_2) is converted into nitrate forms which can be taken up by plants, by different microbes with the help of nitrogenase enzyme (Kim and Rees 1994). These microbes are symbiotic nitrogen-fixing bacteria like *Rhizobium* and *Frankia* and also free-living bacteria like *Azotobacter*, *Azospirillum*, *Azoarcus* (Bhattacharyya and Jha 2012; Bhat et al. 2015; Yadav et al. 2019; Vaishnav et al. 2017). Among the two, free-living bacterial species can only provide a limited quantity of atmospheric nitrogen fixation, while the symbiotic ones are more productive (Jones et al. 2007); nevertheless, both are equally important as symbiotic ones can be only used with legumes with a few exceptions (Yadav et al. 2019). Arbuscular-mycorrhizal fungi are also known for making nitrogen available to plants. They utilize the ammonia present in soil and reduce the production of nitrous oxide (Jansson and Hofmockel 2018).

b. *Phosphorus solubilization*: Soil contains an enormous quantity of phosphorus (P) in both organic and inorganic state but, unfortunately, less than 1% is available for uptake by plants (Bhattacharyya and Jha 2012; Alegria Terrazas et al. 2016). It is an essential nutrient for the plants which is required in macro-quantity. The immobilized form of phosphorus in inorganic forms is mineralised by different acids like formic acid, shikimic acid, gluconic acid, and 2-ketogluconic acid produced by different microbes like *Bacillus*, *Aspergillus*, *Trichoderma*, *Pseudomonas*, *Klebsiella*, *Lactobacillus*, and *Enterobacter* (Hinsinger et al. 2009; Sharma et al. 2013; Hunter et al. 2014; Azeem et al. 2015).

c. *Iron chelation*: Iron is one of the essential micronutrients for the plants which is found in two states, viz: Fe^{2+} and Fe^{3+} of which the latter one is the non-available form of iron to plants and microbes (Colombo et al. 2014; Mimmo et al. 2014). Many soil microbes produce a low molecular weight iron-chelating compounds that have a high affinity to Fe^{3+} ions and aid in the absorption of iron across different membranes (Neilands 1981; Guerinot 1994; Lemanceau et al. 2009; Hider and Kong 2010). The chelated Fe^{3+} ions by the microbial siderophores are also taken up by the plants (Crowley et al. 1988; Walter et al. 1994; Jin et al. 2006; Robin et al. 2008).

d. *Phytohormone modulation*: Phytohormones are known to play various key roles in developmental processes of a plant (Taiz and Zeiger 2006; Glick et al. 2007). The activity and movement of phytohormones in plants are dependent on the versatility of the root system which responds according to nutrients availability (Kloepper et al. 2007). Phytohormones like auxins, cytokinins,

and gibberellins are produced by many PGPRs which affect the plant's root system (Vacheron et al. 2013). The cytokinins and gibberellins produced also have a profound effect on the plant growth and development; however, the exact underlying mechanism is still not precise (Glick 2012). Auxins are also produced by different microbes that are discharged to the external environment (Scagliola et al. 2016) which modulate cell division, cell differentiation, development of vascular bundles, and many other processes in plants (Sachdev et al. 2009; Overvoorde et al. 2010). They also lead to an increased root growth in a plant which alternately provides better access to nutrients and water which relives plants from water stress also (Xie et al. 1996; Mayak et al. 1999; Armada et al. 2015; Lakshmanan et al. 2017). Indole acetic acid (IAA) is one of the auxin molecules which is produced by microbes that are known to act as a signalling molecule and induce different gene expressions in plants and microbes itself (Spaepen and Vanderleyden 2011). PGPRs also produce an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase which catalyses ACC, a precursor of ethylene (Arshad et al. 2007) and thus facilitating plant growth through ethylene modulation (Glick 2014). Ethylene is believed as the stress-hormone (Abeles et al. 2012) and thus its modulation leads to induction of defence responses during stress conditions as well as detrimental responses such as chlorosis, senescence, abscission during prolonged stress condition (Glick 2014). The phytohormone modulation also helps plants to overcome drought stress through accumulation of osmolytes and scavenging of reactive oxygen species (ROS) (Lakshmanan et al. 2017; Vurukonda et al. 2016; Vaishnav et al. 2019).

e. *Disease induction*: Contradictory to the beneficial soil microbes, there are certain other species of microbes which are known for inhibition of plant growth and hampering of plant health, thus, are accordingly called as phytopathogenic microbes or phytopathogens. They are a threat to global food security. The soil-borne phytopathogenic microbes are known to survive in the bulk soil, and the rhizospheric region is also an important niche for them where they live as parasites either on the root surface or inside the roots of plants. The exudates from plant roots activate and attract its pathogenic microbes present in soil towards itself (Agrios 2005). The bacterial phytopathogens are known to enter a plant through natural opening and wounds leading to disease development. Some of them colonize in the xylem vessels and caused wilt in plants like *Ralstonia* spp. (Genin and Boucher 2004), while some of them transmit their nucleic acid into the plant's cells and cause irregular growth of cells as in the case of *Agrobacterium tumefaciens* (Nester et al. 2005). Fungal phytopathogens are more advanced and used different mechanisms for penetrating the root cells and inciting diseases. Most of the soil-borne phytopathogenic fungi are necrotrophic but some of them are biotrophic like *Plasmopara* spp. and *Plasmodiophora* spp. (Friskop et al. 2009) They penetrate the root surface by germ tubes and by infecting the epidermal cells. This is achieved either by cell wall degrading

enzymes or by the action of hydrostatic force. Additionally, both bacterial and fungal phytopathogens produce phytotoxins which are harmful to plants.

2. *Indirect effects*: The major mechanisms of soil microbial complex, which alternatively has indirect effects on the plants, are competition, induced systemic resistance, antibiotics production, and production of lytic enzymes. There has been a very strong agreement among the farm scientific community to utilize these mechanisms in mainstream management practices for controlling phytopathogens instead of chemical pesticides.
   a. *Competition*: It is a type of negative interaction between the soil microbes where they compete for the nutrients and space at intra-specific and inter-specific level. Competition leads to the evolution of superior microbial phenotypes which are able to outcompete and remove incompetent ones. The competition is generally higher during the first encounter between the soil microbes and reduces over time due to partitioning of niche or spatial separation or competitive exclusion; thus, they are able to coexist stably (Ghoul and Mitri 2016). During the competition, different microbes compete for niches or nutrients or both. PGPRs are known to compete for the phytopathogens in the rhizospheric region of soil and thus reduce the incidence and severity of diseases. PGPRs also produce siderophores in higher quantities than the phytopathogens. The siderophores solubilize different micronutrients like iron, making them unavailable for the phytopathogens. This ultimately leads to hampered capacity of phytopathogens to multiply and provide an indirect benefit to plants (Schippers et al. 1987; Lugtenberg and Kamilova 2009).
   b. *Induced systemic resistance (ISR)*: Many of the non-pathogen soil microbes are known to activate a defence response system in plants which enables them to protect themselves from phytopathogens by acting sooner (van Loon et al. 1998). ISR activates plant's innate defence barriers, thereby enhancing the defence response. This upgradation of the defence system is not just activated at the site of infection but is systemically throughout the plant by jasmonic acid or ethylene signalling pathways (Verhagen et al. 2004; Jain et al. 2017). The different bacterial products which are known to induce ISR are chitin, flagellar proteins, glucans, and surfactants (Annapurna et al. 2013).
   c. *Antibiotics and lytic enzymes production*: Another mechanism of soil microbes which has an indirect effect on plants is the production of certain metabolite that can inhibit the growth and multiplication of other microbes. The phenomenon is known as antibiosis and the metabolites are known as antibiotics (Waksman 1947; Selwyn 1981). Lytic enzymes are sub-group of antibiotics which have the ability to hydrolyse the peptidoglycan layer of microbial cell wall (Fischetti 2010). Lytic enzymes are also known as lysozymes and are majorly produced by bacteriophages and bacteria (Ohbuchi et al. 2001; Loessner 2005; Lortal and Chapot-Chartier 2005; Salazar and Asenjo 2007; Fischetti 2010; Oliveira et al. 2012). The focus on lysozyme production by the filamentous fungi is given very less attention (da Silva et al. 2014). Cellulases, chitinases, proteases, glucanases, and lipases are different lytic enzymes which are produced by soil microbes which have an adverse effect on

phytopathogenic microbes. Many of the antibiotics such as tensin, phenazine, xanthobaccin, pyrrolnitrin, and zwittermicin A are produced by PGPRs which enable them to function as a biocontrol agent, thus providing indirect benefit to plant (Whipps 2001; Haas and Keel 2003; Compant et al. 2005; Mazurier et al. 2009).

19.4 Effects of Soil Microbiome on Soil

Soil health is a very important point which is always addressed in any of the discussion pertaining to sustainable agriculture. Management of soil is fundamentally essential for agricultural systems, but mining activities, climate change, land degradation, growing industrialization and urbanization, etc. are causing a detrimental effect on soil health and thus threatening the sustainable agriculture. Soil microbiome plays an important role in the restoration of soil health and productivity, as shown in Fig. 19.1. Soil plays as a diverse role in sustenance and functioning of the ecosystem such as providing the base for the biogeochemical cycle of various elements that also enriches its health (Aislabie et al. 2013). There are many beneficial soil microbes that have been identified which are utilized for improvement of soil health in addition to plant growth. However, unfortunately, only less than 10% of such soil microbes have been described yet (Callaway 2016). As the global food demand is going to be doubled by 2050, it is thus necessary to deploy these microbes for increasing the resistance to various stresses caused by biotic factors present in soil (Vorholt et al. 2017; Zavala-Gonzalez et al. 2017). These microbes not only provide help in providing resistance to plant and playing a role in various biogeochemical cycles but also enhances the nutrient uptake in a plant by making it in available form.

The microbes enhance soil health by making the various nutrients in available form, orchestrating various biogeochemical cycle of elements, increasing water holding capacity, improving soil structure, carbon storage, and root growth and also by favouring the growth of various flora (Nannipieri et al. 2017). There are certain beneficial microbes that have an antagonistic effect on various soil-borne pathogens, hence increase the crop productivity by providing protection against pest and disease outbreaks as described earlier (Bonanomi et al. 2018). They enhance the growth of the plant by improving the uptake of various macro- and micronutrients by bringing changes in characteristics of soil through degrading the organic matter, mineralization, solubilization, and weathering of rocks (Van Der Heijden et al. 2008). There is a huge number of soil microbes which are found in plants rhizosphere that can act as symbionts, enhancing the growth and production of plants by complementing each other with limiting nutrients (Vyas et al. 2018). Earth's crust is a huge reservoir of organic matter and various minerals where different biological phenomenon by soil microbial complex regulates the store and release of carbon and various minerals (Amundson et al. 2015). The soil microbiome decides the physical property of soil and hence often regarded as bioindicator for soil health (Liu et al.

2019). Arbuscular-mycorrhizal fungi have the capacity to reduce leaching of plant nutrients from the soil as well as phosphorus scavenging, thereby ultimately embellishing the nutrient-use efficiency of soil (Cavagnaro et al. 2015; Kumar et al. 2015).

The soil microbes also help in restoration of soil health by carrying out bioremediation and biodegradation processes. The process of remediation is divided into three groups on the basis of the biological entity involved, namely phytoremediation (carried out by plants), micro-remediation (carried out by microbes), and rhizomediation (carried out by plants in association with rhizospheric microbes). There are many genera of arbuscular-mycorrhizal fungi whose potential has been described for degradation of soil contaminants and toxic materials which are leftover as residues like *Gigaspora* spp., *Glomus* spp., and *Acaulospora* spp. (Khan et al. 2014). Additionally, these fungi also have a role to play as bio-surfactants for removing metal ion contaminations in soil and bring them down below threshold level (Thavasi et al. 2011). As stated earlier also, soil microbes also perform the process of biodegradation in soil and convert the complex organic materials into their monomeric forms, accompanied by the release of carbon dioxide and water (Ramana and Singh 2000). The process of biodegradation is done by various chemical and physical mechanisms of various soil microbes (von Wirén-Lehr et al. 2002). Many soil-borne fungi have been reported to decompose compounds such as hydrocarbons, nitrilases, nitro-reductases, radionuclides, polyaromatic hydrocarbons, polychlorinated biphenyls, and even the chemical pesticide dichloro-diphenyl-trichloro-ethane (DDT) (Patil et al. 1970; Chaudhry et al. 2005; Glick 2010).

There are a majority of soil-borne beneficial microbes which have been well-studied for their positive effects on soil but their application is only limited to disease management. Since these microbes are native to the soil, they are major drivers of soil organic matter and nutrients apart from providing resistance to pest and diseases in plants (Dubey et al. 2019). As we all are aware that the overdependence of the farming community on various inorganic fertilizers and chemical pesticides has hampered the environment and soil health. So, its high time for moving towards the green and sustainable agriculture for which we must have to explore more and more soil microbes and deploy them for achieving sustainability. The utilization of various modern biotechnological tools and its application on microbes has enormous potential to enhance the quality of soil, environment, and sustainable agriculture (Peng et al. 2016). There are some important microbes like arbuscular-mycorrhizal fungi that contain multinucleated genetic system which cannot be engineered. For such microbes, classical method of isolation, selection, and culture should be applied in order to make them operational for soil amelioration (Muleta 2017; Charubin and Papoutsakis 2019).

19.5 Methods of Soil Microbiome Management

With our increasing knowledge about the role of soil microbiome on plants and soil itself, different management practices have been devised for utilisation of such microbiome for beneficial effects. These management practices of manoeuvring soil microbiome form under two categories, namely: direct and indirect manipulation of soil microbiome by bringing changes in agricultural practices. Some of the methods for management of soil microbiome are explained as below:

1. *Organic farming*: The organic farm management practices lead towards achievement of more diverse and stable soil microbiome which has a beneficial effect on both plants and soil and is strictly advised for barren agricultural lands (Chaparro et al. 2012). This type of farming is based on principles of minimising the off-farm inputs which aids in restoring, maintenance, and enhancement of ecological harmony (Gold 2007). Since the use of chemical fertilisers and synthetic pesticides is not done during cultivation of land and growing of the crop, their harmful effect of soil microbial evenness and diversity reduction is completely alleviated (Liu et al. 2007; Crowder et al. 2010; Sugiyama et al. 2010; Krauss et al. 2011). On the contrary, organic farming promotes the use of microbial diversity for providing nutrients to plants and controlling plants diseases and pests (Sugiyama et al. 2010). Soil microbial complexity and richness are also affected by land use pattern, degree of stress and disturbance which are least in organic farming and thus further adding to the benefits (Degens et al. 2000, 2001).
2. *Beneficial microbes as inoculants*: The beneficial soil microbes which can inhabit the rhizospheric region and help plants in counteracting stresses can be used as seed inoculants or as supplement to standing crop in either solid or liquid formulations (Prabha et al. 2019). Seeds or planting materials are the base material which develops into plants, and therefore the application of beneficial microbes on themselves provides plants with an edge over the upcoming stresses. This is also a straightforward method of managing the soil microbiome as we do not have to physically manipulate it during advanced stages of plants. The classical example for this is the use of *Rhizobium* spp., as a seed treatment in leguminous crops for enhanced nitrogen fixation. In present time in addition to *Rhizobium* spp., various other beneficial soil microbes are available in market in different formulations which can be used as biofertilizers and biopesticides in various crops as seed inoculants (Yadav et al. 2019). Various PGPRs are also available in the form of commercial formulations which alleviate plants from drought stress (Kumar et al. 2017).
3. *Carbon sequestration*: Carbon sequestration is a process of reducing the atmospheric carbon levels by converting it into stable and non-gaseous forms by different abiotic and/or biotic processes. Plants are the dominant biological organism which performs most of the carbon sequestration, but there are also certain soil microbes which are autotrophic and perform the function of carbon assimilation (Jansson and Hofmockel 2019). Carbon compounds which are deposited by plants and other organisms are known to stimulate free-living and

symbiotic soil microbes. Soil microbial community is regulated by the carbon compounds as it passes through different microbes in bioavailable forms and lastly taking the unavailable forms. The carbon sequestration ability of soil is dependent on the soil microbial diversity with quantity and form of carbon alternatively regulating the diversity (Lal 2004). A particular soil microbe or consortium of different ones who carry out the reactions of carbon sequestration can be used for making more stable carbon products (Hicks et al. 2017). In a complementary fashion, soil microbiomes can also be manoeuvred through the addition of amendments which embellishes their capacity to consume and store carbon (Jansson and Hofmockel 2019). Root exudates of plants also affect the dynamics and makeup of soil microbes of which mostly are carbon compounds. Hence, the crop plants can be genetically engineered to produce exudates which can incite the beneficial soil microbes having the ability to trap this carbon exudates (Wallenstein 2017; Jansson et al. 2018) and also leading to more microbial diversity.

4. *Crop cover*: It is an age-old agricultural practice to cover the croplands with different crops known as cover crops during the season or time when they are not cultivated. This practice of covering agricultural land with cover crops has now attained a major role in sustainable agricultural practices (Schipanski et al. 2014; Groff 2015). The main reason behind the use of cover crops was to control the growth of weeds either by the mechanism of competition and/or allelopathy (Weston 1996; Brust et al. 2014; Cordeau et al. 2015). Additionally, they also prevent soil erosion, nutrient loss, and modify different properties of soil (Kuo and Sainju 1998; Hubbard et al. 2013). Since it is known already that the plants are major drivers of soil microbial complex, cover can thus be used for modulating the microbial communities in the soil to derive beneficial effects (Bardgett and van der Putten 2014; Schlatter et al. 2015; Vukicevich et al. 2016; Romdhane et al. 2019). Deployment of cover crops over a more extended period can lead to an enhanced nutrients availability in soil and thereby stimulating diversity and abundance of soil microbes (Schmidt et al. 2018; Castellano-Hinojosa and Strauss 2020). Cover cropping with multiple species of plants constituting of at least two legumes or non-legumes has shown to increase soil microbial diversity along with the abundance of many beneficial rhizospheric bacteria like *Pseudomonas* spp., *Azotobacter* spp., *Azospirillum* spp., and *Bacillus* spp. and beneficial mycorrhizal fungus such as *Gigaspora* spp., *Acaulospora* spp., *Scutellospora* spp., and *Archaeospora* spp. (Hamel et al. 2005; Mazzola and Manici 2012; Wortman et al. 2012; Bever et al. 2015). The outcome of increased soil microbial diversity is obvious due to application of multiple cover crop as there is a positive correlation between plant biodiversity and soil microbial diversity (Garbeva et al. 2004; Maron et al. 2011; Fanin et al. 2014; Civitello et al. 2015). Furthermore, removal of cover crop through herbicide application leads to more loss of soil bacterial diversity than through other means of removal (Moreno et al. 2009), therefore, during the cultivation period the cover crops should be terminated using any other means than herbicides.

19.6 Challenges in Shaping Plant–Soil Microbiome Interactions

From the recent microbiome research, the perception about the diverse microbial community and its impact on soil physical structure has been changed. However, we are just at the starting point of understanding the diverse microbial community and their interactions with plant and soil. With increasing need to develop alternate methods for soil health restoration and plant improvement, scientists are looking at insights to understand the dynamic role of soil microbiome and their interactions with plant and soil (Goodrich et al. 2017). As unique microbiome is present in the rhizosphere of every plant, we have to move towards the personalization of microbiome according to the host for taking the advantages from beneficial ones (Lundberg et al. 2012). Hence, for deployment of the potential soil microbiome, firstly, there is a need to develop different approaches to comprehend the diverse functions of that particular soil microbiome (Bashiardes et al. 2018). As it is an established fact that soil microbiomes are a key determiner for better crop growth and production, there is a challenge of how to apply this knowledge from lab to field (Sergaki et al. 2018) and persuade farmers for use of this technology.

Before defining the shape of plant–soil microbiome under any environment, we have a challenging task to assign a specific function to that particular microbiome group. The diverse lifestyle of the microbiome at genus or even at species level makes the task further tougher. Additionally, their nature keeps changing due to change in their genetic makeup either due to mutation or due to horizontal transfer of particular functional gene(s) (Qiu et al. 2009; Hiruma et al. 2016). This can variably bring drastic changes from the desired phenotype of soil microbiome (Lidbury et al. 2016). Although, with the advancement of various technologies like computational or modelling methods, transition from metagenomics to metamorphic and metaproteomic enables us to comprehend the critical function performed by the certain specific responsible taxa (Prosser 2015; Ofaim et al. 2017). However, there are several limitations to these, as these methods require a sufficiently high starting material, correctly assigned peptides or proteins, and appropriate computational power.

When the task of assigning the function of soil microbiome is over, next challenge forward is to specify the application of these soil microbes with assigned functions to different crops and in different soils for obtaining an interaction which should be useful to agriculturists. In order to overcome this challenge, we have to carry out a considerable number of experiments in order to find the perfect combination of soil microbiome, plant type, and soil type, which should additionally also be feasible for the farmers to adopt. Since we are working with more than soil microbes if we are taking in consideration of the microbiome, then we also have to study the interactions between the different microbes as well. For a successful achievement of soil microbiome which can benefit the plant growth and production, the consortium of microbes should have synergism between themselves and should have antagonism between them and the abiotic or biotic stresses. For achieving such elaborative information, a lot of combinational studies is required which is quite laborious and tiresome. After overcoming all these challenges, there is also a tedious

task of making the microbial in the form of a formulation which should have longer shelf-life, more comfortable to apply, compatible with other sustainable agricultural inputs, and should be of spreading characteristic for higher reach in after soil application. Lastly, the adoption of this technique by the farmers is also an area of concern as mostly they tend to adopt a technique which gives immediate results. As the technique of soil microbiome manipulation is based on the medium of soil and microbial manipulation is a comparatively slow process, showing results over a longer time, there would be a low adoption rate by the farmers. Nevertheless, it can also be overpowered by educating the farmers about the benefits of soil microbiome manipulation in the long run and motivating them for adopting this technology.

19.7 Social and Economic Impacts of Soil Microbiome Management

For genuineness and successful implementation of technology, it is essential it should be socially and economically beneficial for humankind and world. Therefore, it is always important to analyse the social and economic impact of all the scientific technologies which are developed. The soil microbial community provides a consequential amount of economic and social insistence to the society and global economy on an annual basis through their role in the regulation of diverse processes and functions of the ecosystem (Sandhu et al. 2010). The global economic growth was predicted to about 3.5% in 2019 and 3.6% in 2020, which would mean that there is going to be high-income growth and therefore lead to more consumption of food. For meeting the increased consumption and food demand of the growing population, adequate measure for improvement and maintenance of soil health is essential (Lal 2009). Soil is the base for most of the ecosystems globally which provides support for both plants and animals and therefore, the soil and its services are essential too. Considering soil and its services, 90% of the soil processes are carried out by the soil microbiome (Coleman et al. 2004), making them a major player of the global agricultural economy. The ecological services which are provided by the soil microbiome are estimated to be approximately about US $1.5 trillion year^{-1} globally, in economic terms (Pimentel et al. 1997). The biological nitrogen fixation process which is carried out by the soil microbes is solely responsible to generate an economic value of US $50–70 billion year^{-1} globally (Sandhu et al. 2010). Nitrogen fertilizers are the synthetic source which is applied by the farmers to meet the nitrogen demands of plants which comes at a high cost. The world consumption of three primary fertilizer nutrients, namely: nitrogen (N), phosphorus (P), and potassium (K) was estimated to be about 186.67 million tons in 2016 with an annual growth of 1.5%, 2.2%, and 2.4%, respectively, from 2015 to 2020 (FAO 2017). The manipulation of soil microbiome along with application of nitrogen fixers and phosphorus solubilizers could decrease the dependence on fertilizer and also provide economic benefit to the farmers together with sustainable agriculture (Altieri 1999). The microbial inoculant industry would also get a boost and provide a new domain of

employment and entrepreneurship for the youth globally. The nutrient cycling value of soil microbes was calculated to be about US $165.62 ha^{-1} $year^{-1}$ in organic farming system and US $142.0 ha^{-1} $year^{-1}$ (Sandhu et al. 2008, 2010).

Many of human communities have been surviving on the natural resources from centuries. Despite huge enhancement in agricultural production during green revolution, many of the small and marginal farmers were unable to procure the seeds and other agricultural inputs due to monetary issues. To this lack of information and technical capabilities, these farmers were not able to ripe the benefits and suffered from impaired productivity. The microbial inoculants come at a relatively cheaper cost which is affordable for these types of farmers. The manipulation of soil microbial complex is a sustainable approach; there is no need for microbial augmentation after a beneficial microbial complex is established. Majority of the farmers in South Asia, Africa, and many developing countries are poor who do not realize the importance of managing soil constraints. Since there is an inevitable linkage of human livelihood and their social well-being with soil health, the social sustainability of these farmers could be achieved by soil health maintenance (Lal 2009; Sandhu et al. 2010). Use of microbial formulation instead of pesticides will undoubtedly help in the alleviation of ill-effects that are caused by the residues of latter, thus ensuring a safer environment and human health. The increased agricultural production, reduced use of machinery, reduced use of synthetic input, reduction of soil erosion, etc. are some of the major benefits which are obtained from soil microbiome management which will help farmers in both social and economic aspects.

19.8 Conclusion

For succeeding in the long-term, it is essential to shift from conventional practices towards sustainable agriculture in order to maintain the soil health and meet the demands of the growing population. A complete insight about the structure and function of soil microbiome would undoubtedly help in increasing the crop production and productivity simultaneously with restoration of soil health. The rhizospheric region of soil is a hotspot for microbial functionality; therefore, isolation, characterization, and use of beneficial microbes from this region will help in the stimulation of plant growth and also protect them from various abiotic and biotic stresses. The knowledge of interactions between plant, soil, and microbes will undisputedly play an important role in achieving sustainable development goals. The manipulation of soil microbiome is a resilient technology which is here to stay, since microbes are insistent and stubborn to climatic vagaries. Some more advanced researches in this area would enable us to understand the interactive functions of soil microbiome and plants which will be pivotal for the utilisation of specific microbes against specific problems. The manipulation of soil microbiome is the most simple and effective method of planning future ecological functions and therefore is a key for ensuring a sustainable planet.

References

Abeles FB, Morgan PW, Saltveit ME Jr (2012) Ethylene in plant biology. Academic, New York
Agrios GN (2005) Plant pathology, 5th edn. Elsevier, Burlington, pp 79–103
Aislabie J, Deslippe JR, Dymond J (2013) Soil microbes and their contribution to soil services. Ecosystem services in New Zealand–conditions and trends. Manaaki Whenua Press, Lincoln, pp 143–161
Allison SD, Martiny JB (2008) Resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci 105(Suppl 1):11512–11519
Altieri MA (1999) The ecological role of biodiversity in agroecosystems. In: Invertebrate biodiversity as bioindicators of sustainable landscapes. Elsevier, London, pp 19–31
Amundson R, Berhe AA, Hopmans JW, Olson C, Sztein AE, Sparks DL (2015) Soil and human security in the 21st century. Science 348:6235
Annapurna K, Kumar A, Kumar LV, Govindasamy V, Bose P, Ramadoss D (2013) PGPR-induced systemic resistance (ISR) in plant disease management. In: Bacteria in agrobiology: disease management. Springer, Berlin, pp 405–425
Armada E, Azcón R, López-Castillo OM, Calvo-Polanco M, Ruiz-Lozano JM (2015) Autochthonous arbuscular mycorrhizal fungi and Bacillus thuringiensis from a degraded Mediterranean area can be used to improve physiological traits and performance of a plant of agronomic interest under drought conditions. Plant Physiol Biochem 90:64–74
Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol 25(8):356–362
Attwood GT, Wakelin SA, Leahy SC, Rowe S, Clarke SM, Chapman DF, Muirhead, R.,& Jacobs, J. M. (2019) Applications of the soil, plant and rumen microbiomes in pastoral agriculture. Front Nutr 6:107
Azeem M, Riaz A, Chaudhary AN, Hayat R, Hussain Q, Tahir MI, Imran M (2015) Microbial phytase activity and their role in organic P mineralization. Arch Agron Soil Sci 61(6):751–766
Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian, S.,& Smith, D. L. (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473
Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32 (6):666–681
Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266
Bardgett RD, Van Der Putten WH (2014) Belowground biodiversity and ecosystem functioning. Nature 515(7528):505–511
Bar-On YM, Phillips R, Milo R (2018) The biomass distribution on Earth. Proc Natl Acad Sci 115 (25):6506–6511
Bashiardes S, Godneva A, Elinav E, Segal E (2018) Towards utilization of the human genome and microbiome for personalized nutrition. Curr Opin Biotechnol 51:57–63. https://doi.org/10.1016/j.copbio.2017.11.013
Beneduzi A, Peres D, Vargas LK, Bodanese-Zanettini MH, Passaglia LMP (2008) Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil. Appl Soil Ecol 39(3):311–320
Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486
Berlanas C, Berbegal M, Elena G, Laidani M, Cibriain JF, Sagües A, Gramaje D (2019) The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. Front Microbiol 10:1142
Bever JD, Mangan SA, Alexander HM (2015) Maintenance of plant species diversity by pathogens. Annu Rev Ecol 46:305–325

Bhat TA, Ahmad L, Ganai MA, Khan OA (2015) Nitrogen fixing biofertilizers; mechanism and growth promotion: a review. J Pure Appl Microbiol 9(2):1675–1690
Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28(4):1327–1350
Bonanomi G, Lorito M, Vinale F, Woo SL (2018) Organic amendments, beneficial microbes, and soil microbiota: toward a unified framework for disease suppression. Annu Rev Phytopathol 56:1–20
Brust J, Claupein W, Gerhards R (2014) Growth and weed suppression ability of common and new cover crops in Germany. Crop Prot 63:1–8
Bulgarelli D, Rott M, Schlaeppi K, van Themaat EVL, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488(7409):91–95
Calderón K, Spor A, Breuil MC, Bru D, Bizouard F, Violle C, Barnard RL, Philippot L (2017) Effectiveness of ecological rescue for altered soil microbial communities and functions. ISME J 11(1):272–283
Callaway E (2016) Devastating wheat fungus appears in Asia for first time. Nat News 532 (7600):421
Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. Proc Natl Acad Sci 104(12):4990–4995
Casanovas EM, Barassi C, Sueldo RJ (2002) Azospirillum inoculation mitigates water stress effects in maize seedlings. JSTOR 30:343–350
Castellano-Hinojosa A, Strauss SL (2020) Impact of cover crops on the soil microbiome of tree crops. Microorganisms 8(3):328
Cavagnaro TR, Bender SF, Asghari HR, van der Heijden MG (2015) The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends Plant Sci 20(5):283–290
Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48(5):489–499
Charubin K, Papoutsakis ET (2019) Direct cell-to-cell exchange of matter in a synthetic Clostridium syntrophy enables CO_2 fixation, superior metabolite yields, and an expanded metabolic space. Metab Eng 52:9–19
Chaudhry Q, Blom-Zandstra M, Gupta SK, Joner E (2005) Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment (15 pp). Environ Sci Pollut Res 12(1):34–48
Civitello DJ, Cohen J, Fatima H, Halstead NT, Liriano J, McMahon TA, Ortega CN, Sauer EL, Sehgal T, Young S, Rohr JR (2015) Biodiversity inhibits parasites: broad evidence for the dilution effect. Proc Natl Acad Sci 112(28):8667–8671
Coleman DC, Crossley DA Jr, Hendrix PF (2004) Fundamentals of soil ecology, 2nd edn. Elsevier, Burlington
Colombo C, Palumbo G, He JZ, Pinton R, Cesco S (2014) Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. J Soils Sediments 14(3):538–548
Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959
Compant S, Van Der Heijden MG, Sessitsch A (2010) Climate change effects on beneficial plant–microorganism interactions. FEMS Microbiol Ecol 73(2):197–214
Cordeau S, Guillemin JP, Reibel C, Chauvel B (2015) Weed species differ in their ability to emerge in no-till systems that include cover crops. Ann Appl Biol 166(3):444–455
Crowder DW, Northfield TD, Strand MR, Snyder WE (2010) Organic agriculture promotes evenness and natural pest control. Nature 466(7302):109–112
Crowley DE, Reid CP, Szaniszlo PJ (1988) Utilization of microbial siderophores in iron acquisition by oat. Plant Physiol 87(3):680–685
Curtis TP, Sloan WT, Scannell JW (2002) Estimating prokaryotic diversity and its limits. Proc Natl Acad Sci 99(16):10494–10499

da Silva CR, Silva MLC, Kamida HM, Goes-Neto A, Koblitz MGB (2014) Lytic enzyme production optimization using low-cost substrates and its application in the clarification of xanthan gum culture broth. Food Sci Nutr 2(4):299–307

De Angelis KM, Brodie EL, DeSantis TZ, Andersen GL, Lindow SE, Firestone MK (2009) Selective progressive response of soil microbial community to wild oat roots. ISME J 3 (2):168–178

Degens BP, Schipper LA, Sparling GP, Vojvodic-Vukovic M (2000) Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. Soil Biol Biochem 32(2):189–196

Degens BP, Schipper LA, Sparling GP, Duncan LC (2001) Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biol Biochem 33 (9):1143–1153

Dubey A, Malla MA, Khan F, Chowdhary K, Yadav S, Kumar A, Khan ML (2019) Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv 28(8-9):2405–2429

Fanin N, Hättenschwiler S, Fromin N (2014) Litter fingerprint on microbial biomass, activity, and community structure in the underlying soil. Plant Soil 379(1-2):79–91

FAO (2017) World fertilizer trends and outlook to 2020

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15(10):579

Fischetti VA (2010) Bacteriophage endolysins: a novel anti-infective to control gram-positive pathogens. Int J Med Microbiol 300(6):357–362

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G (2007) Endophytic bacteria in sunflower (Helianthus annuus L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 76(5):1145–1152

Friskop A, Markell SG, Gulya T (2009). Downy mildew of sunflower

Frisli T, Haverkamp THA, Jakobsen KS, Stenseth NC, Rudi K (2013) Estimation of metagenome size and structure in an experimental soil microbiota from low coverage next-generation sequence data. J Appl Microbiol 114(1):141–151

Fuhrman JA, Cram JA, Needham DM (2015) Marine microbial community dynamics and their ecological interpretation. Nat Rev Microbiol 13(3):133–146

Garbeva PV, Van Veen JA, Van Elsas JD (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270

Genin S, Boucher C (2004) Lessons learned from the genome analysis of Ralstonia solanacearum. Annu Rev Phytopathol 42:107–134

Ghoul M, Mitri S (2016) The ecology and evolution of microbial competition. Trends Microbiol 24 (10):833–845

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28(3):367–374

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:1–15

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169(1):30–39

Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. In: New perspectives and approaches in plant growth-promoting Rhizobacteria research. Springer, Dordrecht, pp 329–339

Gold MV (2007) Organic production/organic food: information access tools. Organic Production and Organic Food: Information Access Tools

Goodrich JK, Davenport ER, Clark AG, Ley RE (2017) The relationship between the human genome and microbiome comes into view. Annu Rev Genet 51:413–433. https://doi.org/10.1146/annurev-genet-110711-155532

Griffiths BS, Philippot L (2013) Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol Rev 37(2):112–129

Groff S (2015) The past, present, and future of the cover crop industry. J Soil Water Conserv 70 (6):130–133
Guerinot ML (1994) Microbial iron transport. Annu Rev Microbiol 48(1):743–772
Guttman DS, McHardy AC, Schulze-Lefert P (2014) Microbial genome-enabled insights into plant–microorganism interactions. Nat Rev Genet 15(12):797–813
Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing Pseudomonas spp. and relevance for biological control of plant disease. Annu Rev Phytopathol 41(1):117–153
Hamel C, Vujanovic V, Jeannotte R, Nakano-Hylander A, St-Arnaud M (2005) Negative feedback on a perennial crop: Fusarium crown and root rot of asparagus is related to changes in soil microbial community structure. Plant Soil 268(1):75–87
Hicks N, Vik U, Taylor P, Ladoukakis E, Park J, Kolisis F, Jakobsen KS (2017) Using prokaryotes for carbon capture storage. Trends Biotechnol 35:22–32
Hider RC, Kong X (2010) Chemistry and biology of siderophores. Nat Prod Rep 27(5):637–657
Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321(1-2):117–152
Hiruma K, Gerlach N, Sacristán S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramirez D, Bucherr M, O'Connel RJ, Schulze-Lefert P (2016) Root endophyte Colletotrichum tofieldiae confers plant fitness benefits that are phosphate status dependent. Cell 165(2):464–474
Hodgson D, McDonald JL, Hosken DJ (2015) What do you mean, 'resilient'? Trends Ecol Evol 30 (9):503–506
Hubbard RK, Strickland TC, Phatak S (2013) Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. Soil Tillage Res 126:276–283
Hunter PJ, Teakle G, Bending GD (2014) Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular reference to Brassica. Front Plant Sci 5:27
Jain S, Vaishnav A, Kumari S et al (2017) Chitinolytic bacillus-mediated induction of jasmonic acid and defense-related proteins in soybean (Glycine max L. Merrill) plant against Rhizoctonia solani and Fusarium oxysporum. J Plant Growth Regul 36:200–214. https://doi.org/10.1007/s00344-016-9630-1
Jansson JK, Hofmockel KS (2018) The soil microbiome—from metagenomics to metaphenomics. Curr Opin Microbiol 43:162–168
Jansson JK, Hofmockel KS (2019) Soil microbiomes and climate change. Nat Rev Microbiol 2019:1–12
Jansson C, Vogel J, Hazen S, Brutnell T, Mockler T (2018) Climate-smart crops with enhanced photosynthesis. J Exp Bot 69(16):3801–3809
Jiao S, Chen W, Wei G (2019) Resilience and assemblage of soil microbiome in response to chemical contamination combined with plant growth. Appl Environ Microbiol 85(6):e02523
Jin CW, He YF, Tang CX, Wu P, Zheng SJ (2006) Mechanisms of microbially enhanced Fe acquisition in red clover (Trifolium pratense L.). Plant Cell Environ 29(5):888–897
Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC (2007) How rhizobial symbionts invade plants: the Sinorhizobium–Medicago model. Nat Rev Microbiol 5(8):619–633
Kaestli M, Schmid M, Mayo M, Rothballer M, Harrington G, Richardson L, Hill A, Hill J, Tuanyok A, Keim P, Hartmann A (2012) Out of the ground: aerial and exotic habitats of the melioidosis bacterium *Burkholderia pseudomallei* in grasses in Australia. Environ Microbiol 14(8):2058–2070
Kamal S, Prasad R, Varma A (2010) Soil microbial diversity in relation to heavy metals. In: Soil heavy metals. Springer, Berlin, pp 31–63
Kapilan R, Vaziri M, Zwiazek JJ (2018) Regulation of aquaporins in plants under stress. Biol Res 51:4
Kenrick P, Crane PR (1997) The origin and early evolution of plants on land. Nature 389 (6646):33–39

Khan A, Sharif M, Ali A, Shah SNM, Mian IA, Wahid F, Jan B, Adnan M, Ali N (2014) Potential of AM fungi in phytoremediation of heavy metals and effect on yield of wheat crop. Am J Plant Sci 5:1578–1586

Khan AL, Asaf S, Abed RM, Ning Chai Y, Al-Rawahi AN, Mohanta TK, Al-Rawahi A, Schachtman DP, Al-Harrasi A (2020) Rhizosphere microbiome of arid land medicinal plants and extra cellular enzymes contribute to their abundance. Microorganisms 8(2):213

Kim J, Rees DC (1994) Nitrogenase and biological nitrogen fixation. Biochemistry 33(2):389–397

Kingston-Smith AH, Davies TE, Edwards JE, Theodorou MK (2008) From plants to animals; the role of plant cell death in ruminant herbivores. J Exp Bot 59(3):521–532

Kloepper JW, Gutierrez-Estrada A, McInroy JA (2007) Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria. Can J Microbiol 53(2):159–167

Köhl L, Oehl F, van der Heijden MG (2014) Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Ecol Appl 24(7):1842–1853

Krauss J, Gallenberger I, Steffan-Dewenter I (2011) Decreased functional diversity and biological pest control in conventional compared to organic crop fields. PLoS One 6(5):e0019502

Kumar A, Sharma S, Mishra S, Dames JF (2015) Arbuscular mycorrhizal inoculation improves growth and antioxidative response of Jatropha curcas (L.) under Na_2SO_4 salt stress. Plant Biosyst 149(2):260–269

Kumar G, Patel JS, Maharshi A, Mukherjee A, Keswani C, Singh SP, Singh HB, Sarma BK (2017) PGPR-mediated defence responses in plants under biotic and abiotic stresses. Adv PGPR Res 2017:427

Kuo S, Sainju UM (1998) Nitrogen mineralization and availability of mixed leguminous and non-leguminous cover crop residues in soil. Biol Fertil Soils 26(4):346–353

Lakshmanan V, Selvaraj G, Bais HP (2014) Functional soil microbiome: belowground solutions to an aboveground problem. Plant Physiol 166(2):689–700

Lakshmanan V, Ray P, Craven KD (2017) Toward a resilient, functional microbiome: drought tolerance-alleviating microbes for sustainable agriculture. In: Plant stress tolerance. Humana Press, New York, pp 69–84

Lal R (2004) Soil carbon sequestration impacts on global climate change and food security. Science 304(5677):1623–1627

Lal R (2009) Soils and food sufficiency: a review. In: Sustainable agriculture. Springer, Dordrecht, pp 25–49

Lambers H, Mougel C, Jaillard B, Hinsinger P (2009) Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. Plant Soil 321(1-2):83–115

Lebeis SL (2015) Greater than the sum of their parts: characterizing plant microbiomes at the community-level. Curr Opin Plant Biol 24:82–86

Lebeis SL, Rott M, Dangl JL, Schulze-Lefert P (2012) Culturing a plant microbiome community at the cross-Rhodes. New Phytol 196(2):341–344

Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Dangl JL (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science 349(6250):860–864

Lemanceau P, Expert D, Gaymard F, Bakker PA, Briat JF (2009) Role of iron in plant–microbe interactions. Adv Bot Res 51:491–549

Lidbury ID, Murphy AR, Scanlan DJ, Bending GD, Jones AM, Moore JD, Goodall A, Hammond, J. P.,& Wellington, E. M. (2016) Comparative genomic, proteomic and exoproteomic analyses of three Pseudomonas strains reveals novel insights into the phosphorus scavenging capabilities of soil bacteria. Environ Microbiol 18(10):3535–3549

Liu B, Tu C, Hu S, Gumpertz M, Ristaino JB (2007) Effect of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight. Appl Soil Ecol 37(3):202–214

Liu F, Hewezi T, Lebeis SL, Pantalone V, Grewal PS, Staton ME (2019) Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. BMC Microbiol 19(1):1–19

Loessner MJ (2005) Bacteriophage endolysins—current state of research and applications. Curr Opin Microbiol 8(4):480–487

Lortal S, Chapot-Chartier MP (2005) Role, mechanisms and control of lactic acid bacteria lysis in cheese. Int Dairy J 15(6-9):857–871

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Del Rio TG, Edgar RC (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488(7409):86–90

Maharshi A, Kumar G, Mukherjee A, Raghuwanshi R, Singh HB, Sarma BK (2019) Arbuscular mycorrhizal colonization and activation of plant defense responses against phytopathogens. In: Microbial interventions in agriculture and environment. Springer, Singapore, pp 219–240

Mahmud K, Makaju S, Ibrahim R, Missaoui A (2020) Current progress in nitrogen fixing plants and microbiome research. Plants 9(1):97

Maier MR, Pepper LI, Gerba PC (2009) Environmental microbiology, 2nd edn. Elsevier, London

Maron JL, Marler M, Klironomos JN, Cleveland CC (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. Ecol Lett 14(1):36–41

Mayak S, Tirosh T, Glick BR (1999) Effect of wild-type and mutant plant growth-promoting rhizobacteria on the rooting of mung bean cuttings. J Plant Growth Regul 18(2):49–53

Mazurier S, Corberand T, Lemanceau P, Raaijmakers JM (2009) Phenazine antibiotics produced by fluorescent pseudomonads contribute to natural soil suppressiveness to Fusarium wilt. ISME J 3 (8):977–991

Mazzola M, Manici LM (2012) Apple replant disease: role of microbial ecology in cause and control. Annu Rev Phytopathol 50:45–65

Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332(6033):1097–1100

Mimmo T, Del Buono D, Terzano R, Tomasi N, Vigani G, Crecchio C, Pinton R, Cesco S (2014) Rhizospheric organic compounds in the soil–microorganism–plant system: their role in iron availability. Eur J Soil Sci 65(5):629–642

Mirete S, De Figueras CG, González-Pastor JE (2007) Novel nickel resistance genes from the rhizosphere metagenome of plants adapted to acid mine drainage. Appl Environ Microbiol 73 (19):6001–6011

Moe LA (2013) Amino acids in the rhizosphere: from plants to microbes. Am J Bot 100 (9):1692–1705

Moreno B, Garcia-Rodriguez S, Cañizares R, Castro J, Benítez E (2009) Rainfed olive farming in south-eastern Spain: long-term effect of soil management on biological indicators of soil quality. Agric Ecosyst Environ 131(3-4):333–339

Mukherjee A, Gaurav AK, Singh S, Chouhan GK, Kumar A, Das S (2019) Role of potassium (K) solubilising microbes (KSM) in growth and induction of resistance against biotic and abiotic stress in plant: a book review. Clim Change Environ Sustainability 7(2):212–214

Mukherjee A, Singh B, Verma JP (2020) Harnessing chickpea (Cicer arietinum L.) seed endophytes for enhancing plant growth attributes and bio-controlling against Fusarium sp. Microbiol Res 2020:126469

Muleta D (2017) Legume response to arbuscular mycorrhizal fungi inoculation in sustainable agriculture. In: Microbes for legume improvement. Springer, Cham, pp 227–260

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2017) Microbial diversity and soil functions. Eur J Soil Sci 68(1):12–26

Naylor D, Coleman-Derr D (2018) Drought stress and root-associated bacterial communities. Front Plant Sci 8:2223

Neilands JB (1981) Iron absorption and transport in microorganisms. Annu Rev Nutr 1(1):27–46
Nester E, Gordon MP, Kerr A (2005) Agrobacterium tumefaciens: from plant pathology to biotechnology. American Phytopathological Society (APS Press)
Nkongolo KK, Narendrula-Kotha R (2020) Advances in monitoring soil microbial community dynamic and function. J Appl Genet 2020:1–15
Ofaim S, Ofek-Lalzar M, Sela N, Jinag J, Kashi Y, Minz D, Freilich S (2017) Analysis of microbial functions in the rhizosphere using a metabolic-network based framework for metagenomics interpretation. Front Microbiol 8:1606
Ohbuchi K, Hasegawa K, Hamachi M, Ozeki K, Kumagai C (2001) Isolation of a new lytic enzyme for hiochi bacteria and other lactic acid bacteria. J Biosci Bioeng 91(5):487–492
Oliveira H, Azeredo J, Lavigne R, Kluskens LD (2012) Bacteriophage endolysins as a response to emerging foodborne pathogens. Trends Food Sci Technol 28(2):103–115
Overvoorde P, Fukaki H, Beeckman T (2010) Auxin control of root development. Cold Spring Harb Perspect Biol 2(6):a001537
Parray JA, Jan S, Kamili AN, Qadri RA, Egamberdieva D, Ahmad P (2016) Current perspectives on plant growth-promoting rhizobacteria. J Plant Growth Regul 35(3):877–902
Patel J, Teli B, Bajpai R, Meher J, Rashid M, Mukherjee A, Yadav SK (2019) Trichoderma-mediated biocontrol and growth promotion in plants: an endophytic approach. In: Role of plant growth promoting microorganisms in sustainable agriculture and nanotechnology. Woodhead Publishing, Cambridge, pp 219–239
Patel JS, Yadav SK, Bajpai R, Teli B, Rashid M (2020) PGPR secondary metabolites: an active syrup for improvement of plant health. In: Molecular aspects of plant beneficial microbes in agriculture. Academic, New York, pp 195–208
Patil KC, Matsumura F, Boush GM (1970) Degradation of endrin, aldrin, and DDT by soil microorganisms. Appl Environ Microbiol 19(5):879–881
Peay KG, Kennedy PG, Talbot JM (2016) Dimensions of biodiversity in the earth mycobiome. Nat Rev Microbiol 14(7):434–447
Peng J, Ji W, Ma Z, Li S, Chen S, Zhou L, Shi Z (2016) Predicting total dissolved salts and soluble ion concentrations in agricultural soils using portable visible near-infrared and mid-infrared spectrometers. Biosyst Eng 152:94–103
Pereyra MA, García P, Colabelli MN, Barassi CA, Creus CM (2012) A better water status in wheat seedlings induced by Azospirillum under osmotic stress is related to morphological changes in xylem vessels of the coleoptile. Appl Soil Ecol 53:94–97
Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11(11):789–799
Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C (2015) Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol Fertil Soils 51(4):403–415
Pimentel D, Wilson C, McCullum C, Huang R, Dwen P, Flack J, Tran Q, Saltman, T.,& Cliff, B. (1997) Economic and environmental benefits of biodiversity. Bioscience 47(11):747–757
Pires AC, Cleary DF, Almeida A, Cunha Â, Dealtry S, Mendonça-Hagler LC, Smalla K, Gomes NC (2012) Denaturing gradient gel electrophoresis and barcoded pyrosequencing reveal unprecedented archaeal diversity in mangrove sediment and rhizosphere samples. Appl Environ Microbiol 78(16):5520–5528
Prabha R, Singh DP, Yadav SK (2019) Seed biopriming with potential microbial inoculants as sustainable options for stress management in crops. In: Microbial interventions in agriculture and environment. Springer, Singapore, pp 211–224
Prosser JI (2015) Dispersing misconceptions and identifying opportunities for the use of 'omics' in soil microbial ecology. Nat Rev Microbiol 13(7):439–446
Qiu X, Kulasekara BR, Lory S (2009) Role of horizontal gene transfer in the evolution of Pseudomonas aeruginosa virulence. In: Microbial pathogenomics, vol 6. Karger Publishers, Basel, pp 126–139

Quiroga G, Erice G, Aroca R, Chaumont F, Ruiz-Lozano JM (2017) Enhanced drought stress tolerance by the arbuscular mycorrhizal symbiosis in a drought-sensitive maize cultivar is related to a broader and differential regulation of host plant aquaporins than in a drought-tolerant cultivar. Front Plant Sci 8:1056

Ramana KV, Singh L (2000) Microbial degradation of organic wastes at low temperatures. Def Sci J 50(4):371

Robin A, Vansuyt G, Hinsinger P, Meyer JM, Briat JF, Lemanceau P (2008) Iron dynamics in the rhizosphere: consequences for plant health and nutrition. Adv Agron 99:183–225

Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AK, Kent AD, Daroub SH, Camargo FAO, Farmerie WG, Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 1(4):283–290

Romdhane S, Spor A, Busset H, Falchetto L, Martin J, Bizouard F, Bru D, Breuil MC, Philippot, L., & Cordeau, S. (2019) Cover crop management practices rather than composition of cover crop mixtures affect bacterial communities in no-till agroecosystems. Front Microbiol 10:1618

Roper MM, Gupta VVSR (1995) Management-practices and soil biota. Soil Res 33(2):321–339

Rout ME, Southworth D (2013) The root microbiome influences scales from molecules to ecosystems: the unseen majority. Am J Bot 100(9):1689–1691

Sachdev DP, Chaudhari HG, Kasture VM, Dhavale DD, Chopade BA (2009) Isolation and characterization of indole acetic acid (IAA) producing Klebsiella pneumoniae strains from rhizosphere of wheat (Triticum aestivum) and their effect on plant growth. Indian J Exp Biol 47(12):993–1000

Salazar O, Asenjo JA (2007) Enzymatic lysis of microbial cells. Biotechnol Lett 29(7):985–994

Sandhu HS, Wratten SD, Cullen R, Case B (2008) The future of farming: the value of ecosystem services in conventional and organic arable land. An experimental approach. Ecol Econ 64 (4):835–848

Sandhu HS, Gupta VV, Wratten SD (2010) Evaluating the economic and social impact of soil microbes. In: Soil microbiology and sustainable crop production. Springer, Dordrecht, pp 399–417

Scagliola M, Pii Y, Mimmo T, Cesco S, Ricciuti P, Crecchio C (2016) Characterization of plant growth promoting traits of bacterial isolates from the rhizosphere of barley (Hordeum vulgare L.) and tomato (Solanum lycopersicon L.) grown under Fe sufficiency and deficiency. Plant Physiol Biochem 107:187–196

Schipanski ME, Barbercheck M, Douglas MR, Finney DM, Haider K, Kaye JP, White C (2014) A framework for evaluating ecosystem services provided by cover crops in agroecosystems. Agric Syst 125:12–22

Schippers B, Bakker AW, Bakker PA (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annu Rev Phytopathol 25(1):339–358

Schlaeppi K, Bulgarelli D (2015) The plant microbiome at work. Mol Plant-Microbe Interact 28 (3):212–217

Schlatter DC, Bakker MG, Bradeen JM, Kinkel LL (2015) Plant community richness and microbial interactions structure bacterial communities in soil. Ecology 96(1):134–142

Schloss PD, Handelsman J (2006) Toward a census of bacteria in soil. PLoS Comput Biol 2:7

Schmidt R, Gravuer K, Bossange AV, Mitchell J, Scow K (2018) Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLoS One 13(2): e0192953

Selwyn S (1981) Microbial interactions and antibiosis. In: Skin microbiology. Springer, New York, pp 63–74

Sergaki C, Lagunas B, Lidbury I, Gifford ML, Schäfer P (2018) Challenges and approaches in microbiome research: from fundamental to applied. Front Plant Sci 9:1205

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2(1):587

Shi S, Richardson AE, O'Callaghan M, DeAngelis KM, Jones EE, Stewart A, Firestone MK, Condron LM (2011) Effects of selected root exudate components on soil bacterial communities. FEMS Microbiol Ecol 77(3):600–610

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect 3(4):a001438

Spence C, Alff E, Johnson C, Ramos C, Donofrio N, Sundaresan V, Bais H (2014) Natural rice rhizospheric microbes suppress rice blast infections. BMC Plant Biol 14(1):130

Sugiyama A, Vivanco JM, Jayanty SS, Manter DK (2010) Pyrosequencing assessment of soil microbial communities in organic and conventional potato farms. Plant Dis 94(11):1329–1335

Taiz L, Zeiger E (2006) Plant physiology, 4th edn. Sinauer Associates, Sunderland

Taulé C, Mareque C, Barlocco C, Hackembruch F, Reis VM, Sicardi M, Battistoni F (2012) The contribution of nitrogen fixation to sugarcane (Saccharum officinarum L.), and the identification and characterization of part of the associated diazotrophic bacterial community. Plant Soil 356 (1-2):35–49

Teixeira LC, Peixoto RS, Cury JC, Sul WJ, Pellizari VH, Tiedje J, Rosado AS (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. ISME J 4(8):989–1001

Teplitski M, Warriner K, Bartz J, Schneider KR (2011) Untangling metabolic and communication networks: interactions of enterics with phytobacteria and their implications in produce safety. Trends Microbiol 19(3):121–127

Terrazas RA, Giles C, Paterson E, Robertson-Albertyn S, Cesco S, Mimmo T, Pii Y, Bulgarelli D (2016) Plant–microbiota interactions as a driver of the mineral turnover in the rhizosphere. In: Advances in applied microbiology, vol 95. Academic, New York, pp 1–67

Thavasi R, Jayalakshmi S, Banat IM (2011) Application of biosurfactant produced from peanut oil cake by Lactobacillus delbrueckii in biodegradation of crude oil. Bioresour Technol 102 (3):3366–3372

Torres-Cortés G, Millán V, Fernández-González AJ, Aguirre-Garrido JF, Ramírez-Saad HC, Fernández-López M, Martínez-Abarca F (2012) Bacterial community in the rhizosphere of the cactus species Mammillaria carnea during dry and rainy seasons assessed by deep sequencing. Plant Soil 357(1-2):275–288

Torsvik V, Øvreås L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5(3):240–245

Turner TR, James EK, Poole PS (2013) The plant microbiome. Genome Biol 14(6):209

Uhlik O, Leewis MC, Strejcek M, Musilova L, Mackova M, Leigh MB, Macek T (2013) Stable isotope probing in the metagenomics era: a bridge towards improved bioremediation. Biotechnol Adv 31(2):154–165

Uroz S, Buée M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2 (2):281–288

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dye, F.,& Prigent-Combaret, C. (2013) Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci 4:356

Vaishnav A, Jain S, Kasotia A, Kumari S, Gaur RK, Choudhary DK (2014) Molecular mechanism of benign microbe-elicited alleviation of biotic and abiotic stresses for plants. In: Gaur R, Sharma P (eds) Approaches to plant stress and their management. Springer, New Delhi. https://doi.org/10.1007/978-81-322-1620-9-16

Vaishnav A, Hansen AP, Agrawal PK, Varma A, Choudhary DK (2017) Biotechnological perspectives of legume–rhizobium symbiosis. In: Hansen A, Choudhary D, Agrawal P, Varma A (eds) Rhizobium biology and biotechnology. Soil biology, vol 50. Springer, Cham. https://doi.org/10.1007/978-3-319-64982-5_12

Vaishnav A, Kasotia A, Choudhary DK (2018) Role of functional bacterial phylum proteobacteria in *Glycine max* growth promotion under abiotic stress: a glimpse on case study. In:

Choudhary D, Kumar M, Prasad R, Kumar V (eds) In silico approach for sustainable agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-13-0347-0_2
Vaishnav A, Shukla AK, Sharma A et al (2019) Endophytic bacteria in plant salt stress tolerance: current and future prospects. J Plant Growth Regul 38:650–668
Van Der Heijden MG, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11(3):296–310
Van der Heijden MGA, de Bruin S, Luckerhoff L et al (2015) A widespread plant–fungal–bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. ISME J 10:389–399
Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic induced resistance by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483
Verhagen BW, Glazebrook J, Zhu T, Chang HS, Van Loon LC, Pieterse CM (2004) The transcriptome of rhizobacteria-induced systemic resistance in Arabidopsis. Mol Plant-Microbe Interact 17(8):895–908
von Wirén-Lehr S, Scheunert I, Dörfler U (2002) Mineralization of plant-incorporated residues of 14C-isoproturon in arable soils originating from different farming systems. Geoderma 105 (3-4):351–366
Vorholt JA, Vogel C, Carlström CI, Müller DB (2017) Establishing causality: opportunities of synthetic communities for plant microbiome research. Cell Host Microbe 22(2):142–155
Vukicevich E, Lowery T, Bowen P, Úrbez-Torres JR, Hart M (2016) Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agron Sustain Dev 36(3):48
Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A (2016) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184:13–24
Vyas P, Kumar D, Dubey A, Kumar A (2018) Screening and characterization of Achromobacter xylosoxidans isolated from rhizosphere of Jatropha curcas L.(energy crop) for plant-growth-promoting traits. J Adv Res Biotechnol 3(1):1–8
Waksman SA (1947) What is an antibiotic or an antibiotic substance? Mycologia 39(5):565–569
Wallenstein MD (2017) Managing and manipulating the rhizosphere microbiome for plant health: a systems approach. Rhizosphere 3:230–232
Walter A, Römheld V, Marschner H, Crowley DE (1994) Iron nutrition of cucumber and maize: effect of Pseudomonas putida YC 3 and its siderophore. Soil Biol Biochem 26(8):1023–1031
Wang Q, Ma M, Jiang X, Zhou B, Guan D, Cao F, Chen S, Li J (2019) Long-term N fertilization altered 13C-labeled fungal community composition but not diversity in wheat rhizosphere of Chinese black soil. Soil Biol Biochem 135:117–126
Wei X, Wang X, Cao P, Gao Z, Chen AJ, Han J (2020) Microbial community changes in the rhizosphere soil of healthy and rusty Panax ginseng and discovery of pivotal fungal genera associated with rusty roots. Biomed Res Int 2020:8018525
Weinert N, Piceno Y, Ding GC, Meincke R, Heuer H, Berg G, Schloter M, Andersen, G.,& Smalla, K. (2011) PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. FEMS Microbiol Ecol 75(3):497–506
Werner GD, Cornwell WK, Sprent JI, Kattge J, Kiers ET (2014) A single evolutionary innovation drives the deep evolution of symbiotic N 2-fixation in angiosperms. Nat Commun 5(1):1–9
Weston LA (1996) Utilization of allelopathy for weed management in agroecosystems. Agron J 88 (6):860–866
Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52 (suppl_1):487–511
Wilpiszeski RL, Aufrecht JA, Retterer ST, Sullivan MB, Graham DE, Pierce EM, Elias DA (2019) Soil aggregate microbial communities: towards understanding microbiome interactions at biologically relevant scales. Appl Environ Microbiol 85(14):e00324–e00319

Wortman SE, Francis CA, Bernards ML, Drijber RA, Lindquist JL (2012) Optimizing cover crop benefits with diverse mixtures and an alternative termination method. Agron J 104 (5):1425–1435

Xie H, Pasternak JJ, Glick BR (1996) Isolation and characterization of mutants of the plant growth-promoting rhizobacterium Pseudomonas putida GR12-2 that overproduce indoleacetic acid. Curr Microbiol 32(2):67–71

Yadav SK, Prabha R, Singh V, Bajpai R, Teli B, Rashid MM, Sarma BK, Singh DP (2019) Microbes-mediated nutrient use efficiency in pulse crops. In: Microbial interventions in agriculture and environment. Springer, Singapore, pp 447–460

Zavala-Gonzalez EA, Rodríguez-Cazorla E, Escudero N, Aranda-Martinez A, Martínez-Laborda A, Ramírez-Lepe M, Lopez-Llorca LV (2017) Arabidopsis thaliana root colonization by the nematophagous fungus Pochonia chlamydosporia is modulated by jasmonate signaling and leads to accelerated flowering and improved yield. New Phytol 213(1):351–364

Exploration of Rhizospheric Microbial Diversity of the Indian Sundarbans: A World Heritage Site

20

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Abstract

The mangrove cover of the Indian Sundarbans has reduced drastically by over 40% from the year 1776 to 2020. This has led various true mangrove species such as *Sonneratia griffithii* Kurz to become critically endangered, *Heritiera fomes* Buch. Ham. to be classified as endangered and *Ceriops decandra* Ding Hou, *Aegialitis rotundifolia* Roxb. and *Phoenix paludosa* Roxb. to be classified as near threatened. The factors affecting vegetation are both ecological and anthropogenic. From the ecological point of view, the tilting of the Bengal basin tectonically has resulted in the accumulation of excess saltwater in the Indian Sundarbans while most of the freshwater is being received in the Bangladesh side of the mangrove belt. As a result major stenohaline species are depleting. From the anthropogenic standpoint, forests are not only being cleared for dwelling but also for agricultural land use. Replantation programs over the years have not been sustainable as the nature of natural succession has not been maintained while planting of the seeds and seedlings in the tidal inundation zones, and as a result even the pioneering species have not been able to survive. We have identified core microbiomes from rhizospheric assemblages of several mangrove plants of which data from few ferns and two true mangroves are discussed here.

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Results of our analysis indicate that core microbiomes are plant specific and do not depend on sites of collection. Even core microbiomes are discernible for environmental soil niches as well. These data suggest that future replantation schemes require the microbiome niche to be maintained if successful restoration is to be achieved either in the form of suitable site-specific plantations or microbial consortium-based supplementations.

Keywords

Mangrove · Indian Sundarbans · Core microbiome · Consortium · Restoration

20.1 Introduction

The Sundarbans are the world's largest mangrove forest belt formed in the Ganga-Brahmaputra Delta on the Indian side and the Meghna Delta in Bangladesh. Closed and open mangrove forests interspersed with habitats and agricultural lands along with mudflats are the essential features of the Indian Sundarbans. The Sundarbans has four protected areas which have been recognized as UNESCO World Heritage Site, namely, Sundarbans National Park, Sundarbans East Wildlife Sanctuaries, Sundarbans South and Sundarbans West (Giri et al. 2007). The total area covered by the Sundarbans mangrove forest is about 10,000 km^2 (3900 sq. mi). Bangladesh's Sundarbans part extends over Khulna Division over a stretch of 6017 km^2 (2323 sq. mi). The Indian Sundarbans cover around 4260 km^2 (1640 sq. mi) spanning over districts of the North 24 Parganas and South 24 Parganas of West Bengal (Pani et al. 2013). The zone possesses sundari (*Heritiera fomes*) and gewa (*Excoecaria agallocha*) as the most abundant tree species. The forests is habitat to a total of 453 macro- and micro-wildlife fauna, which includes 290 avian, 120 piscian, 42 mammalian, 35 reptilian and 8 amphibian species altogether (Iftekhar and Islam 2004). It was designated as a UNESCO world heritage site in the year 1987. Water bodies such as river, canals and creeks occupy about 1700 km^2 (660 sq. mi). The creeks may vary from a few metres to several kilometres in their width). The landscape of the Sundarbans is a complex network of tidal creeks, mudflats and small islands which harbour the salt-tolerant mangrove forests. This is why it requires an expert navigator to reach the corners of the delta.

The Sundarbans is a rete mirabile of tidal waterways, small forested islands and mudflats, with varying degrees of salt tolerance. The delta is highly rich in fertile soil, and its intensive use by humans can be traced back to a few centuries. As a result of the intensive agricultural practice, the ecoregion now has few forest patches remaining. These remaining forests, along with the Sundarbans mangroves, are the important habitat for world's one of the most charismatic megafauna—the endangered royal Bengal tiger. To add to it, the Sundarbans serves a vital function as a natural protective barrier around Khulna and Mongla for the millions of

inhabitants against the floods that result from the cyclones. The Sundarbans mangrove is the world's largest mangrove and a Ramsar site (designated on February1, 2019). The seaward fringe of the delta is formed by the ecoregion (IM 1406) on the coast and with total geographical area coverage of 20,400 km^2 (7900 sq. mi) has been inscribed as UNESCO World Heritage Site under category (ix) and (x) in the year 1987. The ecoregion has its name coined from the local name of the dominant mangrove species *Heritiera fomes* (Bengali: sundri or sundari).

Mangrove forests do not exhibit a great floral variety. Prain (1903) recorded a total 245 genera and 334 plant species. These forests have a thick canopy, and the undergrowth comprises mostly of mangrove seedlings. Besides the sundari, other tree species in the forest include *Nypa fruticans*, *Bruguiera gymnorhiza*, *Avicennia*, *Xylocarpus granatum*, *Sonneratia apetala*, *Xylocarpus mekongensis*, *Aegiceras corniculatum*, *Ceriops decandra* and *Rhizophora mucronata*. Of the world's 50 broad mangrove species, 26 show better relative abundance in the Sundarbans. The commonly identifiable vegetation types in the dense Sundarbans mangrove forests are mangrove scrub, brackish water mixed forest, saltwater mixed forest,, littoral forest, wet alluvial grass forests and wet forest. The mangroves of Sundarbans are dominated by the Malvaceae and Euphorbiaceae, whereas most of the mangroves around the world are characterized by members of the Rhizophoraceae, Avicenneaceae or Combretaceae (Chaudhuri et al. 1994). The Sundarbans flora exhibits prominent abundance of sundari (*Heritiera fomes*), keora (*Sonneratia apetala*), gewa (*Excoecaria agallocha*) and goran (*Ceriops decandra*) of which most exhibit profuse growth. Sundari (*Heritiera* sp.) is the characteristic tree of the forest, from which the name of the forest had probably been derived. The hard wood yielded by the tree is used for building houses and making boats, furniture and other goods. Newly formed forest regions and tidal forests often show floral community domination by keora (*Sonneratia apetala*). Thus it may be a bio-indicator species for newly formed mudbanks and is vital to various species of wildlife, especially herbivores such as the spotted deer (*Axis axis*). Kankra (*Bruguiera gymnorhiza*) and dhundul or passur (*Xylocarpus granatum*) are abundant but with discontinuous distribution. Among palms, golpata (*Nypa fruticans*), and grasses such as *Porteresia coarctata* and khagra (*Phragmites karka*) and spear grass (*Imperata cylindrica*) are distributed well.

A number of factors contribute towards the physical development processes along the coast. These are micro- and macro-tidal cycles, wave motions and long shore currents and are characteristics of a coastal ecosystem. The mangrove vegetation is a remarkable equilibration agent to the variations in these climactic factors and is also responsible for acting as a barrier to cyclonic storms.

The Zoological Society of London in a study conducted in 2012 recorded a loss of coastline by up to 220 m in a year. From their data it was also clear that extensive deforestation [17,179 ha of mangroves within three decades (1975–2010)] has been carried out due to anthropogenic encroachment for agriculture and habitat establishment. Shrimp cultivation is attributed to have destroyed another 7554 ha (18,670 acres). The annual rise in sea level has been estimated to be 8 mm (0.31 in) in 2010, as per reports of research from the School of Oceanographic Studies, Jadavpur

University. It has increased twofold from 3.14 mm (0.124 in) as per records reported in 2000. As a result of the rising sea levels, habitat loss and erosion of the island coasts have led to destruction of natural vegetation of around 7500 ha. Added to the extensive deforestation leading to loss of canopy cover, increased levels of salinity and a 1.5 °C (2.7 °F) rise in surface water temperatures have posed serious problem towards the survivability of the indigenous flora and fauna. An anthropometric and ethnographic assessment in the year 2015 by a group of German researchers revealed that the lack of livelihood options had resulted in the migration of human population from the region by up to 13% in a decade (Foundation, Thomson 2015a, b). As mangrove ecosystems produce resources which have widespread human utilization, these areas are threatened globally.

In the Asia-Pacific region, there is a drastic reduction of these resource bases at a very alarming rate, which is brought into action due to overexploitation of resources, unplanned and non-sustainable utilization, and conversion of forest cover to other land uses such as fish ponds and hatcheries, land acquisition for human settlements, infrastructure development as well as paddy cultivation are the major problems faced (Umali et al. 1987). Sustainable management of the mangrove ecosystem in the Indian Sundarbans is hindered primarily by land acquisition by reclamation for expansion of agriculture and human settlement, which is a direct result of population explosion. This further results into other indirect anthropogenic disturbances.

Five of the 24 true mangrove species in Sundarbans have global conservation importance (Barik and Chowdhury 2014). While *Heritiera fomes* Buch. Ham. is considered endangered, *Sonneratia griffithii* Kurz has been declared critically endangered. The remaining three species, viz. *Ceriops decandra* Ding Hou, *Phoenix paludosa* Roxb. and *Aegialitis rotundifolia* Roxb., are near threatened. However, *Sonneratia caseolaris* Engler has not yet been enlisted in the IUCN Red List.

20.2 Contributions of Mangroves Apart from Serving As Ecological Barrier

For centuries using mangrove plant extracts for treating several health disorders has been a popular method. Owing to their versatile applications, these plant-derived substances have drawn quite a lot of attraction. Mangroves have unique biochemistry and produce a wide array of novel natural products. Biologically active antiviral, antibacterial and antifungal compounds are often synthesized by mangrove and mangrove associates. Few studies in the area of pharmacology reported about mangrove extracts and their effects on some microorganisms like *Pseudomonas* sp., *Shigella* sp. and *Staphylococcus* sp. (Abeysinghe et al. 2012). Also different extraction protocols with various types of solvents including ethanol, chloroform and ethyl acetate have been reported (Ravikumar et al. 2010).

Based on their nutrient potential, mangrove forests play crucial role as to serve as food source for marine organisms as well as for human consumption. In traditional medicine numerous mangrove plants are consumed as medicinal plants for many years (Bandaranayake 2002). Some recent studies threw light on and the medicinal

properties in some mangrove plants, which were consumed in folklore medicine, have been confirmed, for example, in an in vitro cytotoxic assay a compound 3′,4′,5,7-tetrahydroxyflavone isolated from *Sonneratia caseolaris* showed promising inhibition activity against SMMC-7721 human hepatoma cell proliferation (Tian et al. 2009).

Antibacterial and antifungal properties along with various other pharmaceutical potential of mangrove plants have also been reported. As per reports of Abeysinghe and Wanigatunge (Abeysinghe et al. 2012), ethyl acetate extract of *Avicennia marina* mature leaves shows promising antimicrobial activity with methanolic extract of *Excoecaria agallocha* leaves and shoots showing the antimicrobial potential (Chandrasekaran et al. 2009; Premanathan et al. 1999). Methanol extract from trunks of *Excoecaria agallocha* and *Bruguiera gymnorhiza* has been reported to show antifungal activity (Kazuhiko 2002; Premanathan et al. 1999). Antiviral, antibacterial and anti-ulcer properties of mangrove plants have also been reported (Perera et al. 2001; Chandrasekaran et al. 2006; Marrero et al. 2006).

Recent research evidenced presence of antibacterial (Chandrasekaran et al. 2009) and antifungal (Bose and Bose 2008) properties in Indian mangroves. Until now, over 200 bioactive metabolites have been isolated from true mangroves of tropical and semitropical populations (Marrero et al. 2006). Most of the isolated compounds, as exhibited by their chemical structure, belong to triterpenes, saponins, alkaloids, flavonoids, tannins, steroids and phenolics that have a broad spectrum of therapeutic possibilities (Bandaranayake 1998).

Extracts of tender leaves, mature leaves and bark of *Bruguiera sexangula*, *Avicennia officinalis* and *Avicennia marina* have been reported to have antibacterial activity (Wu et al. 2008). Screening of antibacterial activity against pathogenic bacteria species of *Escherichia coli*, *Proteus* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *Shigella* sp. was performed by using agar diffusion technique. When tested for growth inhibition, against the bacterial strains under study, extracts of *A. officinalis*, *A. marina* and *B. sexangula* exhibited varying degree of inhibition.

20.2.1 Significance of Rhizospheric Metagenomics Study

Over the years numerous studies have made an effort to understand the causes behind the depletion of the mangrove cover along the coastal lines. However, very less emphasis has been given to the microbial population and the alterations in the metapopulation dynamics of the microbial communities prevalent along the mangrove forest areas. Rhizosphere has been characterized as the region of occurrence of various important life processes for more than over 100 years. In contrast with non-rooted mass soil, the soil compartment specifically around plant roots and their close vicinity, which defines the rhizosphere, is highly populated by microorganisms. The rhizosphere serves as a zone of active interchange between soil bacteria and plants. Organic carbon is released by plants in high amount. Plants regulate processes as quorum sensing, motility, conjugation, biofilm formation, symbiosis, virulence and various mechanisms involving antibiotic production to

influence the rhizosphere microbiome and also recruit function-specific microbiomes.

Several hypotheses have been raised regarding microbial community assembly, in which the 'niche theory' is very essential to understand the microbial consortium in root rhizosphere (Dumbrell et al. 2010). The niche-based theory predicts that the changes in the community composition are related to environmental variables and that effect the survival aspect of the plant is associated to it directly (Jongman 1995). Various studies about microbial rhizosphere communities have revealed the plant species play a key role in shaping the microbial community assemblage in the rhizosphere which includes *Arabidopsis* (Bulgarelli et al. 2012), tobacco (Robin et al. 2006), Norway spruce (Calvaruso et al. 2009), rice (Knief et al. 2012), potato (Rasche et al. 2006), soybean (Xu et al. 2009), oak (Uroz et al. 2010), wild oats (DeAngelis et al. 2009), etc.

The rhizosphere is a chemical signalling hotspot as it is placed in the proximity of the plant roots. Plant and microbial exudates are a plenty, and it is considered as a hotspot of community dynamics (Philippot et al. 2013). The unique but complex food web prevalent in the rhizosphere utilizes the nutrients released by the plant (e.g. exudates, border cells, mucilage, etc.), which are major regulators of microbial diversity and activity in the immediate vicinity of plant roots (Mendes et al. 2013). The accumulation of organic carbon around the rhizosphere as a result of the secretions of the host plant contributes towards the species richness in the rhizosphere, and so factors that bring about changes in the bulk soil communities, for example, anthropogenic effect, pollution, climate change etc., will have an effect on the assembly and the final composition of rhizosphere communities.

20.3 Materials and Method

20.3.1 Rhizospheric Soil Collection and Metagenomic Sequencing

The 16s rRNA gene that comprises conserved regions interspersed by nine hypervariable regions has played a key role in studying and characterizing the bacterial community of an environmental sample. The present study targets the V3–V4 region and exploits the high variability of these regions to distinguish bacteria subtypes and thus microbial community structure identification.

20.3.2 Sample Preparation

Genomic DNA from rhizospheric soil sample was extracted using an in-house standardized protocol. DNA quality was assessed by NanoDrop and on agarose gel, and quantitative assessment was carried using QUBIT. The library was prepared using Illumina standardized V3–V4 regions of the16S rRNA library protocol. The enriched library then was quantified, and validation was carried out using qPCR and

Agilent Bioanalyzer (DNA 1000 chip). The library that generated V3–V4 amplicons was then sequenced using Illumina MiSeq (300 × 2 PE chemistry).

20.3.3 Bioinformatic Analysis

Using the FASTQC toolkit (http://www.bioinformatics.babraham.ac.uk/projects/fastqc), quality control of raw reads was carried out. QIIME software (qiime.org) was used to cluster the quality processed paired end reads into OTUs (operational taxonomic units) to identify the microbial communities. The identified OTUs were used for taxonomic assignment (Greengenes database), phylogenetic and diversity analysis. Further QIIME (Quantitative Insights into Microbial Ecology) was used to assemble the processed reads into contigs during initial bioinformatics analysis. Following this, PICRUSt and Venny were used to predict the biological pathways and create Venn diagrams to reveal common and unique microbes. The flowchart of the analysis are summarized in Fig. 20.1a, b.

Fig. 20.1 Flowchart of analysis. (**a**) Analysis pipeline for rhizospheric metagenomic study; (**b**) processing pipeline of sequenced data; (**c**) in situ images of collected samples; (**d**) herbarium collection of collected samples

20.4 Sites of Study

The sites of study of were Rangabelia, Sudhanshupur, Kumirmari (Fig. 20.2a) and Burirdabri islands (Fig. 20.3a) of the Indian Sundarbans. Some field images are also provided to give an idea regarding the sites of collection along with the herbarium specimens (Figs. 20.1b, c, 20.2b, c, d, e, f; and 20.3 b, c, d, e).

20.5 Insights into the Rhizosphere Microbial Communities

The common bacterial members identified in this study can be directly correlated to the soil chemical profile as well as the overall habitat dynamics of the region under study. The majority of the sequences analysed by all three methods belonged to the phylum *Proteobacteria*, which includes a number of pathogens, such as *Escherichia*, *Yersinia*, *Helicobacter*, *Salmonella*, *Vibrio* and many others. There are also non-parasitic bacteria, and many of them aid to the nitrogen fixation process. Most abundant class of *Proteobacteria* in the sample turns out to be *Alphaproteobacteria* by analysis of both QIIME and KRAKEN, which include agriculturally important bacteria which induce nitrogen fixation in symbiosis with plants. The rhizosphere soil depicted the high abundance of nitrogen-fixing bacteria. *Heliobacterium*

Fig. 20.2 Collection sites of fern samples. (**a**) Google map locations; (**b**) *Microsorum*; (**c**) *Ceratopteris*; (**d**) *Marsilea*; (**e**) *Drynaria and Pyrrosia*; (**f**) *Acrostichum*

Fig. 20.3 Collection sites of *Nypa* and *Heritiera* samples. (**a**) Google map location; (**b**) *Heritiera sampling*; (**c**) *Collection of juvenile sample of Heritiera*; (**d**) Burirdabri island coast with abundance of *Nypa* (**e**). Profuse abundance of *Nypa* along the Burirdabri sea coast

modesticaldum Ice1 was the most abundant of all the microbial community according to MEGAN analysis which is a well-known nitrogen-fixing bacterium. For other rhizospheres analysis suggested the richness of *Firmicutes* (such as *Clostridiales*, *Lactobacillales*, *Bacillales* and *Thermoanaerobacterales*) and *Proteobacteria* (such as *Enterobacteriales*, *Legionellales*, *Rhizobiales*, *Rhodospirillales* and *Burkholderiales*) in the sample. Many nitrogen-fixing phototrophic bacteria that can grow either photoheterotrophically or chemotrophically were identified. The classification suggested 99% of the sequences belonged to *Bacteria*, while 0.4% belonged to Archaea. In bacterial sequences, the major three groups were *Proteobacteria* (44%), *Terrabacteria (*27%) and PVC (12%) (*Planctomycetes*, *Verrucomicrobia* and *Chlamydiae*) superphyla. The major superphyla *Proteobacteria*, comprised of *Delta/Epsilonproteobacteria* (17%), *Alphaproteobacteria* (12%), *Gammaproteobacteria* (7%) and *Betaproteobacteria* (6%) in that order of abundance, which were found to be rhizosphere specific, provides us with the necessary insights towards the development of an *effective microbial consortium* which can be used *towards habitat restoration* and effective design of mangrove management systems through effective replantation (reforestation) strategies.

20.6 Identification of the Core Microbiome

For terrestrial ferns the core microbiome was limited to *Gemmatimonas* and *Bacillus* (Fig. 20.4a, b, c), while for epiphytic ferns there was more complexity in the core microbiome present in the rhizospheric assemblages (Fig. 20.5a, b, c) with *Escherichia*, *Burkholderia*, *Paenibacillus*, *Pseudomonas* and *Ilumatobacter* making up the key members.

20.6.1 Functional Characterization

As most abundant bacteria are known for their nitrogen fixation activity, OTU contributions for KEGG ortholog K02585, which is nitrogen fixation protein NifB, were done and are summarized in Table 20.1. Apart from bacterial pathways such as streptomycin biosynthesis, vancomycin biosynthesis and beta lactamase biosynthesis, pathways were also predicted from the microbial abundance data which indicates a vibrant rhizosphere assemblage capable of stimulating plant growth as well as initiate chemical sensing and preventive pathways (Fig. 20.6).

For *Nypa fruticans* Wurmb., the analysis revealed a total of 626 microbial genera that are associated with the rhizospheric zone [publicly available at NCBI with the following accession number: SRX5993499]. The profile shows *Woeseia* (6.31%), *Pseudomonas* (2.26%), *Thioalkalivibrio* (2.11%), *Nitrospira* (1.89%),

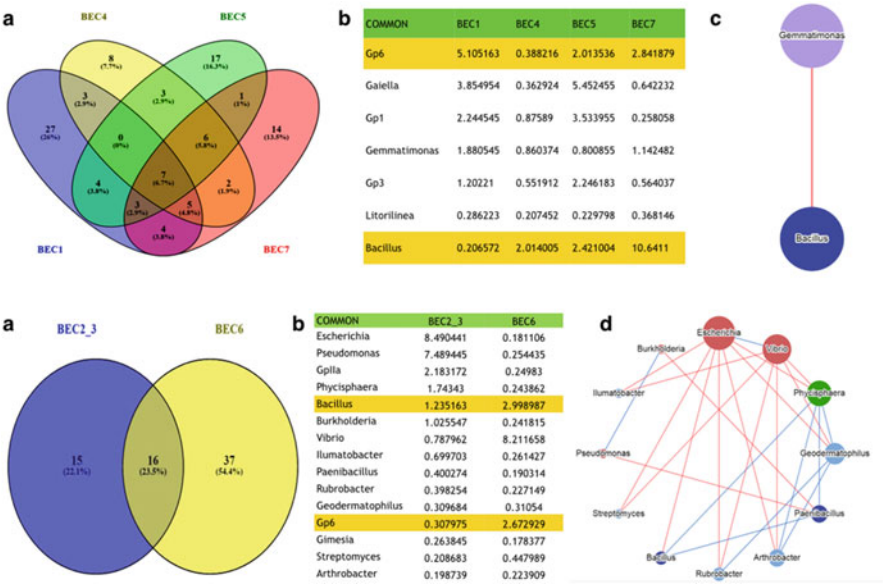


Fig. 20.4 Core microbiome of rhizospheric Microbiome of terrestrial ferns; (**a**) Venn diagram depicting the unique and common members; (**b**) Common members across the rhizospheric assemblages and their abundances; (**c**) Network of overrepresented microbes

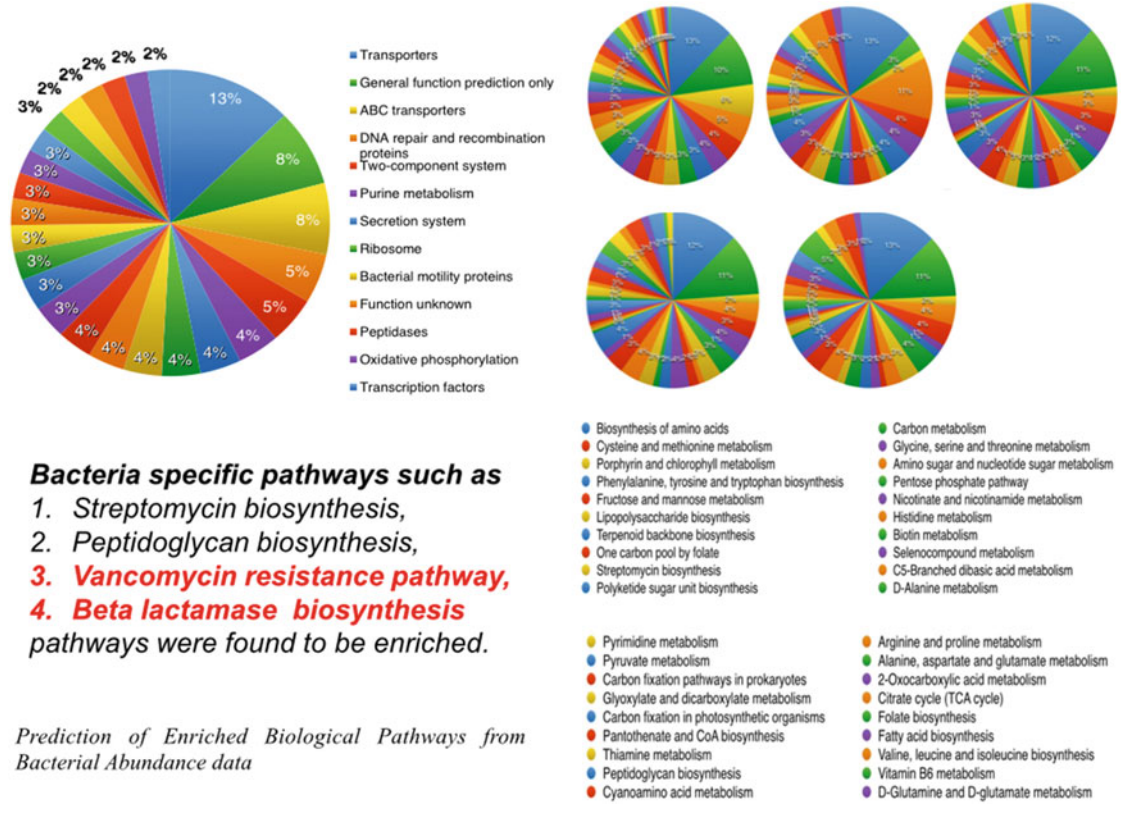


Fig. 20.5 Core microbiome of rhizospheric microbiome of epiphytic ferns *(Drynaria + Pyrrosia and Microsorum)*; (**a**) Venn diagram depicting the unique and common members; (**b**) common members across the rhizospheric assemblages and their abundances; (**c**) network of overrepresented microbes

Table 20.1 Diversity of KEGG (Kyoto Encyclopedia of Genes and Genomes) database resource reported for various pathways genes with accession number

KEGG	Pathways genes
Transporters	7031568
General function prediction only	4516899
ABC transporters	4177049
DNA repair and recombination proteins	2896197
Two-component system	2752492
Purine metabolism	2395112
Secretion system	2298549
Ribosome	2178602
Bacterial motility proteins	2143882
Function unknown	2084832
Peptidases	1978469
Oxidative phosphorylation	1914943
Transcription factors	1899089
Pyrimidine metabolism	1637766
Arginine and proline metabolism	1597341
Amino acid-related enzymes	1572511
Chromosome	1509604
Carbon fixation pathways in prokaryotes	1434044
Pyruvate metabolism	1422486
Amino sugar and nucleotide sugar metabolism	1406874
Glycolysis/gluconeogenesis	1381641
Butanoate metabolism	1360717
Methane metabolism	1346254
Ribosome biogenesis	1307395
Other ion-coupled transporters	1274641

Erythrobacter (1.85%) and *Desulfuromonas* (1.79%) to be the most abundant assemblages of microbial community related of the *Nypa fruticans* Wurmb. rhizosphere, which shows high relative abundance of the species among the other islands. The species is moderately salt tolerant, and the population exhibits a declining trend in the Indian Sundarbans (Alzubaidy et al. 2016; Ellison et al. 2010; Gopal and Chauhan 2006; Ragavan and Mandal 2018; Theerawitaya et al. 2014).

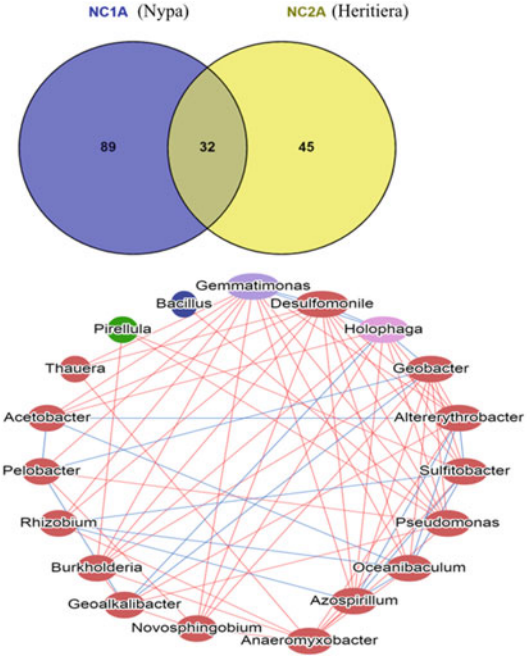
For *Heritiera fomes* Buch. Ham., the profile revealed a total of 442 microbial genera of which the most abundant microbial community assemblages associated with the rhizosphere happen to be *Sphingobium* (6.05%), *Vicinamibacter* (3.17%), *Dechloromonas* (2.88%), *Bacillus* (2.75%), *Thiobacter* (2.73%), *Clostridium* (2.32%), *Ramlibacter* (2.16%) and *Sphingomonas* (2.10%). *Heritiera fomes* Buch. Ham. is a stenoecious species and grows in low salinity and high field capacity. Thus, the plant species serves as a bio-indicator for rising salinity (Banerjee et al. 2017; Hoque et al. 2006; Karim 1988). At present the species is threatened by changes in floristic composition and local extinction as well as by factors such as

hydrology, ocean currents, geomorphology, natural calamities, anthropogenic disturbances and low genetic diversity (Ragavan and Mandal 2018).

The results of comparison of *Nypa* and *Heritiera* are summarized in Fig. 20.7, while the functional characterization reveals a large number of bacterial metabolic pathways abundant such as lipopolysaccharide biosynthesis, recombination and repair pathways, sulphur metabolism, amino acid metabolism, pentose phosphate pathway as well as streptomycin biosynthesis, vancomycin resistance and bacterial chemotaxis pathways (Fig. 20.6). However, none of these pathways were predicted to be abundant in *Heritiera fomes* rhizosphere, which may be due to the lower abundance of the bacterial members in the rhizosphere of the plant under study.

An interesting facet to note is that *Bacillus* represents the only genus that is common to all the plant rhizospheres under study, indicating the essentiality of nitrogen fixation in the soil environment as well as the region as a whole and further establishing the fact that all the plants under study possess a rhizosphere-specific microbial consortium.

Over the years there have been several reports on the soil characters of the Sundarbans area both from the chemical and from the microbial content. This is as a result of the fact that the Sundarbans delta presents a unique transitional zone which also harbours anthropogenic influences. Due to the gradual erosion of the banks of the islands and landmasses of the Indian Sundarbans, more and more deforestation has taken place as the settlers have gradually moved inwards and



SAMPLE	NC1A	NC2A
Acetobacter	0.31719	0.32964
Aciditerrimonas	0.24318	0.61501
Altererythrobacter	0.84849	0.33456
Anaeromyxobacter	0.47579	0.88069
Azospirillum	0.2934	0.41328
Bacillus	0.2564	2.75523
Burkholderia	0.96215	1.19557
CandidatusAlvsiosphae	0.16124	0.17712
CandidatusPhytoplasm	1.65468	1.26937
Clostridium	0.452	2.33456
Desulfomonile	0.46521	0.16236
Desulfuromonas	1.77363	1.57688
Gaiella	0.47579	0.77491
Gemmatimonas	0.22468	0.65929
Gemmatirosa	0.18767	1.20295
Geoalkalibacter	0.7084	0.18204
Geobacter	0.69518	1.31365
Holophaga	0.66875	0.51415
Iamia	0.18503	0.18204
Nitrospira	1.88465	0.23862
Novosphingobium	0.20617	0.48708
Oceanibaculum	0.1586	0.15498
Pelagibius	0.68725	0.29028
Pelobacter	1.66526	0.3567
Pirellula	0.1956	0.2214
Pseudomonas	2.26263	0.89791
Rhizobium	0.17181	0.1722
Sulfitobacter	1.27405	0.20172
Thauera	0.16388	0.2583
Thiobacter	1.21855	2.72817
Vicinamibacter	0.61059	3.17343

Fig. 20.6 Predicted Networks of enriched microbes prevalent in the rhizospheric assembledges

Fig. 20.7 Predicted enriched biological pathways in the rhizospheric assembledges

have cut down large areas of the forest cover. This represents two significant ecological pressures: first since these mangrove forests offer the first barrier for tsunamis and other cyclones, their erosion has resulted in the loss of more and more property. Second, the habitat of important fauna is also lost as a result of the illegitimate felling of trees. Following the declaration of the Indian Sundarbans as the world heritage site, there have been concerted efforts from both the government and local dwellers towards afforestation by planting of true mangroves. Unfortunately the expected success rate has not been achieved as majority of the saplings have died at the initial stages. Numerous workers (Nandy et al. 2009) have attributed this observation to the lack of acclimatization of these newly planted saplings to the environment. However, current ideas regarding the plant rhizosphere associations indicate that each plant possesses a core microbiome which is constant for a particular plant. Thus, it is important that these core microbiomes be identified and used as possible standardized supplements wherever that plant is being replanted (Mendes et al. 2013; Toju et al. 2018). We can further observe that some of the rhizospheric bacteria present in these regions can serve as reservoirs of biologically active compounds which can be explored further in culture-based experiments. Microbial members with reported active nitrogen fixing ability was found to be abundant in the assemblage which is in conformation with the data of rhizospheric soil abundances reported by Ganguli et al. (2017). Generally mangrove species are characterized by the monopoly of pneumatophores or breathing roots (negative

geotropic root), knee roots, stilt roots, xerophilous leaves, viviparous germination and salt excretory glands (Tomlinson 1986). However, several mangrove workers like Tomlinson (1986) have described mangroves prioritizing their ecophysiological attributes (Barik and Chowdhury 2014). During daily high and low tides, they are generally inundated and exposed, respectively, and are nurtured by coastal marine waters mixed with freshwater from rains and land drainage system. Mangrove ecosystems and their widespread resources are constantly threatened by the overutilization for anthropogenic habitat settlements. In the Asia-Pacific region, non-sustainable utilization, overexploitation of resources and forest cover clearing for various human uses have caused drastic decline in the mangrove populations (Umali et al. 1987; Basak et al. 2015). As we have earlier stated, that *Heritiera fomes* Buch. Ham. is considered endangered in the Indian Sundarbans, *Sonneratia griffithii* Kurz has been declared critically endangered, while the remaining three species, viz. *Ceriops decandra* Ding Hou, *Phoenix paludosa* Roxb. and *Aegialitis rotundifolia* Roxb., are near threatened. The microbial population and their associated functions are enriched due to the release of large quantities of organic carbon by plant roots in the rhizospheric zone.

20.7 Conclusion

Global research along with our findings clearly indicates that plants have a specific role in the control of rhizospheric function as reports have demonstrated that processes such as quorum sensing, various mechanisms involving antibiotic production, biofilm formation, conjugation, motility, symbiosis and virulence are maintained by the recruitment and persistence of specific microbial members. The question that remains is whether these bacterial communities also influence plant function as a whole. The presence of core microbiomes associated with individual plant rhizosphere niches probably is an early indication towards this mutualistic control and crosstalk cycle between plants and microbes which is an evidence of the soil-plant continuum. Apart from the changes in the microbial dynamics of the region, till date no study exists which evaluates the alterations in the soundscape of the area and how it changes as a result of anthropogenic and ecotourism activities, since numerous ecological workers in the recent years have attributed the alterations in soundscape to influence the community structure of plants and other species.

We believe that the identification of core microbiomes is an essential prerequisite for understanding of the physiological stability of a particular mangrove species. Our data clearly reveal that mangrove plants independent of their taxonomic position and hierarchy possess a set of core microbiomes, and thus when replantation efforts are undertaken, suitable habitat identification needs to be performed using this set of key microbes or supplementation using these core microbial taxa should be attempted for establishment and proliferation of the saplings.

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References

Abeysinghe PD, Pathirana RN, Wanigatunge RP (2012) Evaluation of antibacterial activity of different mangrove plant extracts. Ruhuna J Sci 1:1

Alzubaidy H, Essack M, Malas TB et al (2016) Rhizosphere microbiome metagenomics of gray mangroves (*Avicennia marina*) in the Red Sea. Gene 576(2):626–636

Bandaranayake WM (1998) Traditional and medicinal uses of mangroves. Mangrove Salt Marshes 2(3):133–148

Bandaranayake WM (2002) Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl Ecol Manag 10(6):421–452

Banerjee K, Gatti RC, Mitra A (2017) Climate change-induced salinity variation impacts on a stenoecious mangrove species in the Indian Sundarbans. Ambio 46(4):492–499

Barik J, Chowdhury S (2014) True mangrove species of Sundarbans delta, West Bengal, eastern India. Check List 10(2):329–334

Basak P, Pramanik A, Roy R, Chattopadhyay D, Bhattacharyya M (2015) Cataloguing the bacterial diversity of the Sundarbans mangrove, India in the light of metagenomics. Genomics Data 4:90–92

Bose S, Bose A (2008) Antimicrobial activity of *Acanthus ilicifolius* (L.). Indian J Pharm Sci 70 (6):821

Bulgarelli D, Rott M, Schlaeppi K et al (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488(7409):91–95

Calvaruso C, Mareschal L, Turpault MP, Leclerc E (2009) Rapid clay weathering in the rhizosphere of Norway spruce and oak in an acid forest ecosystem. Soil Sci Soc Am J 73(1):331–338

Chandrasekaran M, Venkatesalu V, Sivasankari S et al (2006) Antibacterial activity of certain mangroves against methicillin resistant *Staphylococcus aureus*. Seaweed Res Utiln 28:165–170

Chandrasekaran M, Kannathasan K, Venkatesalu V, Prabhakar K (2009) Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant *Staphylococcus aureus*. World J Microbiol Biotechnol 25(1):155

Chaudhuri AB, Choudhury A, Hussain Z, Acharya G (1994) Mangroves of the Sundarbans

DeAngelis KM, Brodie EL, DeSantis TZ et al (2009) Selective progressive response of soil microbial community to wild oat roots. ISME J 3(2):168–178

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. ISME J 4(3):337–345

Ellison J, Koedam NE, Wang Y et al (2010) Nypa fruticans. IUCN Red List Threatened Species 2010:e.T178800A7610085

Foundation, Thomson (2015a) Everyday disasters' driving flight from Sundarbans. trust.org. Retrieved 5 June 2015

Foundation, Thomson (2015b) Poor planning, climate shifts devastating India's Sundarbans. trust.org. Retrieved 5 June 2015

Ganguli S, Rahaman S, Bera AR et al (2017) Rhizospheric metagenome of the terrestrial mangrove fern *Acrostichum* from Indian Sunderbans. Genomics Data 14:53–55

Giri C, Pengra B, Zhu Z, Singh A, Tieszen LL (2007) Monitoring mangrove forest dynamics of the Sundarbans in Bangladesh and India using multi-temporal satellite data from 1973 to 2000. Estuar Coast Shelf Sci 73(1-2):91–100

Gopal B, Chauhan M (2006) Biodiversity and its conservation in the Sundarban mangrove ecosystem. Aquat Sci 68(3):338–354

Hoque MA, Sarkar MSKA, Khan SAKU, Moral MAH, Khurram AKM (2006) Present status of salinity rise in Sundarbans area and its effect on Sundari (*Heritiera fomes*) species. Res J Agric Biol Sci 2(3):115–121

Iftekhar MS, Islam MR (2004) Managing mangroves in Bangladesh: a strategy analysis (PDF). J Coast Conserv 10(1):139–146

Jongman E (1995) Data analysis in community and landscape ecology. Cambridge University Press, Cambridge

Karim A (1988) Environmental factors and the distribution of mangrove in sunderbans with special reference to *Heritiera fomes* Buch Ham

Kazuhiko K (2002) Antifungal activity of mangrove tree. Japan Sci Technol Agency:177–182

Knief C, Delmotte N, Chaffron S et al (2012) Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME J 6(7):1378–1390

Marrero E, Sanchez J, de Armas E et al (2006) COX-2 and sPLA2 inhibitory activity of aqueous extract and polyphenols of *Rhizophora mangle* (red mangrove). Fitoterapia 77(4):313–315

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37 (5):634–663

Nandy P, Dasgupta N, Das S (2009) Differential expression of physiological and biochemical characters of some Indian mangroves towards salt tolerance. Physiol Mol Biol Plants 15 (2):151–160

Pani DR, Sarangi SK, Subudhi HN, Misra RC, Bhandari DC (2013) Exploration, evaluation and conservation of salt tolerant rice genetic resources from Sundarbans region of West Bengal. J Indian Soc Coastal Agric Res 30:45–53

Perera LMS, Ruedas D, Gómez BC (2001) Gastric antiulcer effect of *Rhizophora mangle* L. J Ethnopharmacol 77(1):1–3

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11(11):789–799

Prain D (1903) Flora of the Sundribuns. Allied Book Centre 2:251

Premanathan M, Arakaki R, Izumi H et al (1999) Antiviral properties of a mangrove plant, *Rhizophora apiculata* Blume, against human immunodeficiency virus. Antivir Res 44 (2):113–122

Ragavan P, Mandal RN (2018) Status of Indian mangroves. Oceanogr Fish Open Access J 7 (4):555718. ISSN:2476-0536 Juniper Publishers

Rasche F, Hödl V, Poll C et al (2006) Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. FEMS Microbiol Ecol 56(2):219–235

Ravikumar S, Gnanadesigan M, Suganthi P, Ramalakshmi A (2010) Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens. Int J Med Med Sci 2(3):94–99

Robin A, Mougel C, Siblot S et al (2006) Effect of ferritin overexpression in tobacco on the structure of bacterial and pseudomonad communities associated with the roots. FEMS Microbiol Ecol 58(3):492–502

Theerawitaya C, Samphumphaung T, Cha-um S, Yamada N, Takabe T (2014) Responses of Nipa palm (*Nypa fruticans*) seedlings, a mangrove species, to salt stress in pot culture. Flora-Morphol Distrib Funct Ecol Plants 209(10):597–603

Tian M, Dai H, Li X, Wang B (2009) Chemical constituents of marine medicinal mangrove plant *Sonneratia caseolaris*. Chin J Oceanol Limnol 27(2):288

Toju H, Peay KG, Yamamichi M et al (2018) Core microbiomes for sustainable agroecosystems. Nat Plants 4(5):247–257

Tomlinson PB (1986) The botany of Mangroves. Cambridge University Press, London

Umali RM, Zamora PM, Gatera RR et al (1987) Mangroves of Asia and the Pacific: Status and management. Technical Report of the UNDP/UNESCO Research and Training Pilot Programme on mangrove ecosystems in Asia and the Pacific (RAS/79/002)

Uroz S, Buée M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2 (2):281–288

Wu J, Xiao Q, Xu J et al (2008) Natural products from true mangrove flora: source, chemistry and bioactivities. Nat Prod Rep 25(5):955–981

Xu Y, Wang G, Jin J et al (2009) Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. Soil Biol Biochem 41(5):919–925

Web References

"Cyclone Aila" (2009). https://earthobservatory.nasa.gov/images/38786/cyclone-aila

Indomalayan ecoregions: WWF: Waybackmachine, archived 28 June, 2009. (https://web.archive.org/web/20090628180622/http://www.certisource.co.uk/Press_Articles/Article_Archive/articles/Ecoregions%20profile%20%20pick%20from%20over%20100%20rain%20forests.htm)

Ramsar sites in India. http://wiienvis.nic.in/Database/ramsar_wetland_sites_8224.aspx

UNESCO World Heritage Site List. https://whc.unesco.org/en/list/452

Advances and Challenges in Metatranscriptomic Analysis

21

Anushka Singh, Siddharth Vats, and Prachi Bhargava

Abstract

New-era genomic tools have proved themselves a big boon in showing a path to get an insight to microbial evolution, taxonomic profiling, active members of a community and the genes involved in many metabolic pathways. Shotgun metagenomics helps in random sequencing of genome of the studied microbiome; however, it does not show the exact number of active genes or the functionally active genomic members. This lacuna calls for the role of metatranscriptomics which studies the differential gene expression and has tremendously participated in the unearthing the multifariousness of active genes, quantification of their expression levels and their response to different environmental and biological conditions. This chapter linchpins the various tools and techniques used in metatranscriptomic analysis of any microbial community. We focus on the major headways in this exponentially proliferating field, comparing the various options used in computational bioinformatic analysis of data and the challenges associated with them.

Keywords

Metatranscriptomics · Differential expression analysis · Metagenomics · Functional gene expression

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21.1 Introduction

Microbiomes are widespread and are found in the soil, the ocean and in/on other living organisms. Alteration in the microbiome can influence the health of the environmental habitat in which they dwell. Shortly, after the decoding of human genome, focus has shifted to the huge genomic gene pool of prokaryotes present in the human body that is much beyond that of the eukaryotic human genome, yet its share to human physiology persists ambiguous. Thus, to know more regarding these communities, various attempts on the basis of data taken from many omics have been explored. Both metatranscriptomic and metagenomic sequencing methods are commonly used to associate microbiota with ecological changes and important diseases. Various evaluation methods have also been used to check the functional and taxonomic forms of microbiota throughout individuals or their surroundings. Metatranscriptomic analysis helps provide important understanding of genes activities by analysing gene expression levels and often regulatory mechanisms of microbiota. The initial years of microbiome investigation were greatly determined by use of DNA sequencing depending upon shotgun metagenome sequencing and 16S rDNA, permitting for the clear analysis of genome structure and microbial composition. Although 16S studies solely identify the precise taxonomic form of a microbiota, it is a low-cost alternative to completely captivate biodiversity (calculating the maximum effective gamut of relative exuberance) of various samples utilising minimum sequencing. Figure 21.1 shows the process of metagenomics in a flowchart. A major disadvantage of shotgun metagenomics is its ability of not being able to differentiate between the agile and inactive components of a microbiome and therefore cannot assist in differentiating those that are aiding to

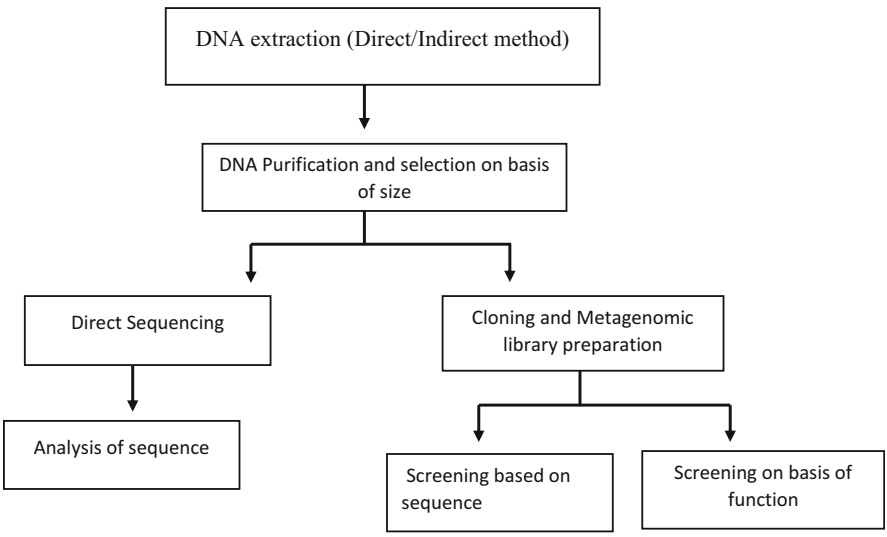

Fig. 21.1 Steps for metagenomics analysis of a sample

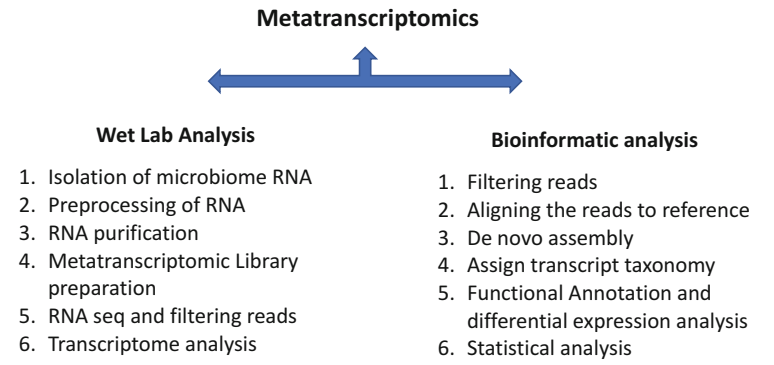

Fig. 21.2 Overview of the different steps in metatranscriptomics

observed ecosystem behaviour from the ones that are just present, apparently waiting for other favourable conditions. Whereas the past few years have seen notable improvements in sequencing techniques that have transformed the way of performing biological experiments, especially in the case of study of complex microbiomes.

The application of NGS in metatranscriptomics is to create big datasets having large degree of complexity that requires to be analysed properly to translate the noninterpretable fresh and naive sequencing reads to biological understandings, in the form of figures and data tables. The onset of RNA-seq and massive parallel sequencing has granted advanced and appealing opportunities in the field of transcriptome analysis, supporting insight and vigorous range that were earlier unimaginable. Technological progress in RNA-seq has lately supplied us with the capability to introspect into the genes that are vigorously expressed in composite bacterial communities, helping in the explanation of the functional variations that edict the microbiome functions at given time and its synergy with the host. Figure 21.2 gives an overall view of the working of metatranscriptomics in a pictorial diagram.

21.2 Experimental Basics of Wet Lab Workflow

Transcriptomics refers to all the RNA molecules present in a cell, whereas metatransriptomics assess the functional genes in any given environment. Initially all the omics technology (Fig. 21.1 metagenomics) started to study the environmental genomes with the help of tools like NGS and whole genome shotgun metagenomics which dealt with both active and inactive genes. To nullify the inactive gene count, metatranscriptomics (Fig. 21.2) and metaproteomics came into light, which study the expression and translation of environmental genes. Microarray chips and EST have played a major role in exploring genome-wide expression though they are very expensive and laborious techniques.

Metatranscriptomics has played a pivotal role in identifying novel genes related to metagenomic functions with zero necessary prior whereabouts avoiding the need to design any probe or primer.

The qualitative approach of metatranscriptomics is used to explore community structure and identify and sort the metabolically active members, whereas the quantitative approach is used to study the functional annotations of a single organism. The presence of a reference metagenome helps us to correlate the gene abundance and its expression levels. The 16s rRNA databases act as repositories for the tools like multiple alignments and phylogenetic distance methods which identify homology sequence reads. Tools like Greengenes are used to classify the overall active members. The quantitative approach helps us to study the real-time gene expression corresponding to dynamic environment and gives an insight to differential gene expression (DGE) which depends on factors like the number of biological replica, read length, etc. Tools like tophat, bowtie and BWA can be used to map NGS sequencing data against references and align single nucleotide polymorphism for each transcript.

Although these factors can be planned accordingly if the reference genome is known, equal sequence coverage should be given to each replica if the reference is unknown. Therefore it is advisable to dedicate a sequence lane to each replica while using tools like Illumina HiSeq2500. A good experimental design brainstorms on factors like the number of replicas to be used and the sequencing depth. NGS assemblers like Celera and Velvet can be used for assembling the reads, and Glimmer and MetaGeneMark tools come handy for ORF predictions.

21.3 Advances and Challenges in Various Steps of Wet Lab

21.3.1 Isolation and Pre-processing of Microbiome mRNA

The isolation of raw material for transcriptomic analysis involves collecting of total RNA from the desired environment. The environmental RNA comprise of both prokaryotic and eukaryotic RNA. Most of the isolated RNA corresponds to ribosomal RNA in environmental NGS metatranscriptomics. Ribosomal RNA has its benefit of helping in determining the whole structure of any community as PCR gives an unbiased insight of the functional taxonomic variability, but it plays the role of a nuisance when it comes to de novo transcript assembly.

Eukaryotic mRNA can be sorted out by manufacturing cDNA using oligo-dT primers as the mRNA has a poly A tail; however, this type of selection is not possible with prokaryotic mRNA which comprises of only 1–5% of total RNA species. Unwanted rRNA can be removed using specific probes attached with magnetic beads wherein the probes anneal to the target rRNA sequences and henceforth removed with the bead. All the methods involved in RNA manipulation have a massive challenge of curbing degradation by contaminating ribonucleases. In comparison to the stable proteins in external environments, mRNA spontaneously gives response to the cellular conditions which keep on changing every nanosecond.

21.3.2 RNA Purification and Metatranscriptomic Library Preparation

The rRNAs are mostly removed in the methods depicted in Fig. 21.3 before sequencing as they usually create upward of major chunk of entire data if not eradicated and do not add to most downstream analyses, like identifying pathways or differentially expressed genes. The major strategies for mRNA enrichment as discussed in the above figure are rRNA separation by means of hybridisation with 16S and 23S rRNA probes or depletion of rRNAs by means of a 5-exonuclease. One of the most challenging steps is mRNA enrichment. rRNA hybridisation based on magnetic microbeads and oligo mixtures which hybridise with 16S and 23S does not require RNA integrity, is sequence specific and does not eliminate all bacteria rRNA. Another shortcoming comes in the form of oligos which sometimes hybridise with some mRNA. Besides it requires very pure RNA as impurities inhibit the exonucleases. Smaller RNA can be agarose gel purified using biotinylated primers eliminating the chances of binding to non-specific sequences.

Besides, rhizosphere niches from where the maximum soil samples are taken pose major hurdles related to plant host-derived RNA with the presence of humic substances. The humic acids can be removed with the help of size separation using Sephadex spin columns or precipitation of nucleic acids using polyethylene glycol so that an enriched population of mRNAs is extracted. These transcriptionally active RNAs are fractioned to synthesize cDNA which is then used to create a library to be amplified and sequenced. Sequence reads are aligned to reference genomes, and the functional genes are identified based on the sequence reads covering these regions. Library making involves the process of RNA fragmentation, synthesis of first and second strand, coupling with adapters and finally validation.

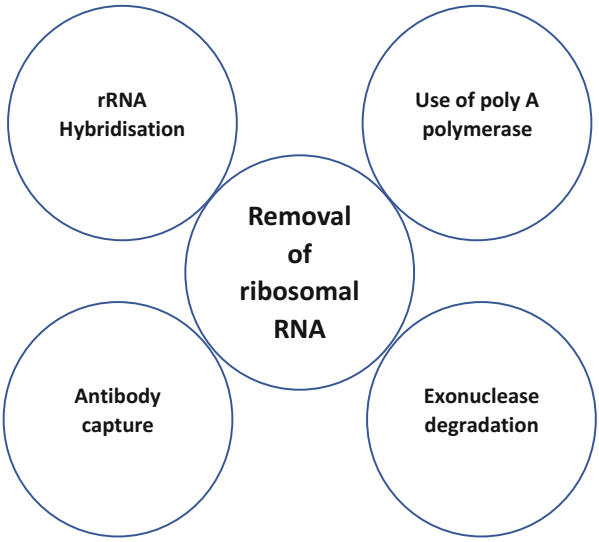

Fig. 21.3 Methods to remove ribosomal RNA

Optimisation of sequencing requires cDNA of a certain size, and enzymes, metals, heat or sonication are used for fragmentation. To maintain the integrity of each sample, the incubation time for fragmentation should also be optimised. The first cDNA strand is synthesised by reverse transcriptase using random hexamer primers. DNA polymerase is then used to synthesise the second strand. The sequencing adapters play a role in providing support for binding and a platform for primer hybridisation. Sometimes they act as markers when linked to a barcode for samples during multiplexing.

Metatranscriptomics pinpointedly focuses on expressed functional genes in the entire microbiome and inherently sheds light on the active functional contour of the microbial community. The roadmap of any standard metatranscriptomic analysis relies either on reading a reference genome as in alignment based methods or converting the reads into transcript contigs by de novo assembly. The first strategy totally relies on the number of database of reference genomes, whereas the second strategy reckons on the potential of the software programmes to assemble contigs correctly from raw reads. As a matter of fact maximum, a number of analysis pipelines were a makeshift or build impromptu to study the functional expression. The tools are categorised on the aforementioned strategy they follow. Those which follow the path of read mapping generally align the metatranscriptomic reads to specialised databases using alignment tools like BLAST, BWA, etc. The outcome is then annotated using software like SWISS PROT and KEGG; thereafter this annotated data is processed further by different downstream analysis for varied desired results.

Many tools conveniently work on bioinformatic data which speed up the pace of metatranscriptomic studies. Efficient Web servers and metatranscriptomic pipelines have been developed for analysis of in silico data to study the dynamic expressions of the microbiome in the past few years. Organism-specific functional profiling for almost all biota, namely, virus, archaea, prokaryotes and eukaryotes, has been facilitated at fast speed with MetaPhlAn2 and ChocoPhlAn pangenome database. MG-RAST, a tool that extracts numerous characteristics for users to determine quality of sequence minimising the impure adapter sequence reads, fakes duplicate reads, thus curbing the error rates up to a high extent. Similarly, HUMAnN2 is a pipeline used in both metagenomics and metatranscriptomics which has simple user interface. It helps in studying community function profiles, expanded database and mapping accelerated reads (Buchfink et al. 2015).

The sequencing platform is chosen which fits in the parameters of cost effectiveness, read length and the sequencing depth. The main players in the field are MiSeq, Life Technologies (PGM, Proton), Illumina's HiSeq (X, 3000/4000, NexSeq, High-Output) and the former 454. The sequence read length ranges from 50 bp in Illumina to 1.5 kb in PacBio. The cost per Mb may vary from USD$ 0.06 (Illumina) to USD$8.72 (454). Similarly the output yield goes from 40 Mb (PacBio) to 300 Gb (Illumina).

To check the quality of short reads originated from Illumina sequencers, various QC tools, viz. FaQCs (Leung et al. 2014a), FastQC (Andrews 2010), Trimmomatic (Bolger et al. 2014) and fastp (Chen et al. 2018), can be used. rRNAs can be analysed

for elimination from downstream analyses by use of tools like barrnap (Seemann 2014) and SortMeRNA (Kopylova et al. 2012) after the sequencing.

Leimena et al. (2013) developed another metatranscriptome analysis pipeline that helps in the study of symbiotic interactions within prokaryotic ecosystems, designed for mapping and function assignment on the basis of provided RNA-seq data. The working of the pipeline has been assessed using data from human small intestine microbiota. Core algorithm/tools used are SortMeRNA, BLASTN (Johnson et al. 2008), MegaBLAST and KAAS (Moriya et al. 2007).

MetaTrans, an open-source pipeline, works with incorporating rRNA removal, quality control and mapping of reads by use of multi-threading computers to stimulate the gene expression and taxonomic assessment of active microbiota (Martinez et al. 2016). Core algorithm/tools like Kraken quoted by Wood and Salzberg in 2014, SortMeRNA (Kopylova et al. 2012), UCLUST (Edgar 2010) and SOAP2 help immensely in the study protocol. The Simple Annotation of Metatranscriptomes by Sequence Analysis (SAMSA) is a broad pipeline for analysis of metatranscriptome operating in conjunction with MG-RAST annotation server, granting an ability to completely determine the expression activity in microbiota. The various phases on which it works are preprocessing which deals with the raw material, annotation, aggregation and lastly the analysis of the data worked upon. This has been used to study gut microbiome by Westreich et al. (2016).

The high-throughput metatranscriptomic data has been worked upon using Comprehensive Metatranscriptomics Analysis (COMAN) which is a very competent Web-based tool. Functional identification and simultaneously its comprehensive analysis converting basic reads to functional assignments are the key feature of this tool. The assignments are then used to compare and analyse the statistical data and co-expressed network data, and conclusions can be drawn considering the functional variations and parameters. COMAN is very user friendly when used with easy-to-handle interfaces and is popular with experimentalists as it can be used without programming instructions and the inconvenience of altering tools/working environments for resolving their biologically permissible questions. Further, more software can be integrated in the pipeline to work on the vital data which is also supplied in form of table (Ni et al. 2016).

21.3.3 RNA-Seq and Filtering Reads

A common metatranscriptome dataset consists of plenty of sequenced mRNA molecules, named RNA-seq reads, and there is a dire need to get an insight of the biological interpretation from these datasets to narrow down the sample size and increase the efficiency (Gosalbes et al. 2011; Korf 2013). Analysis suites like MG-RAST52 and HUMAnN51 have been designed recently to give an end-to-end solution to filter the reads (Glass et al. 2010; Abubucker et al. 2012). These are used by combining specific in silico tools (e.g. GEM54 and BOWTIE53 for mapping, CuffDuff56 for differential gene expression and Trimmomatic55 for quality filtering) to attain the similar overall objective of concluding the levels of gene expression

and alterations in it, from the raw sequenced mRNA reads (Langmead and Salzberg 2012; Marco-Sola et al. 2012; Bolger et al. 2014; Ghosh and Chan 2016). Few analytical steps are important in this process and thus exist invariably in all metatranscriptome analysis which comprise of the separation of non-mRNA reads along with the host reads, filtering and trimming of low-quality nucleotides and reads, open reading frame analysis, mapping of the contigs reads to a known reference database finally normalisation and estimations of the gene expression levels along with other summarising statistics (Wang et al. 2009). However, one of the avoidable analytic steps which is not mandatory is the assembly of the reads into contigs and can be performed later. If performed, the assembly step is succeeded by mapping the contigs to reference genomes, when these are present. This step is computationally difficult to perform as it needs high-quality experimental sequencing data and helps in unravelling the information related to the gene expressions like the relation between start and stop sites and the adjacent genes. Experimentally, to facilitate the assembly, deeper sequencing is needed, and, thus, usually only highly abundant regions can be assembled from a larger set of reads (Morgan and Huttenhower 2014).

Another major challenge in the analysis and interpretation of biological facts by using metatranscriptomics data is integrating the analysis of both RNA-seq data and whole DNA data. It is highly recommended to evaluate these two data types at the same time for a sample to have a comparative study between the absolute expressed genes vs the possibly existing genes. Irrespective of the presence of the assembly step, at the termination of the RNA-seq analysis and the postnormalisation process, an outline of the data is changed into relative gene expression values and can later be analysed further like the statistical analyses observed in 16S and metagenomic sequencing.

21.4 Advances and Challenges in Bioinformatic Analysis

Bioinformatics complement the wet lab analysis in minimising and sorting the data to be processed in wet lab. Omics technology, be it related with genomics, proteomics or bolomics, cannot be successful with in silico analysis. Bioinformatic analysis during metatranscriptomics involves the following pathway:

(a) Filtering reads (QC and rRNA)
(b) Aligning reads to a reference (known gene)
(c) De novo assembly (unknown gene)
(d) Assigning transcript taxonomy
(e) Functional analysis or annotation
(f) Differential expression analysis

21.4.1 Statistical Analysis

(a) **Filtering reads (QC and rRNA)**: The library is split into individual files based on the barcode sequence, and the process is called de-multiplexing. The adapters are then removed, and sequence trimming is done. Each sequence base has its own quality value called the Phred score. The overall quality of the sequence is visualised via boxplots. Finally the rRNA is sorted out with rRNA database and MegaBLAST. Interpolated models like SSU-align come in handy at this stage. The FASTA files can be used as input for quality control and plot qualities by Fast_toolx. Similarly, Galaxy servers can be used to perform all the above said functions in one go.

(b) **Aligning reads to a reference**: Tools like Bowtie and BWA are used to map the reads to a reference sequence. Galaxy under NGS mapping can also be used with the libraries Rsamtools, summarizeOverlaps, and featureCounts of BioConductor for downstream analysis

(c) **De novo Assembly**: If the reference sequences are not known for the metatranscriptomic reads, then one has to go for de novo assembly. High-quality preprocessed reads can be put together into supposed transcripts using de novo assemblers. As the majority of the microbial communities are usually not characterised with reference genomes, de novo assemblers play a major role in supplying a reference scaffold exhibiting longer, expressed genome segments which can contribute a reference set of genes. This helps to easily identify the homologs and build a taxonomic origin which helps as a reference for mapping to aid expression analysis. Some metagenomic assemblers, viz. IDBA-UD, MEGAHIT and metaSPAdes, have proved their mettle in handling complex metagenome possessing some sequence similarity within highly conserved regions. These regions may however vary in terms of relative abundance in that microbiome affecting the strain level population variation (Peng et al. 2012; Li et al. 2015; Nurk et al. 2017). The responsibility of using metagenomic assemblers on metatranscriptomic datasets falls on the shoulders of the user as the parameters like distinct isoforms, presence of introns/exons and shorter non-coding RNAs (ncRNA) influence the efficacy of these assemblers immensely. People are using specific metatranscriptomic de novo assemblers like IDBA-MT, IDBA-MTP and Transcript Assembly Graph (TAG) (Leung et al. 2013, 2014b; Ye and Tang 2016). All these assemblers have been designed to consider the exclusive characteristics of both transcripts and the intricate nature of microbiome. IDBA-MT assembler is fabricated upon IDBA-UD to minimise the rate of mis-assemblies by employing many *k*-values simultaneously in a de Bruijn graph during accounting for characteristic features linked with mRNAs like common repeat patterns and uneven sequencing depth. IDBA-MT was further improvised in the form of IDBA-MTP to gather lowly expressed mRNAs. It applies the valuable information of recognised and identified protein sequences to lead the assembly by initiating with smaller *k*-values to form mRNA sequences that are then included depending on their homology and similarity with an identified protein set. TAG is a relatively new assembler

that also uses a de Bruijn graph, however, to assemble the corresponding metagenome that is further utilised as a reference to map the transcriptome reads and recreate mRNA sequences by spanning the metagenome assembly graph along with mapped transcriptome reads. As it considers genes are contiguous (without splicing), this specific tool is inefficient to be used in microbiomes that also include eukaryotes. There is a dire need to come up with more effective de novo assemblers for metatranscriptomic datasets. Currently very few tools are available which are created exclusively for metatranscriptomics, and their efficacy on diverse datasets has also not been tested thoroughly. The experimentalists still face a challenge to establish their worth in terms of exploring community complexities and their memory to cope with hardware and data volume.

(d) **Assigning transcript taxonomy:** After de novo assemblage of the transcript, one has to recognise the taxonomic profiling of the reads or contigs. Tools which help in taxonomic profiling of shotgun metagenomic data can be used to identify the members which are actively expressing RNA. Some tools can sort such members focusing exclusively on ribosomal RNA; however, during the process of preprocessing and purifying RNA, major chunk of rRNA is removed. Some tools focus on short reads and rely on nucleotide matches. GOTTCHA, Kraken and MetaPhlan2 are some read-based taxonomy classification tools whose potency is limited to the microbial communities having nearby neighbours in existing sequence databases (Wood and Salzberg 2014; Freitas et al. 2015; Truong et al. 2015; Neves et al. 2017). Kraken2 and centrifuge are useful in dealing with longer contigs and full-length transcripts which help in unravelling bigger members of any community (Wood and Salzberg, 2014; Kim et al. 2016). However these tools lack the efficacy in terms of processing the huge volumes of data and somehow are better with short sequences. Therefore, many tools can work only with a subset of accessible genomes like prokaryotes and cannot focus on eukaryotic database. More and more efforts have been put in to incorporate eukaryotic genomes that pose much complexity within their databases, viz. kaiju (Truong et al. 2015) and MetaPhlan2 (Menzel et al. 2016), but their potency in characterising eukaryotes has not been tested fully. Moreover, it is usually difficult to anticipate and sort out low abundance hits from prevailing false-positive hits, which is an inherent issue with microbiome studies. Our common lack of knowledge on complete microbiome and in any biological system being studied can also prevent the applicability of taxonomy classification tools.

(e) **Functional Expression or Annotation:** The advantage of metatranscriptomics over metagenomics lies in determining the functional activity of a microbiome. The tool came into picture to correlate the expressed transcript and the real phenotype taking into account the function of the transcript. Annotation of assembled transcripts progresses in a similar way to the annotation of genomes and metagenomes. The assembled reads or contigs are functionally annotated by read-based functional profilers like HMM-GRASPx, MetaCLADE and UProC (Meinicke 2015; Zhong et al. 2016; Ugarte et al. 2018). These profilers feed

upon tool-specific databases in the form of predicted ORFs which are provided by tools like FragGeneScan (Rho et al. 2010). One of the efficient tools is MetaCLADE that takes into account database containing a majestic number of two million probabilistic models from 15,000 Pfam domains; hence it works on hundreds of models depicting any single domain, to encircle the diversity of every domain across the tree of life. A search performed against this database appears in large numbers of hits per read which are later filtered on the basis of probability, redundancy and bit scores (Ugarte et al. 2018). Starting with genes, the initiation is done by programmes like FragGeneScan and Prodigal (Rho et al. 2010; Hyatt et al. 2010) which helps in finding genes; thereafter, functional assignment is given depending on similarity searches where tools like DIAMOND (Buchfink et al. 2015) come as a boon to search against functional databases like NCBI RefSeq, KEGG, UniProt (Kanehisa and Goto 2000; O'leary et al. 2016; UniProt Consortium 2019), etc. Additional pipelines, platforms and tools include a range of bioinformatics utilities (including annotation and gene finding) such as EDGE Bioinformatics described by Li et al. in 2017, Prokka by Seemann in 2014 and MG-RAST by Wilke et al. (2016), which integrate a number of similarity searches across various databases or can also couple assembly, annotation and gene calling via similarity searches. Once annotations are completed, enzymatic functions may also be mapped to determine metabolic pathways, with the help tools like iPath (Yamada et al. 2011) or MinPath (Ye and Doak 2009).

(f) **Differential Expression Analysis:** The benefits of metatranscriptomics do not end with finding the active members of any community, but the science also deals in exploring the gene expression in relation to time and their effect on each other which helps to explore community dynamics over time. Some tools are specially designed to focus on single genome and its differential gene expression. They take ample amount of data per gene (transcript) and per sample taking into account the expression under a unit condition at a unit time. Amplification or number of data is achieved by including few forms of read alignments a reference genome/gene set/assembly. DeSeq2 (Robinson et al. 2010), EdgeR and limma (Love et al. 2014) are some popularly used R packages which use the abundance information, to determine and identify genes that are significantly statistically differentially expressed with respect to condition and time among a number of samples (Ritchie et al. 2015). According to Luo et al. (2009), tools like Generally Applicable Gene-Set/Pathway Analysis (GAGE) may be used to recognise metabolic pathways which show clear upregulation in any specific condition over another. Complications such as shared genes amidst closely related organisms and alterations in the taxonomic composition of transcripts can lead to inaccurate determination of gene expression profiles which makes transcriptomic analysis quite challenging (Tarazona et al. 2015). To reduce the effect of taxonomic diversity in the sample, some people have tried normalisations in terms of count data based on taxonomic compositions but that is also not unbiased (Klingenberg and Meinicke 2017).

(g) **Statistical Analysis:** For statistical analysis a count matrix needs to be build which is done by counting the occurrence of aligned reads in each sample/experiment. The count matrix is transformed using tools like regularised-logarithm transformation (rlog). This helps to normalise the data between experiments, samples, and replicas, diminishing the importance and dependence of mean values with the help of further downstream process software like R's Bioconductor package DESeq2 and its function RNAseqGene (Love et al. 2014). Heatmap packages are used to assess sample similarity and dissimilarity and calculate the distance on the r log transformed data. Statistical analysis is done using null hypothesis, and p value is calculated. MetagenomeSeq which is available as part of Bioconductor and a standalone Web server (metastats) can be used to calculate FDR and corrected p-values. The most abundant features are connected to its annotation. The known and annotated genes are made sense for their gene expression under tested circumstances. Thus the whole dataset of significant genes can be divided into genes with known functions and genes with unknown functions. Most of the functional analysis is done on known annotated genes as they are easy to work upon, but the unknown transcripts are the most suitable candidates to discover newer members or members showing newer functions, viz. mutants or heterologous expression. The process of gene function data mining for the genes with known functions is done with starting points like the Protein Data Bank, UniProt, KEGG, EcoCyc and STRING which gives an overall knowledge about the protein. More benefits can be gained if the crystal structure and phylogenetic distribution are also known. Omics technology related to transcript shows us the functions of all the genomic sequences and its applications in varied fields, be it healthcare, agriculture or environment. Figure 21.4 depicts the different areas where metatranscriptomics has played a revolutionary role in exploring new depths and helping the mankind to fight adversities.

21.5 Conclusion

Every technique grows to cover up its loopholes. Although contemporary metatranscriptomic techniques are propitious, it is not free of its challenges and limitations which hold them back to serve on a larger scale and horizons. The NGS revolution was started while keeping metagenome in mind though its arms were extended to study the gene expression of the transcript. However, the various goals don't attain full success owing to the lack of proper reference genomes. These known genomes are the guiding lights to characterise the functions of the genes. The procedure of metatranscriptomics is full of hurdles starting with harvesting of RNA as the raw material. The total RNA comprises of mRNA and rRNA. Studies suggest that most of the garnered RNA comes from ribosomal RNA, and its overshadowing amount in the raw material can dramatically reduce the availability

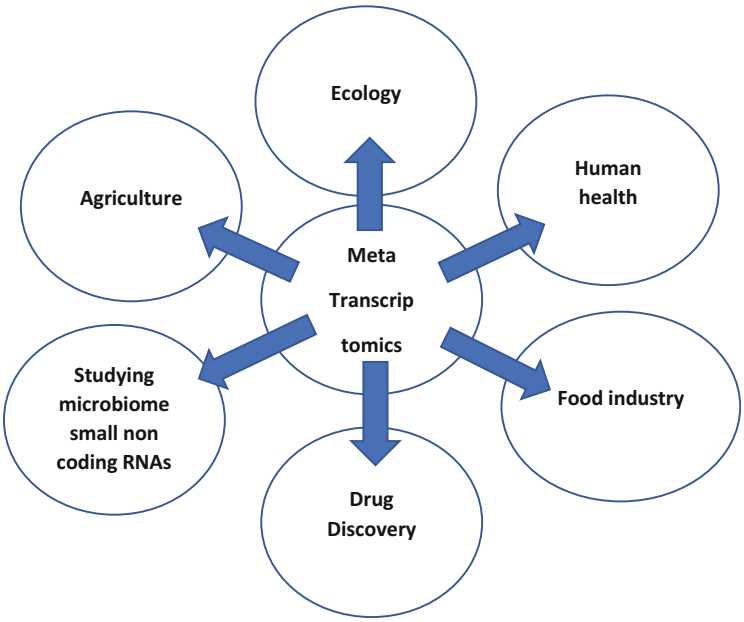

Fig. 21.4 Applications of metatranscriptomics

of mRNA which actually plays a pivotal role in metatranscriptomic studies. Although some attempts have been made scrupulously to remove the rRNA from the collected RNA pool, it somehow happens to be a failure as mRNA is tremendously unstable which leads to suspicion of integrity of the raw sample even before sequencing. This creates another challenge to differentiate between the host and microbial RNA. The problem has been dealt either with the use of enrichment kits available commercially or through in silico tools only if a reference genome is accessible as this is also one big challenge as the transcriptome reference databases are very limited in their range.

The half-life of mRNA is very short; therefore, the samples should be stored at very low temperature to give better results and maintained in an RNA preservation solution. During the isolation process of RNA from soil or other environment, most of the time humic acids and fulvic acids get co-precipitated. Therfor it is practised to use calcium chloride or calcium carbonate for the pretreatment of the soil to remove humic acids and fulvic acids. However, the enrichment methods comprising of size separation using gel electrophoresis, use of exonucleases, bacterial mRNA enrichment kits or subtractive hybridisation come very handy to solve the problem up to some extent.

Another problem which limits the pace of metatranscriptomics is the fact that the transcription and translation take place simultaneously in prokaryotes. This means that the transcripts which we work on may be partial. To overcome this loophole an amplification step may be performed which may lead to the enhancement of the

quantity of RNA. Moreover direct RNA sequencing can help to curb the issue of error generation during reverse transcription to make cDNA. Only the expression of RNA at a given time which is one of the major limitation of RT PCR and microarray. Besides both the methods have a prerequisite for sequence of the desired gene in order to design matching probes or primers. The efficacy of the system is hampered by the paucity of proper reference genomes that can lead to a suboptimal portion of reads from several datasets from being taxonomically or functionally characterised.

Although the new-generation sequencing insurgence came into being to facilitate the study of cultured and uncultured genomes, it got seasoned to the functional studies and helped in understanding the depth of the dynamics of intricate biological systems.

The lack of concomitant availability of samples and experimental metadata to study the complex datasets is a big drawback in doing the global meta-analysis of various essential pathways.

According to Yilmaz et al. (2011), minimum information regarding any sequence or MIxS is essential to a set of standards for inclusion of adequately structured metadata when depositing metatranscriptomic (or any omics) datasets which would permit such all-inclusive analyses.

A large number of reads or data points are required to explore the wide dynamic range of members present in any community and the functional expression of any gene in any organism at a given time. The high-throughput short read technology has an edge over the long read ones; however, the latter one is promising in analysing taxonomy determination, resolving polycistronic operons and studying transcript isoforms with high similarity.

In this chapter, we have tried to highlight the advances and challenges related to some popular ways of analysing metatranscriptomics data and the specific bioinformatics tools used during the process. The intricacy of real microbiomes and the inadequate knowledge of the metagenomes have always played a devil's role in performing crucial benchmark experiments. Apart from the previous ad hoc metrics to check performance using sequencing data and real samples, there is a dire need to develop user-friendly and efficient tools that are actually able to imitate real sequencing datasets. A generally agreed framework for benchmarking new tools would benefit the field progress and perhaps unite towards appropriate and accurate workflows. Despite facing some very big challenges, the science of metatranscriptomics is ceaselessly developing accessed with new algorithms and tools for the analysis of the complex data and shows great potential in improving our outlook of the biologically active part of microbiomes and the appropriate pathways involved.

References

Abubucker S, Segata N, Goll J, Schubert AM, Izard J, Cantarel BL, Rodriguez-Mueller B, Zucker J, Thiagarajan M, Henrissat B, White O (2012) Metabolic reconstruction for metagenomic data and its application to the human microbiome. PLoS Comput Biol 8(6):34–38

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data
Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15):2114–2120
Buchfink B, Xie C, Huson DH (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12(1):59–63
Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34(17):i884–i890
Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26 (19):2460–2461
Freitas TAK, Li PE, Scholz MB, Chain PS (2015) Accurate read-based metagenome characterization using a hierarchical suite of unique signatures. Nucleic Acids Res 43(10):e69–e69
Ghosh S, Chan CKK (2016) Analysis of RNA-Seq data using TopHat and cufflinks. In: Plant bioinformatics. Humana Press, New York, pp 339–361
Glass EM, Wilkening J, Wilke A, Antonopoulos D, Meyer F (2010) Using the metagenomics RAST server (MG-RAST) for analyzing shotgun metagenomes. Cold Spring Harb Protoc 2010 (1):pdb-prot5368
Gosalbes MJ, Durbán A, Pignatelli M, Abellan JJ, Jiménez-Hernández N, Pérez-Cobas AE, Latorre A, Moya A (2011) Metatranscriptomic approach to analyze the functional human gut microbiota. PLoS One 6(3):23–27
Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ (2010) BMC bioinformatics [electronic resource]. BMC Bioinform 11:119–120
Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL (2008) NCBI BLAST: a better web interface. Nucleic Acids Res 36(suppl_2):W5–W9
Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30
Kim D, Song L, Breitwieser FP, Salzberg SL (2016) Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Res 26:1721–1729
Klingenberg H, Meinicke P (2017) How to normalize metatranscriptomic count data for differential expression analysis. PeerJ 5:e3859
Korf I (2013) Genomics: the state of the art in RNA-seq analysis. Nat Methods 10(12):1165–1166
Kopylova E, Noé L, Touzet H (2012) SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28(24):3211–3217
Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9 (4):357
Leimena MM, Ramiro-Garcia J, Davids M, van den Bogert B, Smidt H, Smid EJ, Kleerebezem M (2013) A comprehensive metatranscriptome analysis pipeline and its validation using human small intestine microbiota datasets. BMC Genomics 14(1):530
Leung HCM, Yiu S-M, Parkinson J, Chin FYL (2013) IDBA-MT: de novo assembler for metatranscriptomic data generated from next-generation sequencing technology. J Comput Biol 20:540–550
Leung HC, Yiu SM, Chin FY (2014a) IDBA-MTP: a hybrid metatranscriptomic assembler based on protein information. In: International conference on research in computational molecular biology. Springer, Cham, pp 160–172
Leung HC, Yiu SM, Chin FY (2014b) IDBA-MTP: a hybrid metatranscriptomic assembler based on protein information. Res Comput Mol Biol 22(5):367–376
Li H, Pan Y, Zhang SC, Du R, Li P, Li S (2015) Transcriptomic analysis of clostridium thermocellum in cellulolytic consortium after artificial reconstruction to enhance ethanol production. Bioresources 10(4):7105–7122
Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550
Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ (2009) GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinf 10:161

Marco-Sola S, Sammeth M, Guigó R, Ribeca P (2012) The GEM mapper: fast, accurate and versatile alignment by filtration. Nat Methods 9(12):1185

Martinez X, Pozuelo M, Pascal V, Campos D, Gut I, Gut M et al (2016) MetaTrans: an open-source pipeline for metatranscriptomics. Sci Rep 6:26447

Meinicke P (2015) UProC: tools for ultra-fast protein domain classification. Bioinformatics 31:1382–1388

Menzel P, Ng KL, Krogh A (2016) Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nat Commun 7:11257

Morgan XC, Huttenhower C (2014) Meta'omic analytic techniques for studying the intestinal microbiome. Gastroenterology 146(6):1437–1448

Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M (2007) KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 35:W182–W185

Neves ALA, Li F, Ghoshal B, Mcallister T, Guan LL (2017) Enhancing the resolution of rumen microbial classification from metatranscriptomic data using Kraken and Mothur. Front Microbiol 8:2445

Ni Y, Li J, Panagiotou G (2016) COMAN: a web server for comprehensive metatranscriptomics analysis. BMC Genomics 17(1):622

Nurk S, Meleshko D, Korobeynikov A, Pevzner PA (2017) metaSPAdes: a new versatile metagenomic assembler. Genome Res 27:824–834

O'leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, Mcveigh R et al (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745

Peng M, Xie Q, Hu H, Hong K, Todd JD, Johnston AW, Li Y (2012) Phylogenetic diversity of the dddP gene for dimethylsulfoniopropionatedependent dimethyl sulfide synthesis in mangrove soils. Can J Microbiol 58(4):523–530

Rho M, Tang H, Ye Y (2010) FragGeneScan: predicting genes in short and error-prone reads

Ritchie ME, Phipson B, Wu DI, Hu Y, Law CW, Shi W, Smyth GK (2015) Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43(7):e47

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26(1):139–140

Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30 (14):2068–2069

Tarazona S, Furió-Tarí P, Turrà D, Pietro AD, Nueda MJ, Ferrer A, Conesa A (2015) Data quality aware analysis of differential expression in RNA-seq with NOISeq R/Bioc package. Nucleic Acids Res 43(21):e140–e140

Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E, Tett A, Huttenhower C, Segata N (2015) MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods 12 (10):902–903

Ugarte A, Vicedomini R, Bernardes J, Carbone A (2018) A multi-source domain annotation pipeline for quantitative metagenomic and metatranscriptomic functional profiling. Microbiome 6(1):149–153

UniProt Consortium (2019) UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47 (D1):D506–D515

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10(1):57–63

Westreich ST, Korf I, Mills DA, Lemay DG (2016) SAMSA: a comprehensive metatranscriptome analysis pipeline. BMC Bioinform 17(1):399–404

Wilke A, Bischof J, Gerlach W, Glass E, Harrison T, Keegan KP, Paczian T, Trimble WL, Bagchi S, Grama A, Chaterji S (2016) The MG-RAST metagenomics database and portal in 2015. Nucleic Acids Res 44(D1):D590–D594

Wood DE, Salzberg SL (2014) Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15(3):R46–R49

Yamada T, Letunic I, Okuda S, Kanehisa M, Bork P (2011) iPath2. 0: interactive pathway explorer. Nucleic Acids Res 39(suppl_2):W412–W415

Ye Y, Doak TG (2009) A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. PLoS Comput Biol 5(8):87–92

Ye Y, Tang H (2016) Utilizing de Bruijn graph of metagenome assembly for metatranscriptome analysis. Bioinformatics 32(7):1001–1008

Yilmaz P, Kottmann R, Field D, Knight R, Cole JR, Amaral-Zettler L, Gilbert JA, Karsch-Mizrachi I, Johnston A, Cochrane G, Vaughan R (2011) Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications. Nat Biotechnol 29(5):415–419

Zhong C, Edlund A, Yang Y, McLean JS, Yooseph S (2016) Metagenome and metatranscriptome analyses using protein family profiles. PLoS Comput Biol 12(7):67–71

Metatranscriptomics: A Promising Tool to Depict Dynamics of Microbial Community Structure and Function

22

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Abstract

High-throughput sequencing of metatranscriptomes from various environments has enabled researchers in accessing information about both the known and unknown transcripts expressing in natural communities. Metatranscriptomics allows investigator to retrieve information about whole microbial community, with main emphasis on active functional genes. It investigates entire RNA of microbial community, holding potential to deliver an innovative and new approach to community-specific functional genes and pathways. Metatranscriptome has enabled researchers to probe the active and dynamic microbial populations from different environments such as marine, soil, water, human gut, etc. This chapter will discuss concepts, tools and techniques used to investigate metatranscriptome and will further highlight its application in understanding the microbial structure and function.

Keywords

Metatranscriptome · Sequencing · Ecology · Bioinformatics

22.1 Introduction

Our understanding about microorganisms in natural environments is limited, and thus examining microbial community structure and function in natural environment is important to understand their structure and functioning. From literature, it becomes evident that a gram of soil or residue may comprise 10^{10} bacteria (Torsvik

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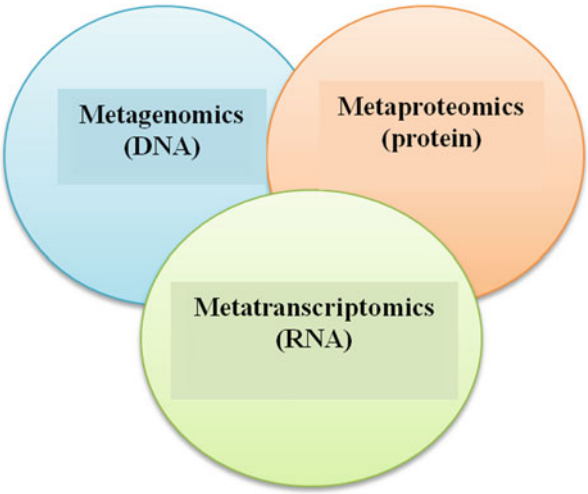

Fig. 22.1 Various strategies of "meta-omics" approaches

et al. 1990; Gans et al. 2005). There are numerous microorganisms which have adapted to almost all the environments. There are microbes that are capable of decomposing all the chemical constituents made by active organisms (Fakruddin and Mannan 2013). Important questions that need to be addressed while studying bacteria under natural environments include how do microbial communities function? How do the environmental changes impact the qualitative distinction in community composition? (Torsvik and Øvreås 2002). Studying microbial metatranscriptome offers the prospective to understand microbial communities structure and function in their native ecological surroundings. It also helps in accessing ample source of genes with biotechnological concern (Bailly et al. 2007).

Meta-analysis of structural and functional genomics has led to huge expansion and understanding of microbial communities. This is result of advances in high-throughput sequencing of DNA, which has enabled investigators to better analyse the microbial population structure and function through high-resolution and culture-independent method (Franzosa et al. 2015). Metatranscriptomics is a quite new technical advancement but has freshly been applied in characterizing functions of a range of microbial communities (Leininger et al. 2006; Frias-Lopez et al. 2008; Shi et al. 2009; Poretsky et al. 2009; Vila-Costa et al. 2010; Ettwig et al. 2010; Helbling et al. 2012). Environmental transcriptomics is a vital method to explore functional gene expression in natural microbial communities. It hold potential to deliver an innovative and new approach to find community-specific functional genes (Poretsky et al. 2005). Meta-sequencing and analysis of genes from an ecosystem (metatranscriptome) can yield information about response of microorganisms under varying environmental conditions. It is one of the important "omics" approaches as shown in Fig. 22.1. Until recently, expression of genes could be measured either by microarray technology or random cloning methodologies. Introduction of high-throughput sequencing technology has enabled researchers in accessing both the known and unknown transcripts from their natural communities

(Gilbert et al. 2008). Recently, metatranscriptomics has allowed investigators to depict functional profile of different microbial communities (Kuske et al. 2015; Bashiardes et al. 2016) and to recognize RNA viruses in a variety of animal samples (Shi et al. 2016, 2017; Zhang et al. 2018; Wille et al. 2018). It could also lead in understanding mycorrhizal communities (Liao et al. 2014; Gonzalez et al. 2018) and in probing several other communities (Marcelino et al. 2019a, b).

22.2 Concepts and Methods

Metatranscriptomics allows investigator to retrieve information about whole microbial community, with main emphasis on active functional genes. Metatranscriptomics studies target entire RNA from microbial community. Furthermore, since mRNAs outnumber bacterial rRNAs in microbial RNA pool, it is essential to enhance microbial mRNAs by reducing rRNA before sequencing (Giannoukos et al. 2012). Process involves conversion of mRNA into cDNA using reverse transcriptase, followed by sequencing by typical methods. Next to sequencing comes the correct barcoding of DNA and cDNA samples. The metatranscriptome sequencing is done in tandem, so that RNA sequencing can be employed as a natural extension for surveys related to microbial community (Franzosa et al. 2015). The workflow for metatranscriptomics studies is presented in Fig. 22.2. Working with mRNAs has always been difficult as it causes hindrance to relate environmental transcriptomics to biogeochemical activity of microorganisms (Liang and Pardee 1992). Various studies have shown that mRNA gets degraded very quickly as it has very short half-life, as short as 30 s (Selinger et al. 2013; Andersson et al. 2006).

Lastly, as pointed above, since rRNA molecules outnumber the mRNA in total RNA extracts, this results in suppression of mRNA signal in the background. Therefore to analyse fractional transcriptomes from environment, researchers have developed certain protocols to analyze mRNA. One such protocol was developed by Poretsky et al. (2005) who developed the following procedure. Firstly, collection of total RNA from the environment, subtractive hybridization to remove rRNA for mRNA enrichment, cDNA synthesis using randomly primed reverse transcription (RT) and generation of cDNA clone libraries by amplifying the templates using PCR. Using the above developed protocol, the author reported analysis of nearly 400 environmental gene transcripts recovered directly from bacterioplankton communities of Sapelo Island, GA, and Mono Lake, CA (Poretsky et al. 2005). Most of the procedures follow the same basic structure while studying metatranscriptome. Targeting genes which are transcribed in different environmental settings reduces resources required by the researcher and explains why considerable efforts are being carried out to develop these strategies. Direct extraction of DNA and RNA from microbes has been reported in a number of studies (Poretsky et al. 2005; Bailly et al. 2007). In these studies, complementary DNA libraries of RNA extracted from environment were prepared followed by sequencing of clones. Interestingly, maximum retrieved sequences compared with the publically accessible

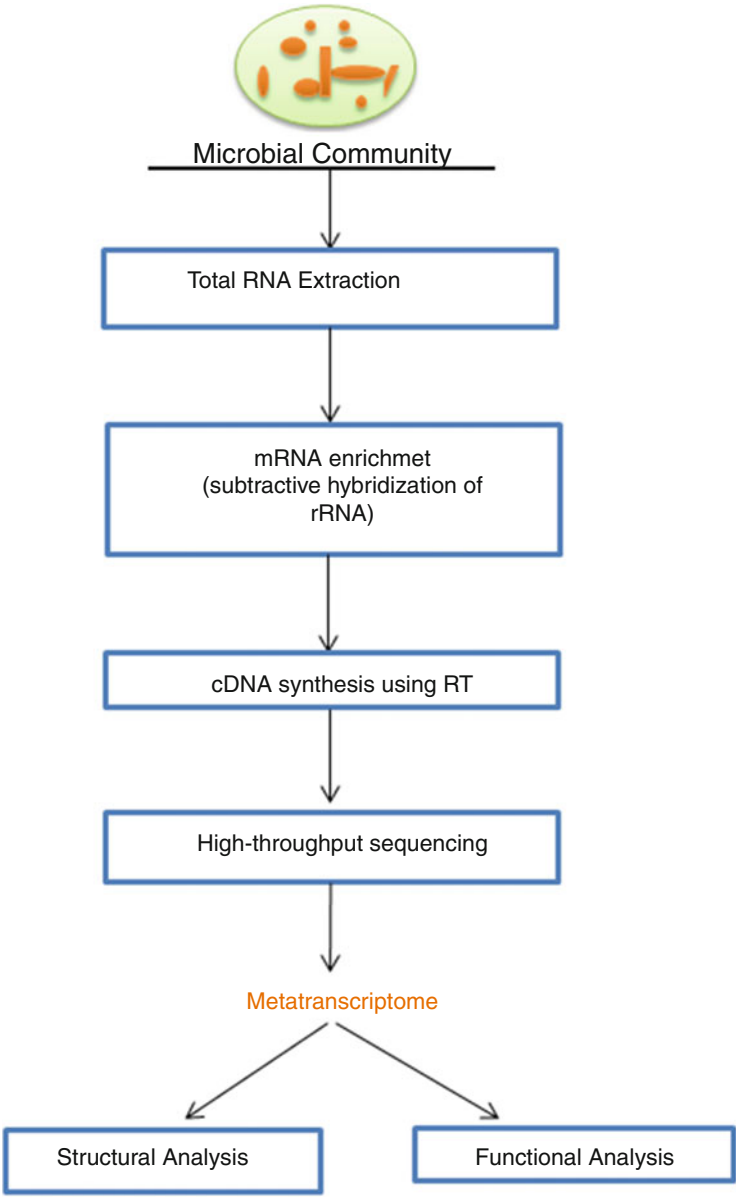

Fig. 22.2 Workflow of metatranscriptomic analysis of microbial communities using high-throughput sequencing

protein databases did not display sequence similarity with protein as reported in the databases. Thus, these studies demonstrate the potential of discovering novel proteins through metatranscriptomics (Warnecke et al. 2009).

Owing to the complication in metatranscriptomic data, widespread analysis is required. The initial raw data consists of millions of discrete reads per sample (Franzosa et al. 2014). Simplifying this data need a software and a keen bioinformatician for accomplishing the complex data analysis. Existing in-house approaches or pipelines need computing command or use of numerous different tools, a number of them were not initially designed for analysis of metatranscriptome (Embree et al. 2014; Gosalbes et al. 2011), and the investigators who perform metatranscriptomic analysis, however, may not have sufficient technical knowledge of bioinformatics. To cope with such problems, many bioinformatics pipelines and data assemblers were developed as described in Table 22.1 to make the metatranscriptomic analysis easy and fast.

22.2.1 Bioinformatic Pipelines Used to Investigate Metatranscriptomic Data

Below, we will provide detailed information about the pipelines used in analysing metatranscriptomics in an easy way.

Leimena Tool

Leimena et al. (2013) developed a reliable and effective pipeline for processing metatranscriptome data generated by Illumina-RNA sequencing by combining sequencing reads with different reference gene databases. It links sequence reads with its predicted functions and phylogeny origin. The data gathered after the processing of information might be used to attain complete biological discernments within biome's activity patterns. This pipeline was used in understanding the activity profiles of the microbiome in the human small intestine. SortMeRNA software was used to remove rRNA/tRNA reads from the distinctive Illumina reads. After removal step, the unique Illumina reads are checked for similarity in ribosomal databases at NCBI and SILVA using BLASTN alignment. Prokaryote genomes available in NCBI are assigned mRNA reads by MegaBLAST followed by BLASTN. After assigning to prokaryote genomes, mRNA reads are classified based on alignment bit scores. The minimum bit score for phylogenetic origin prediction at family and genus level corresponds to 148 and 110, respectively. After that, the reads assigned to the genome are categorized into coding or non-coding protein reads. This is followed by functional annotation by COG, KEGG and metabolic mapping. Further functional assignments are executed for assessment needs by allocating 10% of arbitrarily chosen unassigned reads that have bit score $\leq$74, to the NCBI protein database and then by MetaHIT and SI metagenome databases employing BLASTX tool.

MetaTrans

MetaTrans is a proficient, open-source, downloadable pipeline developed to assess the structural and functional aspects of active microbial populations. It uses powerful computers that support multithreaded applications. This pipeline is basically made to

Table 22.1 Various metatranscriptome analysis pipelines and data assemblers

S. No.	Bioinformatic analysis pipeline	Main features	References
1.	Leimena Bioinformatic Pipeline	Efficient exclusion of rRNA resultant sequences, assertive assignment of the anticipated function and taxonomy origin of the mRNA reads, functional for bacterial metatranscriptome study in any selected environment	Leimena et al. (2013)
2.	MetaTrans	A proficient open-source tool to investigate the structure and function of dynamic microbial populations It executes quality control evaluation, rRNA exclusion, mapping of reads, handles differential gene expression analysis	Martinez et al. (2016)
3.	SAMSA	Eliminate reads having low-quality bases, remove adapter contamination, sequence quality control check, sorting of unique annotations, to test differential expression	Westreich et al. (2016)
4.	COMAN	Controls quality of raw reads, elimination of reads obtained from non-coding RNA, functional annotation of relative statistical analysis, pathway enhancement analysis, analysis of co-expression network, great quality visualization	Ni et al. (2016)
5.	SAMSA2	Stand-alone use on a supercomputing cluster, faster, more flexible and reproducible than SAMSA, availability of illustration input and yield files beside illustrations of master scripts	Westreich et al. (2018)
6.	IMP (Integrated Meta-omic Pipeline)	Reproducible and modular pipeline, large-scale standardized integrated study of joined metagenomic and metatranscriptomic information, integrates vigorous read preprocessing, examines microbial community structure and function, analyses of genomic signature-based visualizations	Narayanasamy et al. (2016)
7.	IDBA-MT	Made for collecting reads from metatranscriptomic information, yields much less chimeric contigs, resolve merged mRNAs using the k-mer multiplicity (local support) at each vertex and paired-end information, produce longer contigs for data with uneven sequencing depth	Leung et al. (2013)
8.	Trans-ABySS	A de novo short-read transcriptome assembly and analysis pipeline, addresses variation in local read densities by assembling read substrings, merges the resulting contigs before analysis	Robertson et al. (2010)
9.	Trinity	A novel method for the efficient and robust de novo reconstruction of transcriptomes from RNA-seq data, combines three independent	Haas et al. (2013)

(continued)

Table 22.1 (continued)

S. No.	Bioinformatic analysis pipeline	Main features	References
		software modules, Inchworm, Chrysalis and Butterfly; partitions the sequence data into many individual de Bruijn graphs; represents the transcriptional complexity at a given gene or locus	
10.	Oases	Designed to heuristically assemble RNA-seq reads in the absence of a reference genome, uses an array of hash lengths, a dynamic filtering of noise, a robust resolution of alternative splicing events, tested on human and mouse RNA-seq data	Schulz et al. (2012)
11.	IDBA-tran	Use a probabilistic progressive approach to iteratively remove erroneous, vertices/edges with local thresholds, able to assemble both high-expressed and low-expressed transcripts	Peng et al. (2013)
12.	Rockhopper	Reference-based transcript assembly, de novo transcript assembly, normalizing data from different experiments, quantifying transcript abundance, testing for differential gene expression, characterizing operon structures, visualizing results in a genome browser	Tjaden (2015)

accomplish dual analysis of paired-end RNA-Seq: 16S rRNA taxonomic analysis and gene expression analysis. It employs the following steps: quality control of reads, elimination of rRNA, mapping reads to various functional databases and performing analysis of differential gene expression. MetaTrans involves many tools such as FastQC tool, Kraken pipeline, SortMeRNA, SOAP2, FragGeneScan and many more; each of them performs different functions. Its effectiveness was validated by studying and examining different data of synthetic pseudo-communities, data from a past study and information produced from 12 human faecal samples (Manichanh et al. 2014). When compared with current Web server, MetaTrans exhibits more proficiency in runtime. It takes about 2 h per million of transcripts. In this way, MetaTrans presents a modified tool to compare gene expression levels. Though the pipeline is tested using human gut microbiome dataset, it also offers a choice to utilize a general database so as to analyze additional environments (Martinez et al. 2016).

SAMSA

Westreich et al. created SAMSA (Simple Analysis of Metatranscriptome Sequence Annotations) pipeline (Westreich et al. 2016), which was designed to entirely evaluate and investigate bacterial metatranscriptome, demonstrating comparative levels of transcription in organism and functional grouping. Overall it has four stages, and every stage involves various tools such as MGRAST for analysis of data. The first phase is the preprocessing phase that involves trimming and

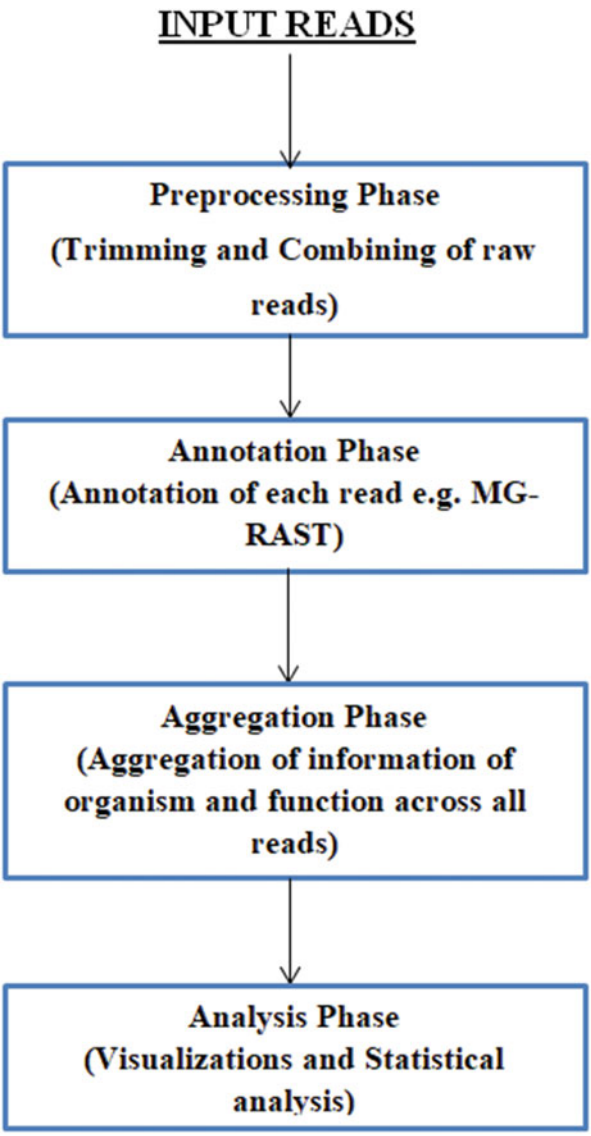

Fig. 22.3 The SAMSA pipeline

combining of reads so that they can be used as input for the annotation phase. The second phase is the annotation which offers annotation for every single read. The third phase is the aggregation in which organism and functional data from all reads is collected. The fourth and last phase is the analysis part which delivers visualizations and statistical analysis. The SAMSA pipeline is shown in Fig. 22.3.

COMAN

The application of new-generation sequencing (NGS) in metatranscriptomics produces massive and complex data which has to be evaluated efficiently for translation of non-explicable raw sequence reads to biological discernments, in the form of data tables and figures. Many relevant techniques or pipelines have been suggested for handling RNA-Seq data as mentioned above. The complete analysis method for this high-throughput information generally consists of several discrete steps, and it needs setting up and execution of extensive sort of software tools, wide computational sources and proficiency in program design and NGS bioinformatics information processing. Leimena et al. (2013) pipeline has talked about providing a well-defined pipeline for analysing metatranscriptomic RNA-seq data of Illumina; however, its implementation as a software system or Web-based server was not successful. MetaTrans software and SAMSA need appropriate setup on a powerful computer and deliver limited functional analysis. Hence, as metatranscriptomic information is nowadays regularly produced, it is difficult for wet lab investigators to evaluate such huge data and create biologically significant data. To eliminate these limitations, COMAN, which is a Web-based server, was designed for functional description and complete exploration of high-throughput metatranscriptomic data. It automatically processes the uploaded raw reads and eventually accomplishes functional assignments. These are then utilized to execute relative statistical analysis, path enhancement and co-expression linkage analysis, to link and compare taxonomy with functional distinctions and to envisage the results. COMAN provides easy user interface and detailed guidelines and can be used by researchers lacking software design experience and eliminating the difficulty of altering tools or operating settings for countering the biologically significant queries. Also the crucial data are given in tabular layout, so users with bioinformatics proficiency might execute further analysis and integration with other different software (Ni et al. 2016). The metatranscriptome analysis pipeline in COMAN is shown in Fig. 22.4.

SAMSA2

The initial studies describing metatranscriptomics analysis represented as workflows failed to offer code or a software platform (Leimena et al. 2013; Davids et al. 2016). MGRAST (Meyer et al. 2008) or COMAN allows operators to analyse metatranscriptome information; however, the level of analysis is limited. Though the above-mentioned approaches enable analysis without the necessity for local computing systems, both COMAN and MGRAST are reliant on a service that might become slow because of oversubscribed and that they do not provide mapping to conventional and standard reference databases (Ni et al. 2016). MetaTrans relies on rRNA for the identification of organism, requiring the absence of biological ribodepletion and therefore decreasing the amount of obtained functional information from mRNA. On the other hand SAMSA2 is completely separate and is meant for group computing. In this, software and databases are totally packed for reproducibility. It uses DIAMOND (Buchfink et al. 2015) to align sequences that significantly escalate its speed in comparison to BLAST tool or other open-source services. It supports end-to-end metatranscriptome analysis by controlling the quality of reads

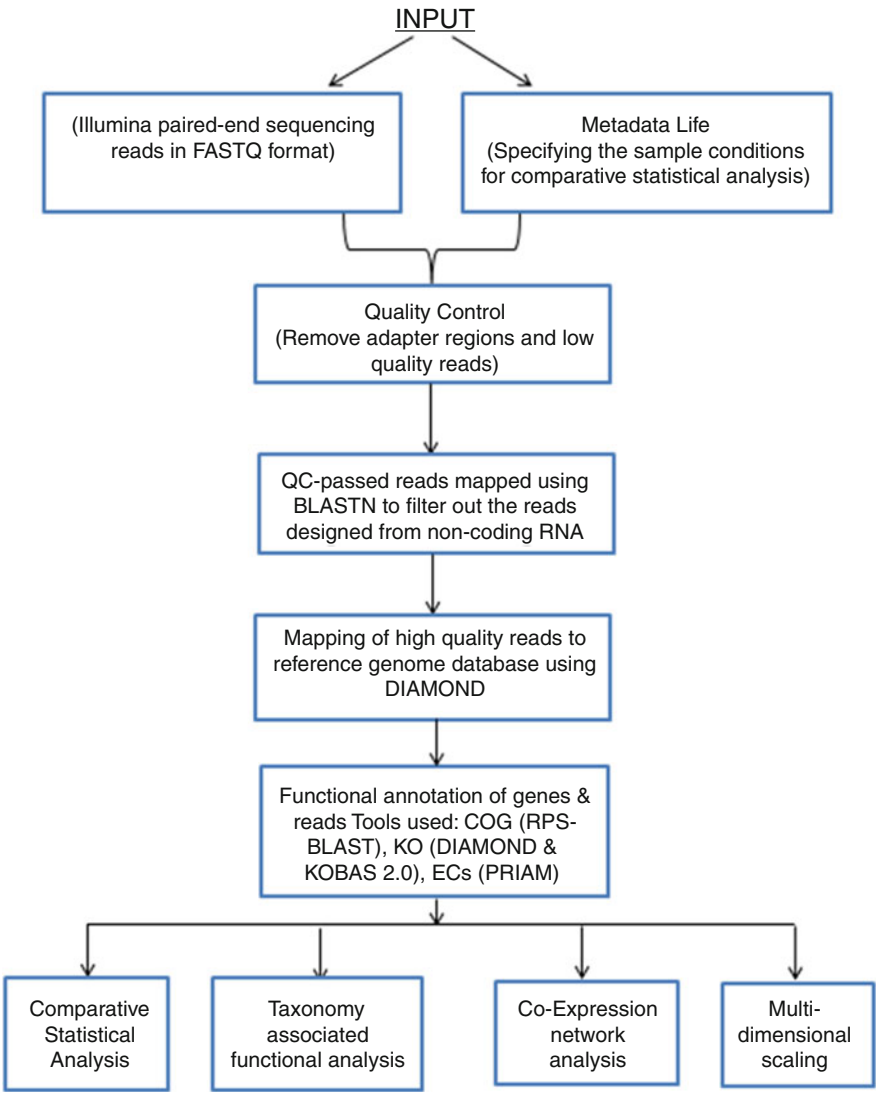

Fig. 22.4 COMAN pipeline

via publication-ready depictions. It also comes with sample information and documentation (Westreich et al. 2018).

22.3 Insights About Active and Dynamic Microbial Community

Initially metatranscriptome studies reported transcripts from *Archaea* and *Bacteria* related to carbon, sulphur and nitrogen cycle from bacterioplankton communities of oceanic and freshwater communities (Poretsky et al. 2005; Cardenas and Tiedje 2008). Poretsky et al. (2005) observed expression of genes from communities of bacterioplankton present in marine and freshwater. Environmental mRNA was enriched using subtractive hybridization approach and was then reverse transcribed using reverse transcriptase. The cDNA obtained was amplified using random primers and cloned, nearly 400 clones were examined, out of which >80% clones explicitly were mRNA derived. The sequences obtained were of varied taxonomic clusters, including both *Bacteria* and *Archaea.* Several transcripts identified were associated with environmentally essential processes like sulphur oxidation (*soxA*), C1 compounds assimilation (*fdh1B*) and polyamine degradation (*aphA*) (Poretsky et al. 2005). Bailly et al. (2007) employed metatranscriptomics approach to investigate eukaryotic microbial communities in soil using an experimental approach involving cDNA library construction and its screening via polyadenylated mRNA. The library obtained was evaluated by sequencing its 18S rDNA gene. The genes were either amplified using reverse-transcribed (RT) RNA or DNA from soil. More than 70% of the total sequences identified belong to either fungi or unicellular eukaryotes (protists); interestingly most characterized class identified belong to metazoa. Calculations based on richness approximation revealed that there might be more than 180 species inhabiting these soil samples. cDNA sequencing of 119 cDNA clones showed that the identified genes have no homology in databases. It also identified genes that code for proteins that are related with various diverse cellular and biochemical processes. RNA-centred metatranscriptomic approach can also be applied to gain simultaneous information about both structure and function of a soil community. Urich et al. (2008) used entire community RNA and cDNA without the need of any polymerase chain reaction (PCR) or cloning. Using pyrosequencing, a huge number of cDNA rRNA tags were yielded and were taxonomically described using MEGAN and two precisely assembled rRNA reference databases encompassing small and large subunit sequences of rRNA. It also generates mRNA tags which resulted in this quantifiable data about the comparative richness of organisms belonging to all three domains of life and covered diverse trophic levels attained in a single experiment. Additionally, in situ activity could be confirmed by presence of mRNA tags specific for enzymes associated with ammonia oxidation and CO_2 fixation. A comparative investigation of microbiota from Arctic peat soil using metatranscriptomics by Tveit et al. (2014) revealed that the number of transcripts encoding cellulose degrading enzyme declined with depth, whereas the number of transcripts encoding debranching of hemicellulose increased with depth. This indicated that the composition of polysaccharides present in the peat was dissimilar in deep and old layers. The annotation of taxonomy revealed dominance of polysaccharide decomposers named as *Actinobacteria* and *Bacteroidetes*. The study further documented that both 16S rRNA and mRNA transcripts of methanogenic *Archaea* increase significantly if one goes deeper into layers. In a

nutshell, linear amplification and sequencing of whole RNA are ideal and important to facilitate high taxonomic resolution and functional analyses of the dynamic microbiota found in soil of Arctic peat. Zhu et al. (2019) reported metabolic characteristics of mycobacterial community found in biofilm of water metre via metagenomics and metatranscriptomics approach. Even though mycobacteria are among the frequently occurring bacterial communities in water metre biofilm, its in situ metabolic patterns are typically unexplored. Coupled metagenomic/metatranscriptomic approach unveiled the metabolic aspects of mycobacteria hence showing its propitious application. Microbial communities structure, function and dynamics were investigated in thermophilic composting, to identify new thermophilic bacteria (Federici et al. 2011; Jurado et al. 2014; Yang et al. 2013) and novel thermostable enzymes, predominantly those associated with biomass degradation offers several advantages for industrial applications (Allgaier et al. 2010; Gladden et al. 2011; Dougherty et al. 2012; D'haeseleer et al. 2013; Habbeche et al. 2014; Mhuantong et al. 2015). Though, several studies have been performed to explore thermophilic composting ecosystems, using culture-dependent (Dees and Ghiorse 2001; López-González et al. 2015) and culture-independent techniques (Partanen et al. 2010; Neher et al. 2013; De Gannes et al. 2013; Martins et al. 2013; D'haeseleer et al. 2013; Tkachuk et al. 2014), there has only a few evidence regarding functional characteristics of related microbiota. Metatranscriptomics however is a valuable tool to identify range of biodegrading microbes and metabolically functionality during thermophilic composting. Martinez et al. (2016) revealed that the São Paulo Zoo harbours considerable microbial diversity, and exploiting this finding they extended the previous work. The key novelty of the study was the combination of three important factors that is time-based sampling through shotgun DNA, amplification of 16S rRNA gene and metatranscriptome sequencing using high-throughput techniques, facilitating first-time comprehensive outlook of microbial population structure, dynamics and function in this ecology (Antunes et al. 2016).

In addition, human gut is the natural habitat for a huge active bacterial community that significantly impacts the human health. Though metagenomics has increased our understanding about gene content and functional and genetic diversity, little is known regarding functional dynamics of bacteria in the gastrointestinal system. A metatranscriptomic study performed in ten healthy volunteers revealed presence of active microbial community of *Lachnospiraceae*, *Bacteroidaceae*, *Ruminococcaceae*, *Prevotellaceae*, and *Rickenellaceae*. The description of mRNAs revealed an even functional pattern in individuals, and key functions identified belong to metabolism of carbohydrate, production of energy and cellular components' synthesis. On the contrary, housekeeping events, for example, lipid and amino acid metabolism, were diminished in the metatranscriptome. These findings offer new insights related to the functioning of the complex microbiota of gut in healthy individuals (Gosalbes et al. 2011). Further, Marcelino et al. (2019a, b) investigated antibiotic resistance pool in birds using metatranscriptome-based approach. In general most of the studies carried out on birds are culture based; therefore, advances in the culture-independent sequencing methods have markedly

extended the understanding of the ecological pool of resistance genes (Zhu et al. 2013; Bengtsson-Palme et al. 2017; Su et al. 2017; Crofts et al. 2017; Surette and Wright 2017; Zhu et al. 2017; Zhao et al. 2018; Munk et al. 2018). Among these methods, sequencing of whole set of transcribed genes through "metatranscriptomics" has infrequently been utilized in exploring antibiotic resistance. In contrast, the DNA-based metagenomics and other high-throughput techniques are not able to differentiate resistance genes that are recently deactivated from their functional lineages. Metatranscriptomics was used to delineate the identification of taxonomy in fungi from a well-defined mock population with high rate of success, while only a few were detected false positives. These results indicate that from metatranscriptome data, it is very likely to achieve precise species- and strain-level identifications in fungi (Marcelino et al. 2019a, b). The above-mentioned studies suggest metatranscriptomics as a promising technique to depict dynamics of microbial community structure and function in different environments.

22.4 Novel Insights About Functional Dynamics of Microbe in Different Environment Niches

Metatranscriptomic methods were first used to assess marine and freshwater microbial communities. These investigations validated that RNA can possibly be utilized to profile structure, function and diversity of community. It further offers opportunity to identify RNA viruses. A metatranscriptome study conducted from marine environment identified large section of the transcripts with novel origin, representing the vast metabolic variety in the oceans (Frias-Lopez et al. 2008). Cardenas and Tiedje in 2008 carried out a soil transcriptome study and found that a large number of transcripts identified code for housekeeping proteins and genes related to various soil processes, while 32% of eukaryotic transcripts retrieved harbour novel genes, and this highlights the presence of unidentified, hypothetical protein (Cardenas and Tiedje 2008).

In addition, a study conducted in the forest revealed the presence of several enzymes from soil fungal communities involved in decomposition of cellulosic biomass. In this study, pyrosequencing of the cDNAs from Avicel and wheat grown in modified soil resulted in 56,084 recognized protein-coding sequence (CDS) of eukaryotic origin and depicts 99% of the total number of recognized CDSs. The function of 9449 eukaryotic CDSs was depicted. Roughly 40% putative CDSs belong to metabolism-linked genes, comprising genes related to carbohydrate, AA and energy metabolism. Among carbohydrate metabolism, 129 sequences encoding glycoside hydrolase enzymes were identified, out of which 47 were found to be putative cellulases belonging to 13 GH families. The study demonstrated that metatranscriptomic sequencing data reported for the fungal communities adapted to Avicel and wheat decomposition can be used as reference to identify novel genes (Takasaki et al. 2013).

Another revelation was made by combined metatranscriptomic and metagenomic sequencing regarding human gut microbial community study. It revealed that there is

underexpression of biosynthesis of molecules like tryptophan and other amino acids which means DNA level is comparatively more than the corresponding RNA. This is caused by bacteria present in this environment as these molecules are easily accessible from the host, and as a result their production by microbes would be dynamically unfavourable (Franzosa et al. 2014).

Franzosa et al. (2015) investigated gut metatranscriptome of healthy individual and found high level of transcriptional activity related to methanogenesis in two genes, i.e. tetA, which is responsible for antibiotic-resistance, and *groEL*, which is a chaperone protein. Remarkably, the transcriptional pattern of *groEL* and other genes coding bacterial ribosomal proteins are very much variable across individuals, and this is consistent with a pattern of subject-specific transcriptional regulation. A summary of recent metatranscriptomic studies is shown in Table 22.2. There have been only few metatranscriptomics studies reported which have explored it towards occurrence of functionally active resistance genes found in natural environments in human and ecological samples (Versluis et al. 2015). This can be further explored using metatranscriptomics. The research carried out by Gilbert et al. (2008) provided additional evidence regarding metatranscriptomic studies. According to the research, study of microbial communities found in natural environments is not only realistic, but, when combined with metagenomic databases, it offers a unique opportunity to discover both structural and functional aspects of microbial communities. The exploration can be enhanced if we are capable to overcome the problems of interpreting the functions of a large number of never-seen-before gene families.

22.5 Future Prospects

Environmental transcriptomics hold considerable potential in identifying novel microbial processes. Tremendously parallel analysis of different "meta-omics" approaches, metagenomics, metatranscriptomics and metaproteomics will permit us to explore deeper aspects of the microbial variety, together with genes having unique or vital functions. The above-stated approaches have the potential to extend the application of metagenomics and provide the chance to examine explicit clusters that were earlier unidentified and uncharacterized. One can at present question about new complicated queries e.g. what is the share of less abundant microbes? can one assess the entire range of the group and estimate its significance. Most importantly, how one can utilize this information to well describe, characterize and produce even better functional diversity? These tools further allow us to identify various functional pathways and genes in complex biotic samples such as seawater. Over all while writing the book chapter, we have concluded that huge fraction of cDNA and DNA sequences were not reported in existing databases as well as in GOS database indicating plethora of unexplored genomes and transcriptomes.

Table 22.2 Some recent studies that employed metatranscriptomics approach

S. No.	Title	Conclusion	References
1.	Metatranscriptomics as a tool to identify fungal species and subspecies in mixed communities	The taxonomy identification of fungi in well-defined mock population is highly successful while eliminating the false positives	Marcelino et al. (2019a, b)
2.	Nitrogen-phosphorus-associated metabolic activities during the development of a cyanobacterial bloom revealed by metatranscriptomics	This study found that nitrogen and phosphorus metabolisms were the top two categories to increase their gene expressions prior to and during a toxic algal bloom	Lu et al. (2019)
3.	Metatranscriptomic exploration of microbial functioning in clouds	Gave many insights into the functioning of microbial cells within cloud droplets, their physiological traits and potential impacts. This specified biological functions of interest, and this should help identifying specific target genes for future investigations	Amato et al. (2019)
4.	Metatranscriptomics reveals a diverse antibiotic resistance gene pool in avian microbiomes	Metatranscriptome study suggested that human generated waste, even after treatment, might be responsible for spread of antibiotic resistance genes into the wild type Revealed the complex factors explaining the distribution of resistance genes and their exchange routes between humans and wildlife	Marcelino et al. (2019a, b)
5.	Metatranscriptomics of the Hu sheep rumen microbiome reveals novel cellulases	It stated metatranscriptomics as an efficient technique to discover unique and novel cellulases which can be good for biotechnological use and found that rumen microbiome of Hu sheep encodes a range of novel cellulose-degrading enzymes	He et al. (2019)
6.	Novel insights into freshwater hydrocarbon-rich sediments using metatranscriptomics: opening the black box	Results provide insight into the microbial communities responsible for hydrocarbon degradation in syntrophic association with methane, sulphate and nitrate reduction within several Athabasca River tributaries	Reid et al. (2018)

(continued)

Table 22.2 (continued)

S. No.	Title	Conclusion	References
7.	Metatranscriptomics analysis of mangrove habitats around Mauritius	Samples showed predominance by *Proteobacteria*, *Bacteroidetes* and *Firmicutes*, with high abundance of sulphate reducers, nitrogen reducers and methanogens. Significant difference was, however, noted at both taxonomic and functional levels among the mangrove species	Rampadarath et al. (2018)
8.	Metatranscriptome sequencing reveals insights into the gene expression and functional potential of rumen wall bacteria	Provided the first insights into the functional potential of rumen wall microbial communities in situ Also provide evidence for nitrogen fixation and sulphate reduction by bacterial communities of the rumen wall and show the presence of archaea and fungi on the rumen wall	Mann et al. (2018)
9.	Metatranscriptome sequencing and analysis of agriculture soil provided significant insights about the microbial community structure and function	Examination of different metabolism discovered that bacteria in soil ecosystem are reliant on organosulfonated composites for their development and growth. Moreover, more richness of transcripts associated with transportation of phosphate occurring in this soil might be related with the phosphate ravenous situation in this atmosphere	Sharma and Sharma (2018)
10.	Metatranscriptomics reveals the functions and enzyme profiles of the microbial community in Chinese nong-flavour liquor starter	Results demonstrated that fungi were the most abundant active community members during the liquor starter production process	Huang et al. (2017)
11.	Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiome-specific functionality	It depicted that complete annotation of metatranscriptome is impacted by microbiome complexity and accessibility of reference genomes Integration of taxonomic and functional annotations in a novel visualization frame exposed the involvement of various taxa in metabolic processes, permitting the recognition of taxa which is responsible for unique functions	Jiang et al. (2016)

References

Allgaier M, Reddy A, Park JI, Ivanova N, D'haeseleer P, Lowry S, Sapra R, Hazen TC, Simmons BA, Vander Gheynst JS, Hugenholtz P et al (2010) Targeted discovery of glycoside hydrolases from a switchgrass-adapted compost community. PLoS One 5(1):e8812

Amato P, Besaury L, Joly M, Penaud B, Deguillaume L, Delort AM et al (2019) Metatranscriptomic exploration of microbial functioning in clouds. Sci Rep 9(1):1–2

Andersson AF, Lundgren M, Eriksson S, Rosenlund M, Bernander R, Nilsson P et al (2006) Global analysis of mRNA stability in the archaeon Sulfolobus. Genome Biol 7(10):R99

Antunes LP, Martins LF, Pereira RV, Thomas AM, Barbosa D, Lemos LN, Silva GM, Moura LM, Epamino GW, Digiampietri LA, Lombardi KC et al (2016) Microbial community structure and dynamics in thermophilic composting viewed through metagenomics and metatranscriptomics. Sci Rep 6:38915

Bailly J, Fraissinet-Tachet L, Verner MC, Debaud JC, Lemaire M, Wesolowski-Louvel M, Marmeisse R et al (2007) Soil eukaryotic functional diversity, a metatranscriptomic approach. ISME J 1(7):632–642

Bashiardes S, Zilberman-Schapira G, Elinav E et al (2016) Use of metatranscriptomics in microbiome research. Bioinform Biol Insights 10:BBI-S34610

Bengtsson-Palme J, DGJ L, Kristiansson E et al (2017) Using metagenomics to investigate human and environmental resistomes. J Antimicrob Chemother 72:2690–2703

Buchfink B, Xie C, Huson DH et al (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12(1):59–60

Cardenas E, Tiedje JM (2008) New tools for discovering and characterizing microbial diversity. Curr Opin Biotechnol 19(6):544–549

Crofts TS, Gasparrini AJ, Dantas G et al (2017) Next-generation approaches to understand and combat the antibiotic resistome. Nat Rev Microbiol 15:422–434

D'haeseleer P, Gladden JM, Allgaier M, Chain PS, Tringe SG, Malfatti SA, Aldrich JT, Nicora CD, Robinson EW, Paša-Tolić L, Hugenholtz P et al (2013) Proteogenomic analysis of a thermophilic bacterial consortium adapted to deconstruct switchgrass. PLoS One 8(7):e68465

Davids M, Hugenholtz F, Martins dos Santos V, Smidt H, Kleerebezem M, Schaap PJ et al (2016) Functional profiling of unfamiliar microbial communities using a validated de novo assembly metatranscriptome pipeline. PLoS One 11(1):e0146423

De Gannes V, Eudoxie G, Hickey WJ et al (2013) Prokaryotic successions and diversity in composts as revealed by 454-pyrosequencing. Bioresour Technol 133:573–580

Dees PM, Ghiorse WC (2001) Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. FEMS Microbiol Ecol 35(2):207–216

Dougherty MJ, D'haeseleer P, Hazen TC, Simmons BA, Adams PD, Hadi MZ et al (2012) Glycoside hydrolases from a targeted compost metagenome, activity-screening and functional characterization. BMC Biotechnol 12(1):38

Embree M, Nagarajan H, Movahedi N, Chitsaz H, Zengler K et al (2014) Single-cell genome and metatranscriptome sequencing reveal metabolic interactions of an alkane-degrading methanogenic community. ISME J 8(4):757–767

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MM, Schreiber F, Dutilh BE, Zedelius J, de Beer D, Gloerich J et al (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464(7288):543–548

Fakruddin M, Mannan K (2013) Methods for analyzing diversity of microbial communities in natural environments. Ceylon J Sci Biol Sci 42(1):19–33

Federici E, Pepi M, Esposito A, Scargetta S, Fidati L, Gasperini S, Cenci G, Altieri R et al (2011) Two-phase olive mill waste composting: community dynamics and functional role of the resident microbiota. Bioresour Technol 102(23):10965–10972

Franzosa EA, Morgan XC, Segata N, Waldron L, Reyes J, Earl AM, Giannoukos G, Boylan MR, Ciulla D, Gevers D, Izard J et al (2014) Relating the metatranscriptome and metagenome of the human gut. Proc Natl Acad Sci U S A 111(22):E2329–E2338

Franzosa EA, Hsu T, Sirota-Madi A, Shafquat A, Abu-Ali G, Morgan XC, Huttenhower C et al (2015) Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. Nat Rev Microbiol 13(6):360–372

Frias-Lopez J, Shi Y, Tyson GW, Coleman ML, Schuster SC, Chisholm SW, DeLong EF et al (2008) Microbial community gene expression in ocean surface waters. Proc Natl Acad Sci U S A 105(10):3805–3810

Gans J, Wolinsky M, Dunbar J et al (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309(5739):1387–1390

Giannoukos G, Ciulla DM, Huang K, Haas BJ, Izard J, Levin JZ, Livny J, Earl AM, Gevers D, Ward DV, Nusbaum C et al (2012) Efficient and robust RNA-seq process for cultured bacteria and complex community transcriptomes. Genome Biol 13(3):r23

Gilbert JA, Field D, Huang Y, Edwards R, Li W, Gilna P, Joint I et al (2008) Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities. PLoS One 3(8):e3042

Gladden JM, Allgaier M, Miller CS, Hazen TC, Vander Gheynst JS, Hugenholtz P, Simmons BA, Singer SW et al (2011) Glycoside hydrolase activities of thermophilic bacterial consortia adapted to switchgrass. Appl Environ Microbiol 77(16):5804–5812

Gonzalez E, Pitre FE, Pagé AP, Marleau J, Nissim WG, St-Arnaud M, Labrecque M, Joly S, Yergeau E, Brereton NJ et al (2018) Trees, fungi and bacteria: tripartite metatranscriptomics of a root microbiome responding to soil contamination. Microbiome 6(1):53

Gosalbes MJ, Durbán A, Pignatelli M, Abellan JJ, Jiménez-Hernández N, Pérez-Cobas AE, Latorre A, Moya A et al (2011) Metatranscriptomic approach to analyze the functional human gut microbiota. PLoS One 6(3):e17447

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD et al (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc 8(8):1494

Habbeche A, Saoudi B, Jaouadi B, Haberra S, Kerouaz B, Boudelaa M, Badis A, Ladjama A et al (2014) Purification and biochemical characterization of a detergent-stable keratinase from a newly thermophilic actinomycete Actinomadura keratinilytica strain Cpt29 isolated from poultry compost. J Biosci Bioeng 117(4):413–421

He B, Jin S, Cao J, Mi L, Wang J et al (2019) Metatranscriptomics of the Hu sheep rumen microbiome reveals novel cellulases. Biotechnol Biofuels 12(1):153

Helbling DE, Ackermann M, Fenner K, Kohler HP, Johnson DR et al (2012) The activity level of a microbial community function can be predicted from its metatranscriptome. ISME J 6(4):902

Huang Y, Yi Z, Jin Y, Huang M, He K, Liu D, Luo H, Zhao D, He H, Fang Y, Zhao H et al (2017) Metatranscriptomics reveals the functions and enzyme profiles of the microbial community in Chinese Nong-flavor liquor starter. Front Microbiol 8:1747

Jiang Y, Xiong X, Danska J, Parkinson J et al (2016) Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiome-specific functionality. Microbiome 4(1):2

Jurado M, López MJ, Suárez-Estrella F, Vargas-García MC, López-González JA, Moreno J et al (2014) Exploiting composting biodiversity: study of the persistent and biotechnologically relevant microorganisms from lignocellulose-based composting. Bioresour Technol 162:283–293

Kuske CR, Hesse CN, Challacombe JF, Cullen D, Herr JR, Mueller RC, Tsang A, Vilgalys R et al (2015) Prospects and challenges for fungal metatranscriptomics of complex communities. Fungal Ecol 14:133–137

Leimena MM, Ramiro-Garcia J, Davids M, van den Bogert B, Smidt H, Smid EJ, Boekhorst J, Zoetendal EG, Schaap PJ, Kleerebezem M et al (2013) A comprehensive metatranscriptome

analysis pipeline and its validation using human small intestine microbiota datasets. BMC Genomics 14:530

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C et al (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442 (7104):806

Leung HC, Yiu SM, Parkinson J, Chin FY et al (2013) IDBA-MT: de novo assembler for metatranscriptomic data generated from next-generation sequencing technology. J Comput Biol 20(7):540–550

Liang P, Pardee AB (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257(5072):967–971

Liao HL, Chen Y, Bruns TD, Peay KG, Taylor JW, Branco S, Talbot JM, Vilgalys R et al (2014) Metatranscriptomic analysis of ectomycorrhizal roots reveals genes associated with P iloderma–P inus symbiosis: improved methodologies for assessing gene expression in situ. Environ Microbiol 16(12):3730–3742

López-González JA, Suárez-Estrella F, Vargas-García MC, López MJ, Jurado MM, Moreno J et al (2015) Dynamics of bacterial microbiota during lignocellulosic waste composting: studies upon its structure, functionality and biodiversity. Bioresour Technol 175:406–416

Lu J, Zhu B, Struewing I, Xu N, Duan S et al (2019) Nitrogen–phosphorus-associated metabolic activities during the development of a cyanobacterial bloom revealed by metatranscriptomics. Sci Rep 9(1):1–1

Manichanh C, Eck A, Varela E, Roca J, Clemente JC, González A, Knights D, Knight R, Estrella S, Hernandez C, Guyonnet D et al (2014) Anal gas evacuation and colonic microbiota in patients with flatulence: effect of diet. Gut 63(3):401–408

Mann E, Wetzels SU, Wagner M, Zebeli Q, Schmitz-Esser S et al (2018) Metatranscriptome sequencing reveals insights into the gene expression and functional potential of rumen wall bacteria. Front Microbiol 9:43

Marcelino VR, Wille M, Hurt AC, González-Acuña D, Klaassen M, Schlub TE, Eden JS, Shi M, Iredell JR, Sorrell TC, Holmes EC et al (2019a) Meta-transcriptomics reveals a diverse antibiotic resistance gene pool in avian microbiomes. BMC Biol 17(1):31

Marcelino VR, Irinyi L, Eden JS, Meyer W, Holmes EC, Sorrell TC et al (2019b) Metatranscriptomics as a tool to identify fungal species and subspecies in mixed communities–a proof of concept under laboratory conditions. IMA Fungus 10(1):8

Martinez X, Pozuelo M, Pascal V, Campos D, Gut I, Gut M, Azpiroz F, Guarner F, Manichanh C et al (2016) MetaTrans: an open-source pipeline for metatranscriptomics. Sci Rep 6:26447

Martins LF, Antunes LP, Pascon RC, de Oliveira JC, Digiampietri LA, Barbosa D, Peixoto BM, Vallim MA, Viana-Niero C, Ostroski EH, Telles GP et al (2013) Metagenomic analysis of a tropical composting operation at the São Paulo Zoo Park reveals diversity of biomass degradation functions and organisms. PLoS One 8(4):e61928

Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A et al (2008) The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9:386

Mhuantong W, Charoensawan V, Kanokratana P, Tangphatsornruang S, Champreda V et al (2015) Comparative analysis of sugarcane bagasse metagenome reveals unique and conserved biomass-degrading enzymes among lignocellulolytic microbial communities. Biotechnol Biofuels 8(1):16

Munk P, Knudsen BE, Lukjancenko O, ASR D, Van Gompel L, REC L et al (2018) Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. Nat Microbiol 3:898–908

Narayanasamy S, Jarosz Y, Muller EE, Heintz-Buschart A, Herold M, Kaysen A, Laczny CC, Pinel N, May P, Wilmes P et al (2016) IMP: a pipeline for reproducible reference-independent integrated metagenomic and metatranscriptomic analyses. Genome Biol 17(1):260

Neher DA, Weicht TR, Bates ST, Leff JW, Fierer N et al (2013) Changes in bacterial and fungal communities across compost recipes, preparation methods, and composting times. PLoS One 8 (11):e79512

Ni Y, Li J, Panagiotou G et al (2016) COMAN: a web server for comprehensive metatranscriptomics analysis. BMC Genomics 17(1):622

Partanen P, Hultman J, Paulin L, Auvinen P, Romantschuk M et al (2010) Bacterial diversity at different stages of the composting process. BMC Microbiol 10(1):94

Peng Y, Leung HC, Yiu SM, Lv MJ, Zhu XG, Chin FY (2013) IDBA-tran: a more robust de novo de Bruijn graph assembler for transcriptomes with uneven expression levels. Bioinformatics 29 (13):i326–i334

Poretsky RS, Bano N, Buchan A, LeCleir G, Kleikemper J, Pickering M, Pate WM, Moran MA, Hollibaugh JT et al (2005) Analysis of microbial gene transcripts in environmental samples. Appl Environ Microbiol 71:4121–4126

Poretsky RS, Hewson I, Sun S, Allen AE, Zehr JP, Moran MA et al (2009) Comparative day/night metatranscriptomic analysis of microbial communities in the North Pacific subtropical gyre. Environ Microbiol 11(6):1358–1375

Rampadarath S, Bandhoa K, Puchooa D, Jeewon R, Bal S et al (2018) Metatranscriptomics analysis of mangroves habitats around Mauritius. World J Microb Biot 34(4):59

Reid T, Chaganti SR, Droppo IG, Weisener CG et al (2018) Novel insights into freshwater hydrocarbon-rich sediments using metatranscriptomics: Opening the black box. Water Res 136:1–1

Robertson G, Schein J, Chiu R, Corbett R, Field M, Jackman SD, Mungall K, Lee S, Okada HM, Qian JQ, Griffith M et al (2010) De novo assembly and analysis of RNA-seq data. Nat Methods 7(11):909–912

Schulz MH, Zerbino DR, Vingron M, Birney E et al (2012) Oases: robust de novo RNA-seq assembly across the dynamic range of expression levels. Bioinformatics 28(8):1086–1092

Selinger DW, Saxena RM, Cheung KJ, Church GM, Rosenow C et al (2013) Global RNA half-life analysis in Escherichia coli reveals positional patterns of transcript degradation. Genome Res 13 (2):216–223

Sharma R, Sharma PK (2018) Metatranscriptome sequencing and analysis of agriculture soil provided significant insights about the microbial community structure and function. Ecol Genet Genom 6:9–15

Shi Y, Tyson GW, DeLong EF et al (2009) Metatranscriptomics reveals unique microbial small RNAs in the ocean's water column. Nature 459(7244):266–269

Shi M, Lin XD, Tian JH, Chen LJ, Chen X, Li CX, Qin XC, Li J, Cao JP, Eden JS, Buchmann J et al (2016) Redefining the invertebrate RNA virosphere. Nature 540(7634):539–543

Shi M, Neville P, Nicholson J, Eden JS, Imrie A, Holmes EC et al (2017) High-resolution metatranscriptomics reveals the ecological dynamics of mosquito-associated RNA viruses in Western Australia. J Virol 91(17):e00680–17

Su JQ, An XL, Li B, Chen QL, Gillings MR, Chen H et al (2017) Metagenomics of urban sewage identifies an extensively shared antibiotic resistome in China. Microbiome 5:84

Surette MD, Wright GD (2017) Lessons from the environmental antibiotic resistome. Annu Rev Microbiol 71:309–329

Takasaki K, Miura T, Kanno M, Tamaki H, Hanada S, Kamagata Y, Kimura N et al (2013) Discovery of glycoside hydrolase enzymes in an avicel-adapted forest soil fungal community by a metatranscriptomic approach. PLoS One 8(2):e55485

Tjaden B (2015) De novo assembly of bacterial transcriptomes from RNA-seq data. Genome Biol 16(1):1

Tkachuk VL, Krause DO, Knox NC, Hamm AC, Zvomuya F, Ominski KH, McAllister TA et al (2014) Targeted 16 S r RNA high-throughput sequencing to characterize microbial communities during composting of livestock mortalities. J Appl Microbiol 116(5):1181–1194

Torsvik V, Øvreås L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5(3):240–245

Torsvik V, Goksøyr J, Daae FL et al (1990) High diversity in DNA of soil bacteria. Appl Environ Microbiol 56(3):782–787

Tveit AT, Urich T, Svenning MM et al (2014) Metatranscriptomic analysis of arctic peat soil microbiota. Appl Environ Microbiol 80(18):5761–5772

Urich T, Lanzén A, Qi J, Huson DH, Schleper C, Schuster SC et al (2008) Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 3(6):e2527

Versluis D, D'Andrea MM, Ramiro Garcia J, Leimena MM, Hugenholtz F, Zhang J et al (2015) Mining microbial metatranscriptomes for expression of antibiotic resistance genes under natural conditions. Sci Rep 5:11981

Vila-Costa M, Rinta-Kanto JM, Sun S, Sharma S, Poretsky R, Moran MA et al (2010) Transcriptomic analysis of a marine bacterial community enriched with dimethylsulfoniopropionate. ISME J 4(11):1410–1420

Warnecke F, Hess M et al (2009) A perspective: metatranscriptomics as a tool for the discovery of novel biocatalysts. J Biotechnol 142(1):91–95

Westreich ST, Korf I, Mills DA, Lemay DG et al (2016) SAMSA: a comprehensive metatranscriptome analysis pipeline. BMC Bioinformatics 17(1):399

Westreich ST, Treiber ML, Mills DA, Korf I, Lemay DG et al (2018) SAMSA2: a standalone metatranscriptome analysis pipeline. BMC Bioinformatics 19(1):175

Wille M, Eden JS, Shi M, Klaassen M, Hurt AC, Holmes EC et al (2018) Virus–virus interactions and host ecology are associated with RNA virome structure in wild birds. Mol Ecol 27 (24):5263–5278

Yang G, Chen M, Yu Z, Lu Q, Zhou S et al (2013) Bacilluscomposti sp. nov. and Bacillusthermophilus sp. nov., two thermophilic, Fe (III)-reducing bacteria isolated from compost. Int J Syst Evol Microbiol 63(8):3030–3036

Zhang YZ, Shi M, Holmes EC et al (2018) Using metagenomics to characterize an expanding virosphere. Cell 172(6):1168–1172

Zhao Y, Su JQ, An XL, Huang FY, Rensing C, Brandt KK, Zhu YG et al (2018) Feed additives shift gut microbiota and enrich antibiotic resistance in swine gut. Sci Total Environ 621:1224–1232

Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD et al (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc Natl Acad Sci U S A 110:3435–3440

Zhu YG, Zhao Y, Li B, Huang CL, Zhang SY, Yu S et al (2017) Continental scale pollution of estuaries with antibiotic resistance genes. Nat Microbiol 2:16270

Zhu J, Liu R, Cao N, Yu J, Liu X, Yu Z et al (2019) Mycobacterial metabolic characteristics in a water meter biofilm revealed by metagenomics and metatranscriptomics. Water Res 153:315–323

A Pipeline for Assessment of Pathogenic Load in the Environment Using Microbiome Analysis

23

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Abstract

The contamination of the environment is taking place at a very fast pace. Industrial pollutants, hospital effluents, domestic and household wastes as well as direct environmental processes such as melting of ice caps around the globe with increase in mean temperature as a result of global warming are contributing towards the continuous alterations in environmental balance. The threats of natural calamities such as cyclones and earthquakes have also challenged the habitability of many areas. As a result pandemics such as COVID-19, Ebola, and at a lesser scale dengue and malaria are affecting a large global population. It is thus important for epidemiologist and public health sectors to come up with suitable prediction models of the pathogenic load of a particular area so as to design and implement suitable mitigation measures. In this work we propose a metagenomics assisted pipeline for estimation of pathogenic load in the

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environment with case studies from rhizospheric soil microbiome, effluent and wastewater microbiome, and human gut microbiome, where we perform a microbiome wide association study with known disease causing microbial datasets and predict the potential pathogenic microorganisms that are prevalent in a particular area or ecological niche. Our pipeline was able to predict the potential pathogenic load of the niche areas under study, which leads us to believe that metagenomics can be utilized at a diagnostic scale and using the dataset obtained we may then predict the pathogenic load of that particular area. This approach has the potential to be utilized for fast prediction of potential disease threats under public health emergencies and should enable proper resource partitioning from suitable stakeholders.

Keywords

Metagenomics · Rhizosphere · Wastewater · Effluent · Gut Microbiome

23.1 Introduction

We are currently in the era of microbes. Permafrost is melting, thus releasing high volumes of unknown microbes into the environment which were trapped into the sub-zero temperatures. We are currently facing an international pandemic with the severity which the world witnesses every 100 years and yet we are doing very little to establish and fast pace diagnostics and monitoring pipeline for pathogenic load that is being released in to the environment. A few repositories have been established by synchronizing the diversity which include the Human Microbiome Project (Micah et al. 2007), the Tara Ocean Project (Karsenti et al. 2011), and the Earth Microbiome Project (Gilbert et al. 2014). Metagenomics have also shown its merit in helping to predict the ancient past from an organisms fossilized DNA (like bones, teeth). While many studies related to ancient DNA focus on the investigations of human endogenous DNA isolated from specimens of ancient ages (Haak et al. 2015, Mallick et al. 2016, Orlando et al. 2013, Schlebusch et al. 2012, Skoglund et al. 2017), associated environmental reconstructions can also be made which throws light on the microbial abundance of the past and also increase our understanding towards the evolution of infectious diseases (Warinner et al. 2017; Key et al. 2017). We know next-generation sequencing is a traditional method to analyze metagenomes, either through amplicon or shotgun sequencing. There are specifically three benefits of using 16S ribosomal RNA amplicon sequencing when compared to shotgun sequencing methodology. Using 16S ribosomal RNA amplicon sequencing is profitable, secondly, data can be analyzed by pre-established bioinformatics channels and the referral databases which are relatively comprehensive (Ranjan et al. 2016; Sedlar et al. 2017). On the other hand, two drawbacks of 16S rRNA amplicon sequencing are, it spots richness of a lower species; and classifies at phylum level, fairly, genus level. Comparatively, shotgun sequencing detects every position of strands of lower species level, and, identifies bacterial species more significantly, by recognizing greater diversity organisms of other kingdoms (Ranjan et al. 2016).

Diagnostics is an emerging area where metagenomics is applied and emphasized. As defined by Pallen, diagnostic metagenomics (Pallen 2014) recognizes and characterizes pathogens using shotgun metagenomic data. In a way, diagnostic metagenomics possess generic potential to ideally and swiftly trace all microbial (includes bacterial, viral, and parasitic) pathogens as well as infections caused due to respective microbes through many samples of feces, urine, meat, blood, etc. The advantage of diagnostic metagenomics is that, it reduces the time consumed from sampling to result to less than 24 h, instead of cultivation of pathogens for several days. There is also an excellent alternative method, i.e., polymerase chain reaction (PCR) enriched with pathogen specificity, but, it requires splitted PCR setup for every targeted pathogen. The features like vulnerability, specificness, capability of quick identification (also quantification in few cases) of pathogens allow metagenomics to play a key role in diagnostics. However, there is still more to explore about complete potential of diagnostic metagenomics at its experimental stages. Considerably, data analysis is an important part of metagenomic studies which is difficult as well as time consuming. The metagenomic sequences which either interpret or assemble are categorized on the basis of taxonomy dependent and taxonomy independent. The first classification, taxonomy dependent is based upon a referral database with sequenced data or only marker genes (Mande et al. 2012; Sedlar et al. 2017; Lindgreen et al. 2016; Menzel et al. 2016). In 2014, Wood and co-authors described the approach of taxonomy dependent used in different classifiers (Wood and Salzberg 2014). In 2015, Ounit et al., used the classifier CLARK (CLAssifier based on Reduced K-mers) (Ounit et al. 2015), simultaneously, in 2017, the usage of metagenomic mapper (MGmapper) was published in 2017 (Petersen et al. 2017). The metagenomic phylogenetic analysis (MetaPhlAn) is an example of a reference-based tool which only emphasize on a defined set of strain-specific marker genes (Segata et al. 2012). Meanwhile, all these taxonomy-dependent classifiers likely provide fewer details concerning the sequences, and the search against the database becomes much faster (Segata et al. 2013). Naccache et al., reported a resolute pipeline of bioinformatics for diagnostic metagenomics, named as, Sequence-based Ultra-rapid Pathogen Identification (SURPI) which is a reference-based pipeline and uses the National Center for Biotechnology Information (NCBI) nucleotide database and the RefSeq non-redundant proteins database in its comprehensive mode (Naccache et al. 2014). The methods classified under taxonomy-dependent metagenomic sequences are frequently used in diagnostic metagenomics because the integrated databases can rapidly detect the causative pathogen which is useful for further treatment of a patient. Presently, the majority of the accessible databases are inadequate and/or distorted to incorporate additional number of human pathogens and model organisms (Segata et al. 2013). Now, these unclassified sequences, raises the risk of false positive results, where non-pathogen with sequences similar to pathogens are detected and finally categorized as pathogens because of incorrect references (Ranjan et al. 2016; Sedlar et al. 2017). Many defined databases and networks are in the process of development by the U.S. Food and Drug Administration (for example, GenomeTrakr) (Allard et al. 2016) and to add to it are European Commission funded collaborative management

platforms (such as, COMPARE project) (Aarestrup and Koopmans 2016) for spotting and analytical studies of re-emerging food borne outbreaks. The second classification of metagenomics sequence is taxonomy independent which is also known as binning, only depends on the sequence composition based data (Sedlar et al. 2017; Sangwan et al. 2016). The approach of taxonomy-independent classification in metagenomic studies was used as compiler, for example, CONCOCT (Clustering Contigs with Coverage and Composition) (Albertsen et al. 2013; Nielsen et al. 2014; Cleary et al. 2015; Alneberg et al. 2014). However, it was reviewed that these studies and programs cannot be applied for diagnostic purposes (Sangwan et al. 2016). The classification of metagenomic sequences are made more precise and correct pre-processing of the shotgun data for quality control include trimming, masking, and assembly. However, assembling reads into contigs makes the analysis better, but, from bioinformatics point of view, it is usually a difficult task (Ranjan et al. 2016; Sedlar et al. 2017; Sangwan et al. 2016). Generally, it is suggested to examine the exposure of the metagenomic data set because higher exposure is helpful to assemble and detect distinctly abundant genes in a more systematic way (Rodriguez-r and Konstantinidis 2014). It was reviewed that many tools are available for end-to-end metagenomic data analysis together with pre-processing (Segata et al. 2013). Yun and Yun, in 2014 reported the comparison of the two pre-processing methods trimming and masking. It was observed that the method of masking is more commendable than trimming, because masking was better in analyzing the rate of false positive data of single nucleotide polymorphism, and, bases of low quality present in the sequence are replaced with "N" which are not detected. On contrary, trimming process is frequently used due to efficient removal of low quality bases (repeated only at the ends of a read) results into a shorter read (Yun and Yun 2014). Andersen et al., in 2017a, b focused on the importance of pointing out the loop holes of the software used for analysis. In a case study, fecal samples of ten-fold dilutions showed data spiked with *Campylobacter jejuni* which were detected by two taxonomic classifiers Kraken and CLARK. It was observed that both classifiers identified false positive reads from negative samples with scarcely present *Campylobacter jejuni* of quantitative polymerase chain reaction (qPCR). It was developed that sorting of Kraken hits can eliminate false positives reads. While sorting of Kraken hits, firstly, the sequences are sorted by assigning each hit a score, then, hits for phage and plasmid DNA are removed using the Kraken and Basic Local Alignment Search Tool (BLAST) among the high scoring hits (Andersen et al. 2017a, b). The study also represented a non-linear correlation between the rising levels and the hits read from metagenomic data.

23.2 General Analysis Pipeline

The pipeline involves the use of advanced next generation sequencing techniques, though traditional methods are also in use, while Table 23.1 summarises the general approaches for pathogen detection from samples.

Table 23.1 Strategy and Detection of Pathogens from different samples (modified from Miller et al. 2013)

Application/ Strategy	Method	Application	Examples	Advantages	Disadvantages
Deep sequencing	rRNA	Identification of prokaryotes and eukaryotes	Human gut microbiome characterization	Highly sensitive	Concerns regarding universality of target gene
		Determination of taxonomic relationships	Similarities of ancient gut with modern rural gut and dissimilar with modern urban gut	Taxonomic classification can be done using fewer reads	Primer bias may be detrimental
	rpoB	Archaeal and bacterial identification*	Applicable to subgroup species	rpoB and cpn-60 offer enhanced taxonomic resolution compared to rRNA	Possibility of variable gene copy numbers among targeted species
	Cpn-60	Taxonomic relationships determination			
	Viral RNA polymerase (RdRP)	Novel virus discovery	Identified novel families of picornaviruses off the coast of British Columbia		
Metagenomics	Shotgun sequencing	Functional and taxonomic characterization	Swine fever virus like sequences were detected(Asfarviridae)	Microorganism wide detection of sequences	Broad specificity may lead to decreased sensitivity
			Stool samples exhibited the presence of unexpected microbes	No a priori knowledge of microorganisms required	Labor intensive detection of sequences
	Subtraction	Functional and taxonomic characterization	Identified divergent regions in non-coding RNAs in Listeria monocytogenes	Potential for bias is reduced by the use of random primers	Bioinformatics analysis is more challenging
			Association of *Fusobacterium nucleatum* with colorectal carcinoma		Relatively expensive as more reads are required

(continued)

Table 23.1 (continued)

Application/ Strategy	Method	Application	Examples	Advantages	Disadvantages
	Virus	Novel virus discovery	Detection of the novel H1N1 influenza from nasopharyngeal swabs		Almost 50% of the generated sequences generally have no significant homology to known proteins in databases (dark matter)

Once the abundance data is obtained then the comparative metagenomics predictions are initiated using tools such as Venny and Comparator. These provide us with a set of unique and common dataset for each of the comparative datasets that are being evaluated. Following the identification of the subset the metagenome-wide association studies are initiated which is mainly focused on taxonomic enrichment analysis. Metagenome-wide association studies have already found strong association of microbes with host health and disease. It also identifies a large number of microbes differentially regulated in various conditions. However, computational methods for analyzing such differentially regulated microbes from microbiome study are limited. TSEA or Taxon Set Enrichment Analysis is a way to identify biologically or ecologically meaningful patterns by analyzing them with context to pre-defined taxon set (microbes sharing some common trait) from a given list of significant features or microbes. These microbes undergo certain significance levels and the obtained results are combined to observe discerned meaningful patterns. In contrast, TSEA directly examines a set of functionally related microbes without any preselected compounds based on arbitrary cutoff threshold. TSEA has potential to identify subtle but consistent changes among a group of related microbes, which may go undetected with conventional approaches.

23.3 TSEA Overview

Taxon set enrichment analysis comprises 4 steps of data assembly—input, processing, analysis, and compilation of results. Microbiome Analyst Different taxon sets are selected on the basis of different input types and supported by three types of taxon sets which are based on the taxonomic resolution of microbes to be analyzed. The taxon name mapping to higher taxonomic level of variety of microbes by using major database identifiers can be performed by users. TSEA offers three algorithms for enrichment analysis with three different data inputs required for following three approaches:

1. A list of microbes are characterized at any possible taxonomic level—entered as a one column data (Mixed-level taxa);
2. A list of microbes are characterized at any species level taxa and enlisted in one column (Species level taxa).
3. A list of microbes names (Binomial Nomenclature Name/GOLD ID/NCBI Taxonomy ID) characterized at any strain level—entered as a one column data (Strain-level taxa)).

23.4 Selection of Taxon Set Library

Our entire list was analysed using a mixed level taxon set. Mixed-level taxon sets associated with Human diseases were used for improved analysis. Over Representation Analysis (ORA) is done by enlisting taxa or microbes found to be abundant in

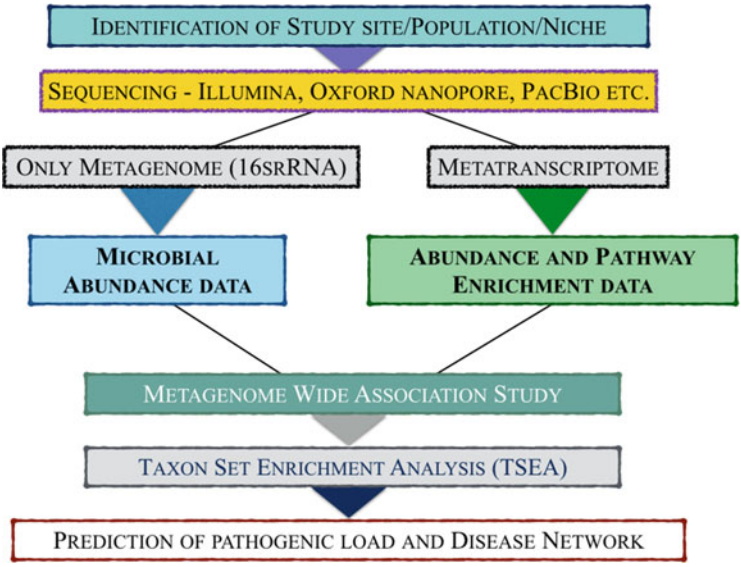

Fig. 23.1 Proposed pipeline for detection of pathogenic load in a an environmental niche

the individual data sets and common to all. The list of microbes can also be obtained through differential abundance testing, or from biomarker analysis or from clustering of algorithm to examine a few biologically meaningful patterns, if present. ORA was implemented using hyper-geometric test to calculate whether a particular taxon set is represented more than expected by chance within the given list. One-tailed p values are generated after adjusting multiple tests. Figure 23.1 summarizes the proposed pipeline for identification of pathogenic load.

23.5 Case Reports

There are numerous microbiome analysis reported on the applications of metagenomics. The following section will focus on a few existing data.

23.5.1 Case Study 1

One of the case studies reported in recent past showed the use of metagenomics in diagnosis of clinical fecal samples. The sampling was done from individuals of two categories, the patients with illness and the patients recovered after 3 months. The data was analyzed by Nucleotide Basic Local Alignment search tool (BLAST) against a reference database. *C. jejuni* was identified as the pathogen from the samples collected from patients suffering from illness because the reads of the analysis aligned to *C. jejuni*. However, the pathogen was detected through

metagenomic data and diagnosed, the study was very limited to the dependency of the samples collected from the patients after 3 months recovery and the method was inapplicable to real-time surveillance situations (Nakamura et al. 2008). Loman et al., performed an experiment with 45 human fecal samples while a breakout in Germany in 2011of Shiga-toxigenic Escherichia coli *O104:H4. Among 45 samples considered for the study, 40 were observed to have pathogen. 45 samples were paired end to end and sequenced with 151 bp to yield total 180 giga base pairs using HiSeq (Illumina). It was likely to retrieve a draft of genome of strains obtained from 27 human fecal samples collected during the outbreak. In addition, genes of* Escherichia coli *O104:H4 were identified from 27 human fecal samples (Loman et al. 2013).*

23.5.2 Case Study 2

In 2016, Schneeberger et al., using shotgun sequencing demonstrated a proof of application of diagnostic metagenomics. For the experiment, 4 fecal samples were collected from patients suffering with persistent diarrhea. The patients were from areas of high occurrence of gastrointestinal infections with asymptomatic carriers and co-infections. The samples were observed to have bacterial, parasitic, and viral infections. The comparison analysis of data was carried out by BLASTn against three reference reads from NCBI databases: nucleotide, genome-specific markers (GSMer), and inclusive antibiotic resistance database (CARD). Each patient with 8–11 different pathogens was detected to be positive, which was more than if diagnosed with conventional methods like, microscopy, cultivation, and multiplex PCR (Schneeberger et al. 2016). The result imposed question on how many infectious pathogens and asymptomatic carriers were detected, and, how many pathogens were detected with false positive hits. Nevertheless, the study showed the potential of taxonomy-dependent method which used the entire genome sequence, markers and Antibiotic Resistance Genes(ARGs) to detect pathogens and co- infections from multiple classes of kingdoms.

23.5.3 Case Study 3

The plant microbiome group has been extensively studied the microbial composition of rhizospheric soils of several plants from the Indian Sunderbans. Indian Sunderbans represent the deltaic region of the rivers Ganga and Brahmaputra (India) and Meghna (Bangladesh). These are uniquely characterized as they are under continuous tidal inundation and agricultural practices are very limited to local landraces of rice and a few leafy vegetables due to the high salt content of the soil. The region is also plagued with geographical challenges and unorganized healthcare facilities. Due to the prevalent high humidity conditions, flu, dengue, as well as other diarrheal diseases are very common. The rhizopsheric microbial abundance was used as the starting data and pathogenic load around human habitats

were predicted as described in the material and method segment. We found that several pathogenic microbes were identified having reported pathogenesis in Colitis and malaria (Ganguli et al. 2017, Rahaman et al. 2019). Apart from that several antibiotic resistance pathways were also found to be upregulated such as beta lactam resistance, vancomycin resistance, and neomycin resistance (Fig. 23.2).

Disease network analysis (Fig. 23.3) revealed the interactions of the causative pathogens and the diseases that share the pathogen as causal agents. However, as it can be observed that complex disease networks are not very prevalent in the analysis which indicates that the pathogenic load of the area under study is moderate.

23.5.4 Case Study 4

The evolution of new strains with antibiotic resistivity is gradually affecting public health with implications on economic and social throughout the world. The infections like pneumonia, typhoid fever, etc., are caused by *Streptococcus*, which are community acquired infections. The infections caused due to methicillin (antibiotic) resistance of *Staphylococcus aureus*, vancomycin resistance of *Enterococci,* and various other Gram-negative bacteria producing beta-lactamase enzyme producing Gram-negative bacteria, are known to be hospital acquired infections. These infections direct additional diseases to patients with their longer stay at hospitals which may cause pressure ulcers (bedsore) and economic burden on the community. The common organisms identified during pressure ulcers are *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*. The emergence of pathogenic bacteria showing resistivity towards most of the currently available antimicrobial agents has really become a critical problem in area of modern medicine, particularly because of the increase in immune suppressed patients. In June 2000, WHO (World Health Organization) warned regarding the increase in the level of resistivity of drugs towards treating common infectious diseases is slowly reaching a crisis point. There are resistant and multi-resistant pathogenic bacteria detected in wastewater, sewage treatment plants as well as in other environment sectors (Singh et al. 2019). Furthermore, in arid regions, wastewater containing antibiotic resistant bacteria is used for irrigation, and sewage sludge serves as fertilizers. Thus, this allows antibiotic resistant bacteria to enter the food chain directly (Singh et al. 2019). Hospital wastewater can be hazardous to public health and ecological balance. Many studies have demonstrated that wastewater from hospitals contribute to high rates of resistant bacteria that are being discharged in the natural environment. Waste effluent from hospitals contains adequate concentration of numerous resistant bacteria and antibiotic residues which inhibit the growth of susceptible bacteria. Hence, as a result, waste effluent of hospitals can also increase the numbers of resistant bacteria in the recipient sewers. In this work we analyzed the microbial composition of urban and rural wastewater which carries hospital waste (Singh et al. 2019) and found that severe disease causing pathogens are abundantly present having a number of antibiotic resistance pathways being overexpressed (Fig. 23.4). When enriched analyses were performed with the

Genera Enriched
Roseovarius; Burkholderia; Anaeromyxobacter
Rhodococcus; Paenibacillus;
Desulfovibrio
Arthrobacter; Acinetobacter; Pseudoalteromonas
Acinetobacter
Marmoricola
Flavobacterium
Lysobacter;Xanthomonas; Burkholderia;
Gemmata; Haliangium; Thioalkalivibrio;
Clostridium
Pseudomonas; Burkholderia;
Desulfovibrio; Desulfohalobium;
Desulfovibrio;
Flavobacterium;
Pseudoxanthomonas
Pseudomonas
Pseudoxanthomonas; Halomonas; Burkholderia;
Methylobacterium;Herbaspirillum
Mesorhizobium; Achromobacter; Stenotrophomonas;
Acinetobacter;
Desulfovibrio;
Desulfobulbus;Pseudomonas;

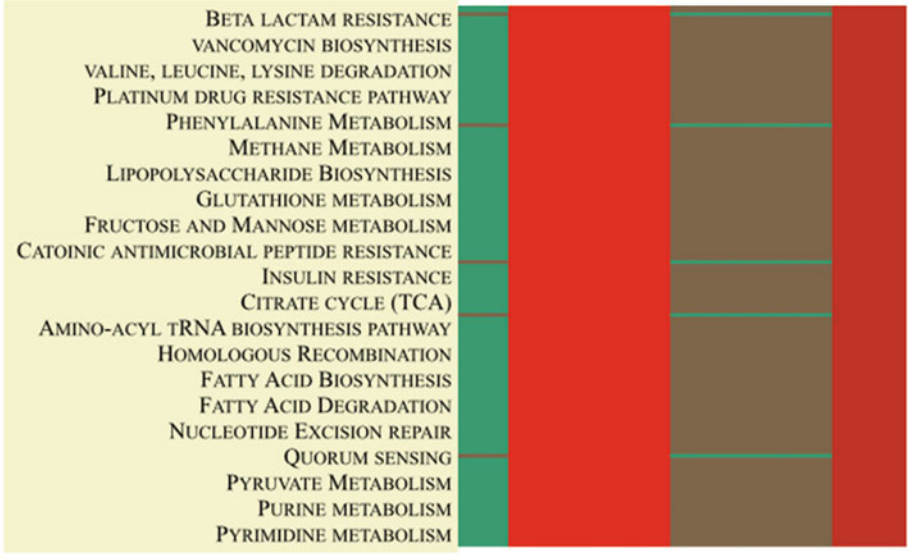

Fig. 23.2 Enriched genera and corresponding enriched pathways in the rhizospheric niche under study

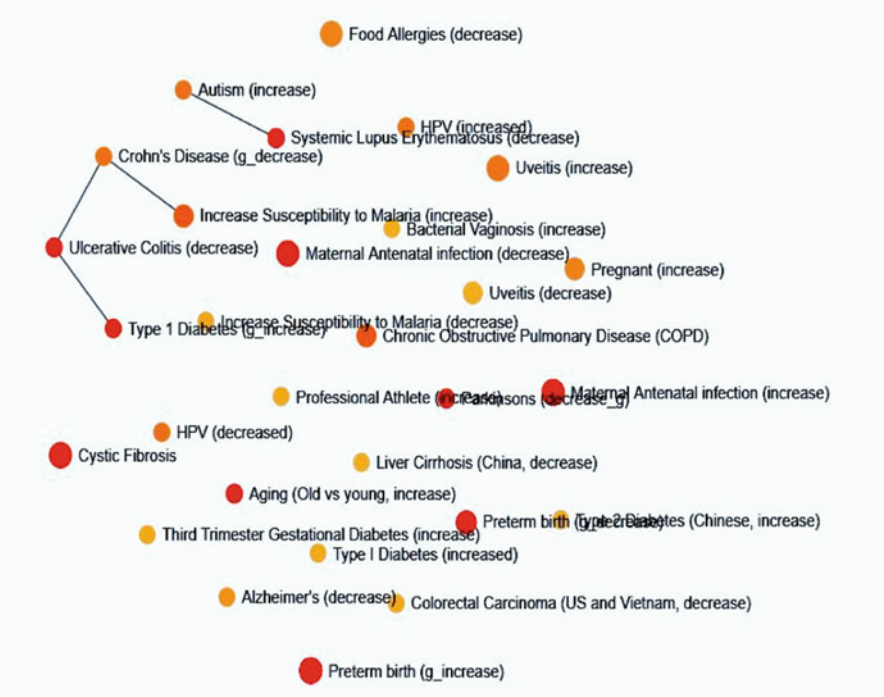

Fig. 23.3 Prediction of disease networks from rhizospheric microbial abundance

common microbes it was found that a complex disease network was prevalent in the area based on predictions (Fig. 23.5). This leads us to conclude that the variety of pathogenic organisms is much higher in both rural and urban wastewater samples, which are direct runoffs of hospital effluents thus increasing the inherent pathogenic load of the area.

23.5.5 Case Study 5

There are trillions of diverse bacteria which inhabit in human gastrointestinal tract and vary among individuals within and between communities. The initial inoculum of bacterium is acquired maternally during birth or inside womb. Subsequently, colonization of bacteria inside human gut depends upon several factors including diet, age, and diseases. The bacterial communities isolated from gastrointestinal exert phenotypic traits of the host by a complex network of interactions among them. Many of such interactions arising from modified gut bacterial profiles (GBP) have been observed to cause diseases in human. Moreover, modern lifestyle of the western countries makes people more prone to inflammatory disorders with altered GBP. The GBP of several population of the world, both with modern and traditional lifestyles have been studied from America, Europe, Africa, Korea, and China. The

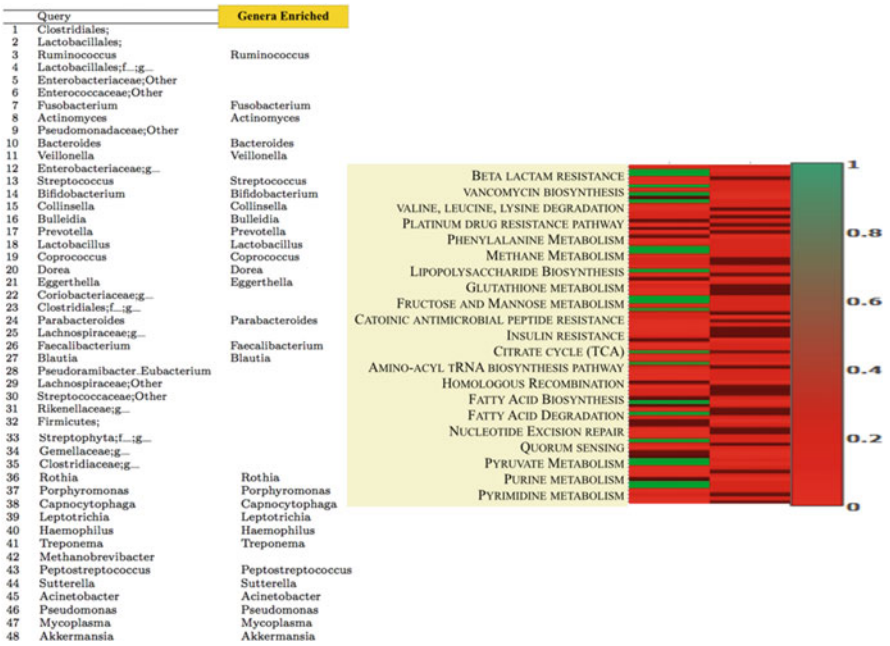

Fig. 23.4 Enriched genera and corresponding enriched pathways in the wastewater niche under study

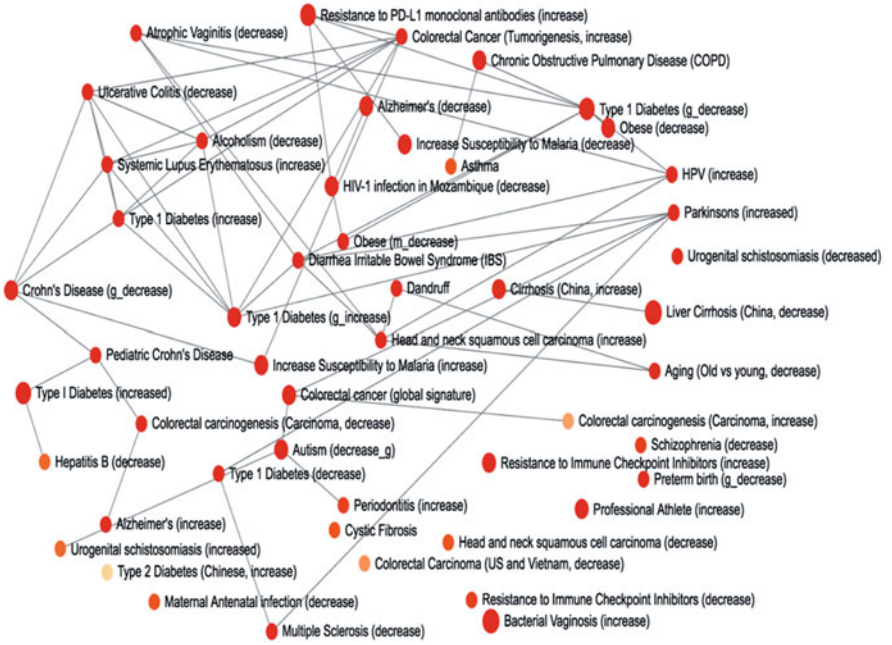

Fig. 23.5 Prediction of disease networks from microbial abundance dative the wastewater niche under study

	Query	Genera Enriched
1	unclassified (derived from Bacteria)	
2	Clostridium	Clostridium
3	unclassified (derived from Verrucomicrobia subdivision 3)	
4	Propionibacterium	Propionibacterium
5	unclassified (derived from Planctomycetaceae)	
6	Bacteroides	Bacteroides
7	Kineococcus	
8	Coptotermes	
9	Lactobacillus	Lactobacillus
10	Bifidobacterium	Bifidobacterium
11	Prevotella	Prevotella
12	Oryza	
13	Candidatus Solibacter	
14	Paenibacillus	Paenibacillus
15	Heliobacterium	
16	unclassified (derived from Alphaproteobacteria)	
17	unclassified (derived from unclassified sequences)	
18	Bacillus	Bacillus
19	Atopobium	Atopobium
20	Porphyromonas	Porphyromonas

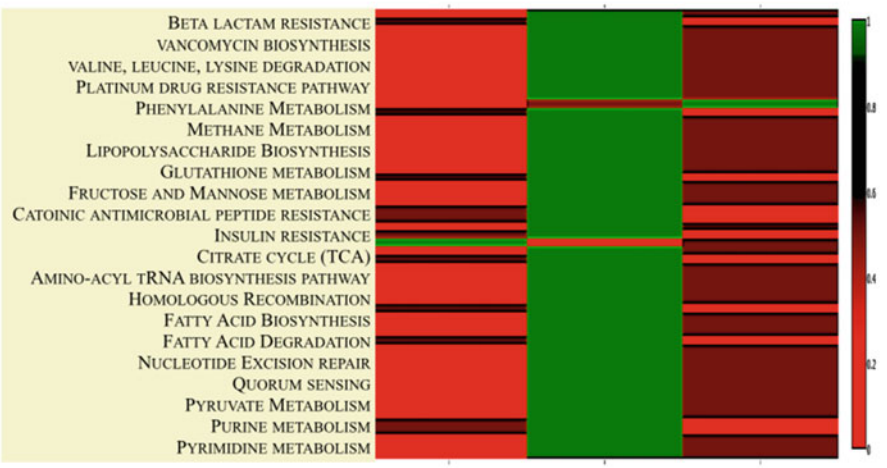

Fig. 23.6 Enriched genera and corresponding enriched pathways in the gut microbiomes under study

large tribal population of India offers a unique scenario for studies on gut bacterial profiles, because India consist of diverse communities who still depend on hunting, agriculture, and fishing along with their own culture, tradition, dietary habits, language, and genetic adaptability (Ganguli et al. 2019).

In this work fecal samples were collected from a tribal family belonging to the Dhrupka Bhutia tribal community and were subsequently sequenced using OXFORD Nanopore Minion sequencing platform for better elucidation of the bacterial members. Results obtained showed heterogenous abundance profiles of the bacterial members with the highest in case of male being *Lactobacillus*, for female: *Enterobacteria* and *Rothia* and for their male kid: *Leuconostoc* and *Fusobacterium*. Interesting observation was no antibiotic resistance pathway was identified in the pathway enrichment analysis (Fig. 23.6) which can be justified by the fact these tribal communities are not exposed to the over the counter medicines

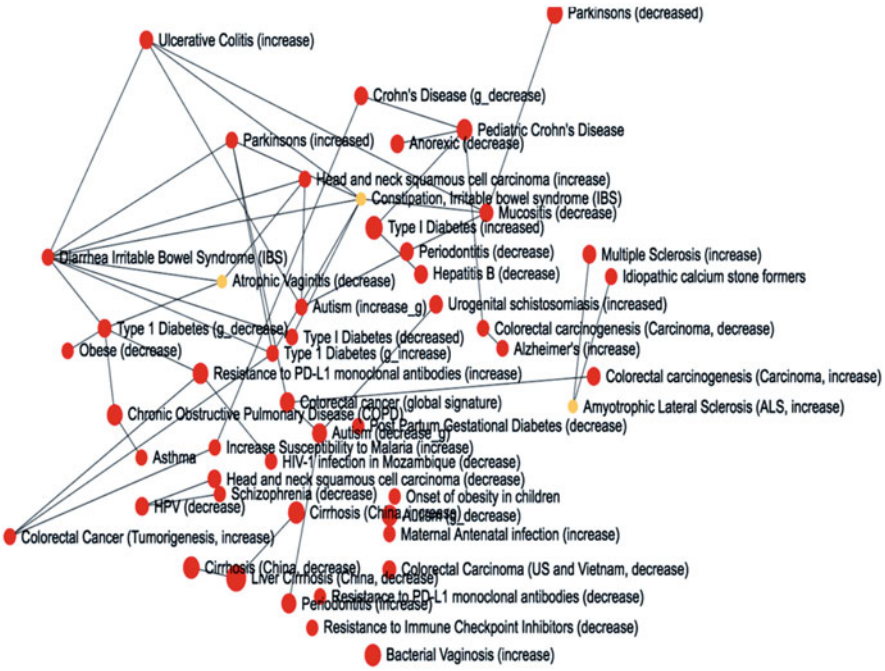

Fig. 23.7 Prediction of disease networks from microbial abundance data of the gut microbiomes under study

due to their remote habitat and extreme environmental conditions. Thus, their gut is still not exposed to antibiotic resistant bacteria, however, the presence of *Exiguo bacterium* in the gut is a clear warning to the threats of microplastics in the diet. Yang et al., have reported the ability of this bacterial strain to utilize plastics (Yang et al. 2014). It is thus alarming that this particular member has established itself as an important member of the gut of even tribal people whose gut is thought to be unadulterated and pristine. The disease network analysis revealed that irritable bowel syndrome and liver cirrhosis were important nodes (Fig. 23.7) which may be attributed to the inclination of these tribal members in having a regular dose of alcohol in their diet.

If we observe closely then all the predictions have their unique pathways which support the inter disease network analysis. While rhizospheric niche possesses microbes for food allergies as there may be several plant associations and exudates in the rhizosphere, hospital wastewater presents a complex biological niche laden with antimicrobial resistance pathways as well as behavioral disease pathways for diabetes, COPD, and vaginitis. The gut microbial datasets from remote tribes also exhibit characteristics features of no resistance pathways and lesser communicable disease pathways. These data further indicate the robustness of the pipeline in successful prediction of pathogenic load and possible disease prevalence in the

areas under study also providing a background insight on diet practices and medicine usage.

23.6 Conclusion

The above case studies indicate that culture-independent mechanism of metagenomics can be utilized properly for predicting the pathogenic load from a variety of samples from different environmental and disease associated niches. The DNA extraction mechanisms are vigorously standardized worldwide in equipped laboratories, where 21 DNA extraction protocols have been evaluated and reported recently. These protocols have contributed to comparison of microbial community and organization of DNA purification steps, with a conclusion that shows the largest outcome of preferably raw sequenced data and associated metadata, which is the ultimate focus for diagnostics. Following the abundance mapping and enrichment analyses steps a clear picture can be predicted which provides us with the necessary information on what pathogenic microbes may be present, what are the enriched biological pathways that are prevalent in the consortium and finally what disease associations can be prevalent. Once all technical and ethical barriers are overcome, we believe that metagenomic guided environment impact assessment, will be the next big area of research in the near future having the potential to alter the policymakers perspective on climate change and associated healthcare.

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References

Aarestrup FM, Koopmans MG (2016) Sharing data for global infectious disease surveillance and outbreak detection. Trends Microbiol 24(4):241–245

Albertsen M, Hugenholtz P, Skarshewski A, Nielsen KL, Tyson GW, Nielsen PH (2013) Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. Nat Biotechnol 31(6):533

Allard MW, Strain E, Melka D, Bunning K, Musser SM, Brown EW, Timme R (2016) Practical value of food pathogen traceability through building a whole-genome sequencing network and database. J Clin Microbiol 54(8):1975–1983

Alneberg J, Bjarnason BS, De Bruijn I, Schirmer M, Quick J, Ijaz UZ, Lahti L, Loman NJ, Andersson AF, Quince C (2014) Binning metagenomic contigs by coverage and composition. Nat Methods 11(11):1144–1146

Andersen SC, Fachmann MSR, Kiil K, Møller Nielsen E, Hoorfar J (2017a) Gene-based pathogen detection: can we use qPCR to predict the outcome of diagnostic Metagenomics? Genes 8 (11):332

Andersen SC, Kiil K, Harder CB, Josefsen MH, Persson S, Nielsen EM, Hoorfar J (2017b) Towards diagnostic metagenomics of campylobacter in fecal samples. BMC Microbiol 17(1):133

Cleary B, Brito IL, Huang K, Gevers D, Shea T, Young S, Alm EJ (2015) Detection of low-abundance bacterial strains in metagenomic datasets by eigengenome partitioning. Nat Biotechnol 33(10):1053

Ganguli S, Rahaman S, Bera AR, Vishal V, Malik S, Roopalakshmi K, Singh PK (2017) Rhizospheric metagenome of the terrestrial mangrove fern Acrostichum from Indian Sunderbans. Genomics Data 14:53–55

Ganguli S, Pal S, Das K, Banerjee R, Bagchi SS (2019) Gut microbial dataset of a foraging tribe from rural West Bengal - insights into unadulterated and transitional microbial abundance. Data Brief 25:103963. https://doi.org/10.1016/j.dib.2019.103963

Gilbert JA, Jansson JK, Knight R (2014) The earth microbiome project: successes and aspirations. BMC Biol 12(1):69

Haak W, Lazaridis I, Patterson N, Rohland N, Mallick S, Llamas B, Brandt G, Nordenfelt S, Harney E, Stewardson K, Fu Q (2015) Massive migration from the steppe was a source for indo-European languages in Europe. Nature 522(7555):207

Karsenti E, Acinas SG, Bork P, Bowler C, De Vargas C, Raes J, Sullivan M, Arendt D, Benzoni F, Claverie JM, Follows M (2011) A holistic approach to marine eco-systems biology. PLoS Biol 9 (10):e1001177

Key FM, Posth C, Krause J, Herbig A, Bos KI (2017) Mining metagenomic data sets for ancient DNA: recommended protocols for authentication. Trends Genet 33(8):508–520

Lindgreen S, Adair KL, Gardner PP (2016) An evaluation of the accuracy and speed of metagenome analysis tools. Sci Rep 6:19233

Loman NJ, Constantinidou C, Christner M, Rohde H, Chan JZM, Quick J, Weir JC, Quince C, Smith GP, Betley JR, Aepfelbacher M (2013) A culture-independent sequence-based metagenomics approach to the investigation of an outbreak of Shiga-toxigenic Escherichia coli O104: H4. JAMA 309(14):1502–1510

Mallick S, Li H, Lipson M, Mathieson I, Gymrek M, Racimo F, Zhao M, Chennagiri N, Nordenfelt S, Tandon A, Skoglund P (2016) The Simons genome diversity project: 300 genomes from 142 diverse populations. Nature 538(7624):201–206

Mande SS, Mohammed MH, Ghosh TS (2012) Classification of metagenomic sequences: methods and challenges. Brief Bioinform 13(6):669–681

Menzel P, Ng KL, Krogh A (2016) Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nat Commun 7(1):1–9

Micah H, Claire FL, Rob K (2007) The human microbiome project: exploring the microbial part of ourselves in a changing world. Nature 449(7164):804–810

Miller RR, Montoya V, Gardy JL et al (2013) Metagenomics for pathogen detection in public health. Genome Med (5):81. https://doi.org/10.1186/gm485

Naccache SN, Federman S, Veeraraghavan N, Zaharia M, Lee D, Samayoa E, Bouquet J, Greninger AL, Luk KC, Enge B, Wadford DA (2014) A cloudcompatible bioinformatics pipeline for ultrarapid pathogen identification from next-generation sequencing of clinical samples. Genome Res 24(7):1180–1192

Nakamura S, Maeda N, Miron IM, Yoh M, Izutsu K, Kataoka C, Honda T, Yasunaga T, Nakaya T, Kawai J, Hayashizaki Y (2008) Metagenomic diagnosis of bacterial infections. Emerg Infect Dis 14(11):1784

Nielsen HB, Almeida M, Juncker AS, Rasmussen S, Li J, Sunagawa S, Plichta DR, Gautier L, Pedersen AG, Le Chatelier E, Pelletier E (2014) Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. Nat Biotechnol 32(8):822–828

Orlando L, Ginolhac A, Zhang G, Froese D, Albrechtsen A, Stiller M, Schubert M, Cappellini E, Petersen B, Moltke I, Johnson PL (2013) Recalibrating Equus evolution using the genome sequence of an early middle Pleistocene horse. Nature 499(7456):74–78

Ounit R, Wanamaker S, Close TJ, Lonardi S (2015) CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. BMC Genomics 16(1):236
Pallen MJ (2014) Diagnostic metagenomics: potential applications to bacterial, viral and parasitic infections. Parasitology 141(14):1856–1862
Petersen TN, Lukjancenko O, Thomsen MCF, Sperotto MM, Lund O, Aarestrup FM, Sicheritz-Pontén T (2017) MGmapper: reference based mapping and taxonomy annotation of metagenomics sequence reads. PLoS One 12(5):e0179778
Rahaman S, Bera AR, Vishal V, Gupta S, Chakraborty W, Ganguli S (2019) Biochemical and metagenomic insight into the impact of climate change on an epiphytic fern of the Indian Sunderbans. Trop Plant Res 6(3):433–437
Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL (2016) Analysis of the microbiome: advantages of whole genome shotgun versus 16S amplicon sequencing. Biochem Biophys Res Commun 469(4):967–977
Rodriguez-r LM, Konstantinidis KT (2014) Estimating coverage in metagenomic data sets and why it matters. ISME J 8(11):2349–2351
Sangwan N, Xia F, Gilbert JA (2016) Recovering complete and draft population genomes from metagenome datasets. Microbiome 4(1):8
Schlebusch CM, Skoglund P, Sjödin P, Gattepaille LM, Hernandez D, Jay F, Li S, De Jongh M, Singleton A, Blum MG, Soodyall H (2012) Genomic variation in seven Khoe-san groups reveals adaptation and complex African history. Science 338(6105):374–379
Schneeberger PH, Becker SL, Pothier JF, Duffy B, N'Goran EK, Beuret C, Frey JE, Utzinger J (2016) Metagenomic diagnostics for the simultaneous detection of multiple pathogens in human stool specimens from Cote d'Ivoire: a proof-of-concept study. Infect Genet Evol 40:389–397
Sedlar K, Kupkova K, Provaznik I (2017) Bioinformatics strategies for taxonomy independent binning and visualization of sequences in shotgun metagenomics. Comput Struct Biotechnol J 15:48–55
Segata N, Boernigen D, Tickle TL, Morgan XC, Garrett WS, Huttenhower C (2013) Computational meta'omics for microbial community studies. Mol Syst Biol 9(1):666
Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C (2012) Metagenomic microbial community profiling using unique clade-specific marker genes. Nat Methods 9(8):811
Singh M, Ganguli S, Ghosh MM (2019) Comparative metagenomic dataset of hospital effluent microbiome from rural and urban hospitals in West Bengal. Data Brief 25:104264
Skoglund P, Thompson JC, Prendergast ME, Mittnik A, Sirak K, Hajdinjak M, Salie T, Rohland N, Mallick S, Peltzer A, Heinze A (2017) Reconstructing prehistoric African population structure. Cell 171(1):59–71
Warinner C, Herbig A, Mann A, Fellows Yates JA, Weiß CL, Burbano HA, Orlando L, Krause J (2017) A robust framework for microbial archaeology. Annu Rev Genomics Hum Genet 18:321–356
Wood DE, Salzberg SL (2014) Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15(3):R46
Yun S, Yun S (2014) Masking as an effective quality control method for next-generation sequencing data analysis. BMC Bioinform 15(1):382
Yang J, Yang Y, Wu WM, Zhao J, Jiang L (2014) Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environ Sci Technol 48 (23):13776–13784

High-Throughput Analysis to Decipher Bacterial Diversity and their Functional Properties in Freshwater Bodies

24

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Abstract

Freshwater ecosystem encompasses varied and rich diversity of habitat conditions and is home to diverse microbial community. Each of these diverse microbial community plays a specific function that contributes to the importance of such an ecosystem in the global carbon cycle through consumption and emission of carbon dioxide and thereby in the regulation of the global climate. However, unlike their counterpart of other ecosystem, viz., terrestrial, the microbial diversity studies of freshwater ecosystem have been comparatively scanty mainly because many of the methods suitable for their identification were only developed recently. The conventional methods of studying microbes that relied on cultivation of the organism for identification in laboratories left out many that were uncultivable under laboratory conditions. Recently, several tools and techniques including both traditional and high-throughput state-of-the-art technologies have been explored to decipher the microbial diversities of freshwater ecosystem. Advancement in tools and techniques related to microbial diversity studies has provided new insights into microbial diversity and their functioning in freshwater ecosystem. In this chapter we discuss the different traditional methods followed by molecular biology techniques that are used to decipher microbial diversity of both cultivable and non-cultivable microbes of freshwater ecosystem. We discuss

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in detail about the cutting-edge high-throughput technologies, viz., metagenomics, metatranscriptomics, and metaproteomics that are aiding in increasing our understanding of the freshwater microbial diversity as well as their functioning.

Keywords

Belowground microbes · Metagenomics · Metatranscriptomics · Metaproteomics · NGS

24.1 Introduction

It is estimated that above 96% of the free water on earth is found in the ocean while the remaining 4% is available as freshwater bodies in the form of lakes, rivers, glaciers, stagnant water bodies, etc. (Durack 2015). Although a very small proportion of free water is available as fresh water, these are reservoirs of biodiversity. Freshwater bodies are considered as one of the most important life-support systems on this planet (Young and Steffen 2009). The freshwater bodies are rich in nutrients, organic sediments, and minerals and supplies diversified flora, fauna, and microorganisms. Not only aquatic organisms, but countless of terrestrial organisms are also linked to the freshwater ecosystems through their food web. Starting from the producers to the decomposers, all individuals of food chains are part of these ecosystems and thus, these ecosystems have been important contributors to the process of evolution since the origin of life.

The energy recycling process is very important for maintenance and alteration of the vegetation in an ecosystem. The decomposers are crucial players of an ecosystem, which recycles the energy by breakdown of complex organics into simpler forms. Microorganisms including bacteria and fungi function as prime units of decomposition of the dead remains and unutilized complex organic matters into usable forms such as phosphorus (in the form of phosphate) and nitrogen (in the form of ammonium) for producers, thereby recycling the ecosystem's energy (Stockner and Porter 1988). Bacterial communities of freshwater ecosystems are exclusively diverse due to the existence of unexpectedly heterogeneous microhabitats differing in size, complexity, and temporal–spatial dynamics. These microhabitats can be classified into diffusion-controlled water phase (DifP), colloidal phase including the nanogels and microgels (ColP), particles such as exudates and aggregates (Par), and the living biosphere including algae, zooplankton, phytoplankton, and fish (Bio) (Zoccarato and Grossart 2019). For each microhabitat, microbial diversity and functionality differ within a particular ecosystem. Likewise, bacterial diversity and functionality also vary among different types of freshwater bodies depending upon geographical location, external environmental factors (such as temperature, soil pH), types of biotic population linked to that ecosystem, and age of the ecosystem (Hartman et al. 2008; Stanish et al. 2016; Shafi et al. 2017). In this chapter, we provide a brief overview of the taxonomic and functional diversity of bacteria

deciphered using various conventional methods as well as high-throughput approaches such as "omics" technology. We also discuss how the bacterial diversity and functionality vary in different freshwater ecosystems to influence the productivity of these water bodies as well as their surrounding terrestrial environments. The chapter also introduces the readers briefly to the emerging technologies for estimating bacterial diversity in any environment.

24.2 Bacterial Diversity in Freshwater Ecosystems and their Functional Attributes

Bacterial diversity in different freshwater ecosystems varies depending on the physical, compositional, and biochemical properties of the freshwater bodies. For example, the bacterial community compositions in a lake differ from those in a river or in agricultural wetlands. More specifically, different lake ecosystems host different bacterial community compositions depending upon the type, structure, and geographical location. Freshwater bacteria have been extensively examined using culture-independent methods, such as metagenomics (Abia et al. 2018; Shen et al. 2019; Samson et al. 2019), fluorescence in situ hybridization (FISH) (Sekar et al. 2003; Lindström et al. 2005), quantitative polymerase chain reaction (qPCR), and terminal restriction fragment length polymorphism (T-RFLP) (Eiler and Bertilsson 2004, 2007; Hu et al. 2016). It is not possible to discuss all of the studies that analyze bacterial diversity in different freshwater ecosystems. However, a detailed list of published literatures regarding analysis of bacterial diversity in different freshwater bodies is mentioned in Table 24.1.

Bacterial communities of aquatic ecosystems play a crucial role in accumulation, transformation, and migration of nutrients and other organic matters leading to energy conversion and recycling of materials (Fenchel and Jørgensen 1977; Cotner and Biddanda 2002; Newton et al. 2011). Apart from the compositional variability, bacterial communities also show high degrees of functional variability (Newton et al. 2011). These variations serve as valuable ecological marker for the study of bacterial community assembly and functions of the ecosystem. Previous studies suggested that specific fundamental attributes can be shared by some distinct taxa, while closely related bacterial species can also show distinctive functional properties (Allison and Martiny 2008; Fierer et al. 2012; Dopheide et al. 2015).

The microbial diversity and functionality are dependent upon the type of microhabitats they occupy in an aquatic ecosystem. The functional properties of bacterial communities vary in each microhabitat. The diffusion-controlled water phase (DifP) associated bacteria rely on the physical and chemical changes of the water phase (Zoccarato and Grossart 2019). Few bacteria are able to recognize the spatial and temporal distribution of dissolved organic compounds through chemotaxis, while other non-motile bacteria are found to be distributed randomly in the water phase and consume those compounds available in the vicinity (Stocker 2012). The consumption of organic matters from water phase by these bacteria is through enzymatic breakdown and concomitant release of inorganic compounds (e.g., N and

Table 24.1 Bacterial diversities in different freshwater ecosystems

Sl. no.	Type of ecosystem	Dominant bacterial taxa	Approach	Reference
1.	River ecosystem; Mississippi River	Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia	16S rRNA amplicon sequencing: Next gen Illumina	(Staley et al. 2013; Payne et al. 2017)
2.	Arctic river ecosystem: Yenisei River	Actinobacteria, Proteobacteria	16S rRNA amplicon sequencing: Next gen Illumina	(Kolmakova et al. 2014)
3.	River ecosystem: Danube river	Actinobacteria, Proteobacteria, Bacteroidetes, Verrucomicrobia	16S rRNA amplicon sequencing: Next gen Illumina	(Savio et al. 2015a)
4.	River ecosystem: River Thames	Bacteroidetes in the headwaters; Actinobacteria-dominated downstream	16S rRNA gene pyrosequencing	(Read et al. 2015)
5.	River ecosystem: River Mandakini Alaknanda and their confluence	*Pseudomonas extremoriental*, *Bacillus licheniformis*, *Paenibacillus glucanolyticus*, *Bacillus badius*, *Pseudomas fulva*, *Pseudomonas azotoforman*, *Paenibacillus thiaminolyticus*	MADI-ToF-MS	(Kumar et al. 2018)
6.	River ecosystem: River Yamuna and its confluence on River Ganga	Proteobacteria, Bacteroidetes, and Firmicutes	Whole metagenome sequencing using MinION (Oxford Nanopore Technologies, Oxford, UK)	(Samson et al. 2019)
	Polluted river ecosystem: Apatlaco River	*Thiomonas, Polaromonas, Pedobacter, Myroides, Pseudomonas, Acinetobacter, Aeromonas,* and *Tavera*	Whole metagenome sequencing using NextSeq500 (Illumina, Inc., San Diego, CA, USA)	(Breton-deval et al. 2020)
7.	Himalayan Lake ecosystem: Pangong Lake	Proteobacteria, Bacteroidetes	Metagenome shotgun sequencing: Illumina	(Rathour et al. 2017)
8.	Sediments of urban lakes	Chloroflexi, Proteobacteria, and Acidobacteria; Bacteroidetes and Proteobacteria	T-RFLP analysis	(Zhao et al. 2012)

(continued)

Table 24.1 (continued)

Sl. no.	Type of ecosystem	Dominant bacterial taxa	Approach	Reference
9.	Freshwater Lake ecosystem: Baikal Lake	Anaerolineaceae (Chloroflexi), Flavobacteriaceae, Cytophagaceae (Bacteroidetes), Coriobacteriaceae (Actinobacteria), and Nitriliruptoraceae (Actinobacteria)	16S rRNA amplicon pyrosequencing	(Kurilkina et al. 2016)
10	Freshwater lakes on Yun-Gui plateau	Cyanobacteria in the eutrophic ecosystems; Actinobacteria, Proteobacteria (alpha-, Beta-, and Gamma-proteobacteria), Verrucomicrobia and Planctomycetes in meso-oligotrophic system	Metagenome shotgun sequencing: Illumina platform (Illumina, Inc., San Diego, CA, USA)	(Shen et al. 2019)
11.	Reshi and Yumthang hot spring lake ecosystems	Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes	Metagenome shotgun sequencing	(Najar et al. 2020)
12.	Freshwater glacier lake Yukidori-Ike	Proteobacteria Alpha-proteobacteria, Beta-proteobacteria, Deltaproteobacteria, and Gamma-proteobacteria, cyanobacteria, Firmicutes	16S rRNA amplicon sequencing	(Chaya et al. 2019)
13.	Recreational freshwaters (east fork Lake, Delaware Lake, and Madison Lake)	Actinobacteria, Cyanobacteria, Firmicutes, and Proteobacteria	Bacterial tag-encoded pyrosequencing	(Noble et al. 2016)
14.	Arsenic contaminated groundwater of Assam	Proteobacteria followed by Bacteroidetes, Planctomycetes, Verrucomicrobia	Metagenome shotgun sequencing: Illumina	(Das et al. 2017)
15.	Agricultural pond ecosystem	Actinobacteria in all time points; Chloroflexi, Firmicutes, Cyanobacteria, and Proteobacteria with seasonal occurrence	16S rRNA amplicon sequencing	(Chopyk et al. 2018)
16.	Municipal drinking waters in the Ohio River basin	*Mycobacterium* spp. (Actinobacteria), MLE1–12 (phylum cyanobacteria), *Methylobacterium* spp., and Sphingomonads	16S amplicon sequencing: Illumina (Illumina, Inc., USA)	(Stanish et al. 2016)

(continued)

Table 24.1 (continued)

Sl. no.	Type of ecosystem	Dominant bacterial taxa	Approach	Reference
17.	Wetland ecosystem	Proteobacteria, Chloroflexi, Bacteroidetes, and Euryarchaeota	16S amplicon sequencing: Illumina (Illumina, Inc., USA)	(He et al. 2015)
18.	Wastewater treatment plants	Proteobacteria, Actinobacteria, Firmicutes, and Chloroflexi	16S rRNA amplicon sequencing	(Osunmakinde et al. 2019)
19.	Constructed wetland	Proteobacteria (50% of all taxa in soil, 65% in water)	Whole metagenome sequencing using Illumina HiSeq2500, (Illumina, Inc., USA)	(Bai et al. 2014)
20.	Acidic peatlands	Members of Acidobacteria and Actinobacteria were most active candidates Members of Acidobacteria and Actinobacteria were most active candidates	Metatranscriptomic analysis using Illumina HiSeq2000, (Illumina, Inc., USA)	(Ivanova et al. 2016)
21.	Wetland sediments in the US geological survey managed Cottonwood Lake	Proteobacteria, Acidobacteria, Chloroflexi, Planctomycetes, Ignavibacteriae, Thaumarchaeota, and the candidate divisions KSB1 and Rokubacteria	Whole metagenome sequencing using Illumina HiSeq2500, (Illumina, Inc., USA)	(Martins et al. 2019)
22.	Freshwater wetland soils	Members of Methanomassiliicoccaceae were the most active group	16S rRNA amplicon sequencing using miSeq platform (Illumina, Inc., USA)	(Narrowe et al. 2019)
23.	Mudflat sediments	Chloroflexi, Acidobacteria, and Bacteroidetes and the classes Delta- and Gamma-proteobacteria, along with the archaeal lineages phylum Bathyarchaeota and the order Thermoplasmatales	16S rRNA gene amplicon sequencing and metatranscriptome sequencing using Illumina MiSeq platform (Illumina, Inc., USA)	(Yan et al. 2018)
24.	Subarctic wetland in Russia	Acidobacteria, Alpha-proteobacteria, Gamma-proteobacteria, Actinobacteria, Planctomycetes, Verrucomicrobia, and Candidatus *Methylospira mobilis*	Amplicon sequencing using Illumina MiSeq platform (Illumina, Inc., USA)	(Danilova et al. 2016)

(continued)

Table 24.1 (continued)

Sl. no.	Type of ecosystem	Dominant bacterial taxa	Approach	Reference
25.	Wetlands on the Qinghai-Tibetan plateau revealed	Proteobacteria, Actinobacteria and Bacteroidetes, Chloroflexi, Acidobacteria, Verrucomicrobia, Firmicutes, and Planctomycetes	16S rRNA pyrosequencing	(Deng et al. 2014)

P). This process of bacterial conversion is called mineralization of the organic matter.

In aquatic systems, the dissolved organic matter (DOM) often undergoes self-assembly to form nanogels and eventually aggregates as microgels of approximately 3–5 mm (Chin et al. 1998a). These microgels, also termed as particulate organic matter (POM), are often found in the marine aquatic systems; however, DOM-POM conversion is also found in freshwater environments such as lakes and rivers (Chin et al. 1998b; Kerner et al. 2003; Pace et al. 2012). The prime source of DOM includes algal exudates, sloppy grazing carried out by zooplankton, and cellular decomposition often performed by bacteria through degradation of phytoplankton, zooplankton, or other bacteria (Carlson 2002). Degradation of phytoplankton, zooplankton or other bacteria may change the composition and concentration of the DOM pool present in an aquatic system. Bacteria has the ability to metabolize the components of DOM pool such as carbohydrates, lipids, proteins, and organic acids and convert them into a simpler form (Kirchman 2003).

24.2.1 Bacterial Community Compositions in Lakes

Lakes may vary in nutrient status, some of which being oligotrophic, while the others being eutrophic. Lakes that are poor in nutrients remain oxic throughout the year, and distinct oxygen stratification usually does not occur due to seasonal temperature variations. In contrast, sedimentation of organic matters in the bottom parts occurs in case of eutrophic lakes. The epilimnion (warmer, upper layer) of a thermally stratified lake is oxic, while the hypolimnion (colder, bottom layer) is usually anoxic (particularly when the lake is rich in nutrients). There is a thermocline separating the epilimnion and hypolimnion, which prevents the mixing of the two layers. Therefore, waters in the bottom layer may become anoxic. This situation is permanent for tropical eutrophic lakes and occurs in the summer in eutrophic lakes of temperate regions (Sharma et al. 2019).

Till a few decades, the diversity analysis of bacterial communities inhabiting the terrestrial and aquatic habitats was quite similar and dependent upon traditional culture-based cultivation techniques (Jones and Rheinheimer 1986). But the emergence of modern tools and techniques such as next-generation sequencing provided

unprecedented access to the community composition and diversity of bacterial species in these distinct habitats (Lozupone and Knight 2007). Llirós et al. (2014) analyzed bacterial community composition (BCC) in three freshwater reservoirs with varying physical and chemical properties and distinctive trophic status through 16S rRNA gene amplicon 454 pyrosequencing. Those reservoirs had BCC similar to that of natural freshwater lakes. The dominant bacterial groups in those reservoirs were Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Cytophaga–Flavobacteria–Bacteroidetes (CFB), and Verrucomicrobia (Llirós et al. 2014). In an independent study, previously reported 689 bacterial 16S rRNA gene sequences from 11 freshwater lakes were analyzed to decode their phylogeny, and identified ten freshwater phyla with 34 supposed clusters (monophyletic branches of a phylogenetic tree) freshwater bacteria. Among those, at least two sequences (with $\geq$95% gene identity among them) were linked to more than one freshwater environment (Zwart et al. 2002).

In a recent study, Shen et al. (2019) employed a metagenomics approach to assess the correlations between trophic status and planktonic microbiota in the freshwater lakes of Yun-Gui Plateau, China. Distinct community structures and metabolic potential were recorded among the eutrophic and mesotrophic-oligotrophic lake ecosystems. Cyanobacterial species were found to be dominant in the eutrophic ecosystems, whereas Actinobacteria, Proteobacteria (Alpha-, Beta-, and Gamma-Proteobacteria, Verrucomicrobia, and Planctomycetes were dominant communities in the mesotrophic-oligotrophic ecosystems (Shen et al. 2019). The bacterial diversity in the deepest freshwater lake Baikal was assessed using next-generation pyrosequencing. The 16S rRNA based metagenomic sequencing identified 1693 operational taxonomic units (OTUs) belonging to different phyla, *viz*., Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Acidobacteria Firmicutes, and Cyanobacteria (Kurilkina et al. 2016).

24.2.2 Bacterial Community Compositions in Rivers

The physical and biological properties of a river ecosystem are somewhat different than a lake ecosystem. Unlike the lakes, the river water achieves a fluid motion and thus physical properties change from upstream to downstream. Such fluid ecosystem may display changes in the composition of microbiome from one region to another along the flow path (Crump et al. 2007; Savio et al. 2015b). Fluid ecosystem such as rivers that are major confluences of rapid running waters and long stretches of slow, gradual change between these interfaces are potential sites for analyzing the microbial diversity. Such analysis may provide a better understanding of how abrupt supplementated distinct and external microbiomes adapt in comparison to adaptation of microbiomes selected gradually across the moving river. The 16S rRNA-gene amplicon sequencing based analysis of bacterial diversity revealed the dominance of Actinobacteria and Proteobacteria in Upper Mississippi River (USA) (Staley et al. 2013), the Yenisei River (Russia) (Kolmakova et al. 2014), the River Thames (UK) (Read et al. 2015), and the Danube River (Europe) (Savio et al. 2015b). Staley

and colleagues (Staley et al. 2013) first reported the occurrence of a persistent and ubiquitous "core bacterial community" throughout a river stretch. The variation pattern in diversity of the microbiome in Mississippi river was also examined by Payne et al. (2017). Their study revealed distinct and dominant bacterial phyla composition and proportional abundance in free-living and particle-associated cells along the entire river, except for substantial but transient disturbance near the city of Memphis, Tennessee. Samples collected from free-living samples along the Mississippi River contained higher abundance of Actinobacteria while in particle-associated samples, Proteobacteria were dominant (Payne et al. 2017).

A high-throughput metagenomic study was conducted to decipher the BCC of Ganga River, India and the transient influence of Yamuna River on it (Samson et al. 2019). Whole metagenome sequencing of the metagenomic DNA from the sediment samples revealed differences in their relative abundance across the confluence. It was reported that the site in the Yamuna River (G15Y) and at immediate downstream of confluence of Ganges (G15DS) had higher abundance of Proteobacteria and lower abundance of Bacteroidetes and Firmicutes compared to the upstream, confluence, and distant downstream of confluence (Samson et al. 2019).

24.2.3 Bacterial Community Compositions in Wetlands

Wetlands, comprising of about 5–8% of the earth's land surface (Mitsch et al. 2013) and nearly 45% of the total natural ecosystems globally (Costanza et al. 1997), are considered as one of the most essential aquatic cum terrestrial ecosystems and distributed throughout the world. The bacterial communities mediate crucial role in the functional characteristics of the wetland ecosystems. Microbes inhabiting the rhizosphere of wetland plants are important for nutrient cycling, carbon sequestration, contaminant elimination, and ecosystem functioning. Terminal restriction fragment length polymorphism technique was adopted to determine the BCC in the rhizosphere of three wetland plants, viz., *Acorus calamus*, *Typha latifolia*, and *Phragmites karka* (Singh and Singh 2018). Firmicutes were reported to be most dominant phylum in the rhizosphere of these plants, which was followed by Proteobacteria and Actinobacteria. The bacterial groups Chloroflexi, Acidobacteria, Deferribacteres, and Thermotogae also reported from the rhizosphere of *P. karka* and *T. latifolia* but were not detected in *A. calamus* (Singh and Singh 2018).

Specific bacterial taxa may perform important functions in different wetland ecosystems. Loktak Lake in Manipur, India is a freshwater wetland known for its floating natural vegetations known as floating islands or *Phumdi*. Salkar and his coworkers reported that the bacterium *Enterobacter tabaci* isolated from the *Phumdi* of Loktak Lake possesses multiple PGP traits like IAA production, siderophore production, HCN production, ammonia production, phosphate solubilization, and nitrogen fixation (Salkar et al. 2018). Recent study using metagenomics approach has detected the presence of several plant growth promoting bacterial taxonomic units in the soil samples collected from Loktak Lake. Like the wetland ecosystems, bacterial isolates from rivers and other ecosystems were also reported to show plant

growth promoting properties. In a recent study, *Chryseobacterium salivictor* sp. nov. with plant growth promoting properties has been characterized (Kim and Yu 2020a). Genomic data suggested the ability of that bacterial isolate to encode several PGP enzymes (Kim and Yu 2020b).

24.3 Determination of Microbial Diversity

Microbial diversity in a particular ecosystem can be addressed through the genetic diversity within species, the species diversity, and the ecological diversity of the community (Harpole 2010). The innate ability of microorganisms to not only adapt and survive in different niches but also to maintain the balance in ecosystems is a very important feature and thus it is relevant to study their diversity as it exists. The inability to visualize them with the unaided eye made effective classification a difficult task in the past. However, with the advent of modern tools and techniques classifications of microorganisms have become much efficient and accurate. Using various tools and techniques microorganisms could be classified into various taxonomical units and the classification is growing based on the availability of new tools and data. In the following sections, we discuss on the types of microbial diversity and the techniques being used to determine the microbial diversity.

24.4 Taxonomic Diversity and Functional Diversity

Diversity is the variation among the microorganisms at the taxonomic level that accounts for species composition and abundance or at the functional level which accounts for the ecological traits of species. Taxonomy is the theory and practice of classifying groups of biological organisms on the basis of common characteristics into subspecies, species, genera, families, and higher orders (Ohl 2015). Taxonomic diversity refers to the numbers of different taxonomic groups present in an ecosystem. Functional diversity refers to a range of functional traits of microorganisms prevailing in an ecosystem, i.e., how they affect and interact with each other in a native environment and under changing environmental conditions (Petchey and Gaston 2006; Laureto et al. 2015).

24.5 Techniques to Evaluate Bacterial Taxonomic and Functional Diversity

The different techniques used for evaluating bacterial diversity (Taxonomic and Functional) are based on the fact that all bacteria are not culturable. Therefore, the techniques used to decipher the diversity of cultivable bacteria would vary from those which are uncultivable. Culture-dependent methods of determining bacterial diversity rely on the pure cultures of organisms present in an environmental sample followed by determination of their taxonomic and functional characteristics through

the application of conventional/biochemical methods as well as modern molecular tools. The conventional/biochemical methods of microbial diversity assessment are straightforward in revealing taxonomic and functional diversity and involve plating of environmental samples on appropriate nutrient media, followed by analysis of the colonies formed. Although this method is rapid and economical, it requires the knowledge of suitable growth media, optimum growth conditions, and other parameters (Trevors 1998; Tabacchioni et al. 2000). Other methods include BIOLOG based carbon source utilization profile and community level physiological profile (CLPP). These methods provide the initial idea of the physiological profile such as the nutritional profile and the nature of the products produced by the organism. This information also gives an idea about the functions of a particular group of bacteria in that habitat from where it was isolated. However, methods based on the biochemical analysis sometime fail to give the actual taxonomic identity of bacteria.

Therefore, the polyphasic system of taxonomy has been adopted to verify the taxonomic diversity of cultivable bacteria (Vandamme et al. 1996). Polyphasic taxonomy groups bacteria based on the information obtained at phenotypic, genetic, and phylogenetic level (Colwell 1970). Phenotypic information include various data such as cell wall composition, cellular fatty acid composition, isoprenoid quinones, polyamines, etc., and other expressed characters whereas the genotypic characteristics are based on the data obtained from nucleic acids (DNA and RNA) such as the sequences of 16S rDNA, %GC content, and DNA–DNA relatedness, etc. (Vandamme et al. 1996). These techniques help identify bacteria up to genus or species level (Vandamme et al. 1996). In addition, different genetic fingerprinting techniques, serological typing, ribotyping, phage typing, have been used to identify bacteria up to species and strain levels (Vandamme et al. 1996; Prakash et al. 2007). Some advanced techniques such as Fourier transform infrared spectroscopy, pyrolysis mass spectrometry, and UV resonance Raman spectroscopy have also used to assess cell wall composition of bacteria (Magee 1993; Vandamme et al. 1996; Prakash et al. 2007). The phylogenetic approach uses the evolutionary data to group uncharacterized bacteria based on the established data obtained from known bacteria. Basically this approach relies on obtaining partial or complete DNA sequence information of unknown bacterial species followed by comparing the unknown sequences with the partial/total DNA sequences of similar known member of the bacterial community (Srivastava et al. 2019). The 16S rDNA-based phylogenetic studies have been routinely applied by researchers to classify or group bacteria isolated from different environment (Parveen et al. 2016; Goswami et al. 2017a, b; Fatima et al. 2018; Chowdhury et al. 2018; Deka et al. 2019; Hazarika et al. 2019, 2020). However, the difficulties in culturing approximately 99% of bacteria present in the natural habitats is the biggest bottleneck of these techniques (Hugenholtz 2002). Therefore, it is important to develop culture techniques which can cultivate bacteria which are previously uncultivable to get better insight into their physiological processes which may be beneficial in developing different biotechnological products (Giovannoni and Stingl 2005).

24.6 Culture-Independent Methods to Determine Taxonomic and Functional Diversity of Bacteria

It is estimated that approximately 5×10^5 microbes inhabit the earth, but only 1% of the total microbes that exist in the environment are cultivable (Amann et al. 1995; Rappé and Giovannoni 2003). Due to this most of the microbes inhabiting the environment have not been studied or described. So, there is a great probability that the unknown and uncultivable bacteria have the potential to become warehouse of novel industrial enzymes, natural compounds to produce novel bioprocesses and technology for diagnosis and medicine and agriculturally important resources (Kimura 2018). To explore the taxonomic diversity as well as functional characteristics of bioresources, culture-independent technologies such as phospholipid and fatty acid analysis (PLFA) (Cotter et al. 2000; Buyer and Sasser 2012; Mrozik et al. 2014), DNA microarrays (Wagner et al. 2007), fluorescence in situ hybridization (FISH) (Ivanov et al. 2003), quantitative real-time polymerase chain reaction (qRT-PCR) (Fierer et al. 2005), different genetic fingerprinting techniques, viz., denaturing-gradient gel electrophoresis or temperature-gradient gel electrophoresis (DGGE/TGGE) (Muyzer et al. 1993), single-strand conformation polymorphism (SSCP) (Schwieger and Tebbe 1998), amplified ribosomal DNA restriction analysis (ARDRA) (Uchiyama et al. 2002; Lagacé et al. 2004), terminal restriction fragment length polymorphism (T-RFLP) (Liu et al. 1997), and ribosomal intergenic spacer analysis (RISA) (Fisher and Triplett 1999; Fechner et al. 2010). However these techniques are not described as these are not in the scope of this chapter.

The rapid advances in molecular techniques have aided the development of cutting-edge technology such as metagenomics, metatranscriptomics, and metaproteomics which have enabled rapid analysis of large number of samples, profiling of multiple communities, deciphering genetic diversity, and functional characteristics (Daniel 2005; Gilbert et al. 2008; Shi et al. 2009; Langille et al. 2013; Kolmeder et al. 2015; Abia et al. 2018; Hayden et al. 2018; Russo et al. 2019; Samson et al. 2019). The techniques are discussed in the following sections.

24.7 Metagenomics

The term metagenomics was coined in 1998 (Handelsman et al. 1998). Metagenomic analysis is generally used to explore complex microbial communities of different environment directly without the need of culturing or isolating any organism from the environmental samples and allows researchers to assess the species present in the community and also provides insights into the metabolic and functional activities of the microbes/bacteria in the environmental sample (Langille et al. 2013). Metagenomic approaches answer fundamental questions such as which and how many types of organisms are present in a particular environmental sample (taxonomic diversity) and what are the roles the different organisms perform (functional metagenomics) (Vieites et al. 2009). The two most commonly used high-throughput methods to decipher bacterial diversity are amplicon sequencing method which

includes sequencing of 16S ribosomal RNA gene or other specific bacterial genes and whole metagenomic shotgun sequencing (Ghosh et al. 2019).

The amplicon based method is based on the PCR amplification of a target gene from the metagenomic DNA and the resulting amplicons are sequenced using any sequencing platform. In case of bacterial taxonomy profiling sequencing of the whole 16S rRNA gene or its different variable regions (V1-V9) are widely used. However the target gene may vary based on the aim of the experiment. If the aim is to identify a particular group of organism with special metabolic activity, then genes related to metabolism of such activities are selected (e.g., *celB*, for organisms having cellulose degrading activity (Štursová et al. 2012)), *dxnA*, *dfdA* for analysis of communities with aromatic hydrocarbons degrading ability (Penton et al. 2013), *nifH*, *amoA*, *nirS,* or *nirK* for organisms involved in nitrogen cycling (reviewed in Levy-Booth et al. 2014). The16S rRNA gene sequencing has been widely used to analyze microbial diversity of various environmental samples. The limitation of this method is that organisms with same16S rRNA gene sequence may be classified as the same species in a 16S analysis, even if they are from different species. Thus 16S rRNA based analysis is not always accurate to distinguish between the closely related species and strains. The 16S rRNA based sequence analysis groups the identical sequence (>97%) into OTUs which are analyzed at each taxonomic level, but defining at species level is not precise (Ranjan et al. 2016).

Shotgun metagenomic sequencing is a method of sequencing the total DNA present in a given environmental sample. Shotgun metagenomic approach is used for complete genome sequencing of microbes present in an environmental sample and has the ability to identify the majority of the organisms present in the environmental sample (Sharpton 2014). Shotgun metagenomic sequencing with the help of next-generation sequencing (NGS) platform, many samples can be sequenced in a single sequencing run with high sequence coverage per sample and eventually it will help detect very low abundance members of the microbial community (Ghosh et al. 2019). Shotgun metagenomic studies can be performed through two approaches: (1) Sequence-based screens that give idea of the microbial diversity and genomes present in as a particular environmental sample and (2) functional screens that identify the functional gene products do not reveal the bacterial species that express the functional gene products (Madhavan et al. 2017). The bacterial diversity and relative abundance of species in environmental samples vary tremendously based on the type of the samples. Therefore, it is very important to have rough probable bacterial diversity and relative abundance of species in an environmental sample before initiating whole metagenomic study as it will help determine the required sequencing depth and data generation for proper coverage. Higher sequencing depth provides better detection of rare taxa (Sharpton 2014).

24.8 Metagenomics Workflow

The metagenomic workflow depends on the sequencing platform being used to sequence the metagenomic DNA library. Metagenomic analysis is a well-designed process that involves series of steps. The first step is the sampling process which is very essential for the downstream applications. After that total DNA was extracted from the samples under investigation using a suitable DNA extraction protocol. The choice of DNA extraction depends on the physicochemical properties of each sample. For example, soils encompass many components (such as humic and fulvic acids) that are also extracted with the genomic DNA and reported to create problems during downstream experiments (Young et al. 2014). Therefore, the DNA extraction methods have to be optimized for each type of environmental samples to get good quality total DNA (Finley et al. 2016; Lim et al. 2016; Gupta et al. 2017). Nowadays various commercial metagenomic DNA extraction kits are available that take care of the probable inhibitors and allow the extraction of almost pure metagenomic DNA. After that metagenomic DNA library is prepared by fragmenting the DNA into different sizes (for easy cloning into suitable vector) followed by attaching specific adaptors to the DNA fragments (van Dijk et al. 2014). There are two different approaches of library construction, viz., meta-pair library consisting of long fragment insert and the paired-end libraries that are characterized by short fragment insert (Simon and Daniel 2017). The DNA fragments obtained are cloned into the proper cloning vector. For small DNA fragments plasmid vectors are usually used, whereas up to 40 kb fragments may be cloned into cosmid or fosmid vectors. When the fragments size exceeds 40 kb, cloning is performed using bacterial artificial chromosome (BAC) vectors (Simon and Daniel 2017). The libraries are sequenced using appropriate sequencing platform. As the sequencing technologies are improving day by day, the procedure for library preparation has also been modified to match the technology requirements. For example, sequencing of metagenomic DNA in a next-generation sequencer doesn't require vector-based cloning procedure; instead, the library can be prepared directly from the metagenomic DNA and hence reduces the chance of DNA cross-contamination (Mardis 2008). In addition, each next-generation sequencing technology/platform uses different procedure to prepare library and their subsequent sequencing.

24.9 Sequencing Platforms

The first-generation sequencing techniques, viz., chain termination (Sanger and Coulson 1975), and chemical sequencing approaches (Maxam and Gilbert 1977) were developed during the 1970s. But, only the Sanger sequencing method has got immense applications and is sustaining till date because of its simplicity and having options to scale up (Schadt et al. 2010). Although the Sanger sequencing has been used widely by researchers, this approach still has some limitations such as high cost and low throughput (Metzker 2010). Because of certain shortcomings of Sanger sequencing technique, the next-generation sequencing techniques have emerged in

2005 (Varshney et al. 2009) which improved the metagenomic sequencing process many fold. Microbial diversity and their functional relationships to other microbial communities can be understood through metagenomic approaches using NGS platform. The next-generation sequencing has paved the way for identifying organisms directly from the environments without any further preparation (Sogin et al. 2006). The NGS technology has made possible to generate large sequencing reads in parallel bypassing the conventional steps that involve vector-based cloning procedure. This method decreases the probability of the DNA getting contaminated with other organisms (Mardis 2008). The numerous advantages of next-generation sequencing platforms have led to the development of several platforms including Roche 454, Illumina®, Applied Biosystems SOLiD sequencer, and Ion Torrent. These next-generation sequencing platforms utilize optical sensors or semiconductors that detect luminescent/fluorescent signal produced when a new base is incorporated during the new strand synthesis (Garrido-Cardenas et al. 2017). The basic workflow of metagenomic analysis using NGS includes DNA extraction, library construction, and automated sequence analysis (Vincent et al. 2017). However, NGS is limited by several issues including short-read length, PCR biasness that is introduced by clonal amplification and detection issues of the fluorescent-based signaling (Schadt et al. 2010). The advent of third-generation sequencing (TGS) or single-molecule-sequencing technologies (SMS) has eliminated many of these limitations by omitting the PCR before sequencing and generation and capturing of the signal in real time by monitoring the enzymatic reaction (Schadt et al. 2010; Korlach et al. 2010). The different TGS platforms include Helicos biosciences (HeliScope) PacBio technology/SMRT sequencer, and Oxford Nanopore technology (Shuikan et al. 2020).

24.10 Metagenomic Data Analysis

A number of bioinformatics tools are currently available to analyze the metagenomic data. However the choice of tools varied depending on the type of analysis required. Some of the common tools used for bioinformatics analysis of metagenome data are discussed here. Microbial taxonomy and phylogeny continue to be analyzed with the most common approach of using 16S rRNA gene sequence strategy (amplicon sequencing). Several bioinformatics tools are available for the analysis of 16S rRNA gene sequences, viz., QIIME, MOTHUR, DADA2, UPARSE, and minimum entropy decomposition (MED) (Niu et al. 2018). The QIIME software is designed for the analysis of data obtained from Illumina or other NGS platforms. It analyzes NGS data using graphics and statistics. The steps include demultiplexing and quality filtering of raw data, OUT picking, taxonomic assignment, and phylogenetic reconstruction, and diversity analysis and visualization (Schloss et al. 2009; Caporaso et al. 2010). Generation of operational taxonomic units (OTUs) from the NGS data can be done using the UPARSE tool (Edgar 2013). The UPARSE software filters the low quality reads and trims reads into equal lengths, removes singleton reads, and then clusters the remaining high quality reads (Edgar 2013). Analysis of community

sequence data can be achieved through a flexible and comprehensive software package called MOTHUR. Several metagenomic data analysis software are available including MetaPhlAn2 (Truong et al. 2015), Kraken (Wood and Salzberg 2014), CLARK (Ounit et al. 2015), FOCUS (Silva et al. 2014), SUPERFOCUS (Silva et al. 2016), and MG-RAST (Aziz et al. 2008; Meyer et al. 2008) to analyze the metagenomic data to species-level. These software are programmed to profile organisms in environmental samples and to determine their abundance.

The metagenomic approach not only provides detailed knowledge of the uncultured microorganism but also allows gene profiling along with the microbiome membership (Shakya et al. 2019). These approaches have provided a greater insights into diverse world of microbiome by providing information about the presence of different organisms or genes but failed to provide information on the active members of the microbiome (Shakya et al. 2019). To get a better insights into how a microbial community interacts with their varying environmental conditions at different time point, what roles they play in a particular ecosystem, and more specifically, their interactions with biotic and abiotic environmental factors, scientists have adopted other "omics" strategies, viz., metatranscriptomics and metaproteomics (Rechenberger et al. 2019; Sujun et al. 2019; Li et al. 2019; Salazar et al. 2019; Amato et al. 2019; Wang et al. 2020). A combination of these meta "omics" tools can provide information on the complex microbial communities (Gude 2015). These two "omics" techniques are discussed briefly in the following sections.

24.11 Metatranscriptomics

Metatranscriptomics is the study of the complete set of transcripts in terms of their expression and function in environmental samples under certain conditions. Metatranscriptomic sequencing provides direct access to culturable and non-culturable microbial transcriptome information by large-scale, high-throughput sequencing of transcripts from all microbial communities in specific environmental samples (Li et al. 2019). It offers an opportunity to understand the regulation of complex processes in microbial communities and provide new insights into poorly known biological systems (Shakya et al. 2019).

24.12 Workflow of Metatranscriptomic Sequencing

In simple terms metatranscriptome sequencing consists of the following steps: total RNA extraction from the sample, removal of rRNA, quality testing, fragmentation, library preparation and its quality testing. The qualified library is sequenced using NGS platform (mostly Illumina sequencing platform). The raw data obtained by sequencing is analyzed using appropriate bioinformatics tools (Peimbert and Alcaraz 2016).

Due to the highly complex nature of the microbial communities of different environment, the metatranscriptomic studies require high-throughput sequencing

data and most preferably short sequence reads (e.g., generated from Illumina sequencing technology) with proper sequencing depth to produce quality results (Peimbert and Alcaraz 2016). However, to decide the right parameters such as depth of sequencing for metatranscriptomics is highly difficult as the most of the information about the sample including microbial composition, relative abundance of different microbial community, size of genomes, and relative expression of the genes of diverse microbial communities are unknown (Shakya et al. 2019). The advantage of long read sequencing technique is the ability to generate full-length or almost full-length mRNAs which may be used to select the different gene isoforms. However, till date the various long read sequences are basically used as supporting reads for metatranscriptome studies or other genomics studies (Pollard et al. 2018).

The analysis of NGS data starts with quality control (QC). Quality control of NGS data means removal or trimming of low quality reads in order to reduce errors in the later downstream analysis. For this purpose different tools, viz., FastQC (Andrews 2010), FaQCs (Lo and Chain 2014), fastp (Chen et al. 2018), and Trimmomatic (Bolger et al. 2014) are mostly employed. As stated earlier, almost 90% of the extracted total RNA is occupied by rRNA, it is therefore necessary to remove these rRNA prior to library preparation. However, if not removed during library preparation, the rRNA sequences can also be removed after sequencing by applying tools like SortMeRNA (Kopylova et al. 2012) and barrnap (Seemann 2014).

The preprocessed, high-quality reads may be assembled using de novo assemblers into putative transcripts and can provide a reference set of genes of microbial communities that are inadequately characterized based on reference genomes. This also allows direct detection of homologous genes, determines taxonomic origin, and also helps in expression analysis by providing the reference sequence. A number of assemblers such as MEGAHIT (Li et al. 2015), IDBA-UD (Peng et al. 2012), and metaSPAdes (Nurk et al. 2017) have been developed and are being used to assemble complex metagenomes. However, the effectiveness of these assemblers in reconstructing transcripts has yet to be reported (Shakya et al. 2019). Some assemblers such as Trinity (Grabherr et al. 2011), Oases (Schulz et al. 2012), Metavelvet (Namiki et al. 2012), which were actually developed to assemble transcripts from a single organism have also been used to assemble metatranscriptome sequence data (Celaj et al. 2014; Shakya et al. 2019). Some examples of de novo assemblers that are designed precisely to assemble metatranscriptome sequence data include IDBA-MT (Leung et al. 2013), IDBA-MTP (Leung et al. 2014), and Transcript Assembly Graph (Ye and Tang 2016). These tools consider the complex nature of microbial communities as well as the unique features of transcripts (Shakya et al. 2019). To assemble mRNAs with very low expression, IDBA-MTP tool can be used (Leung et al. 2014).

At present, the de novo assembly for metatranscriptomic data sets is under developmental stage and only few tools have been dedicatedly developed for metatranscriptomics data analysis. Their efficacy on various datasets, hardware requirements, or memory depending on the community complexities and data volume has yet to be established (Shakya et al. 2019). The assembled data is then

used to get taxonomic profile, functional annotation, and differential gene expression (Peimbert and Alcaraz 2016).

The tools that are used for the taxonomic profiling of shotgun metagenomic data can also be used to perform taxonomic assignments of the metatranscriptomics data. Taxonomic profiling metatranscriptomes data with short reads or contigs may be performed using certain tools (Neves et al. 2017) such as Kraken (Wood and Salzberg 2014), GOTTCHA (Freitas et al. 2015), and MetaPhlan2 (Truong et al. 2015). Tools like Centrifuge (Kim et al. 2016a) and Kraken2 (Wood et al. 2019) can be applied to get taxonomic profile of long reads or full-length transcripts. To evaluate the functional activity of the microbes present in the environmental samples is one of the principal objectives of metatranscriptomics as the expressed transcripts display a mirror image of the actual phenotype (Shakya et al. 2019). Functional annotation of the assembled transcripts is done using functional profilers such as MetaCLADE (Ugarte et al. 2018), HMMGRASPx (Zhong et al. 2016), and UProC (Meinicke 2015). But these software demand predicted open reading frames as input which are obtained using tools like FragGeneScan (Rho et al. 2010). These profilers then perform functional assignment of the input reads using tools such as DIAMOND (Buchfink et al. 2014) which carries out similarity searches against functional databases like KEGG (Kanehisa and Goto 2000), NCBI RefSeq (O'Leary et al. 2016), UniProt (The UniProt Consortium 2019), etc. Other software tools or integrated platforms such as Prokka (Seemann 2014), EDGE Bioinformatics (Li et al. 2017; Philipson et al. 2017), and MG-RAST (Wilke et al. 2016) can be used to do the same. These integrated tools have the capacity to perform a number of similarity searches against different databases, carry out assembly, gene calling, and also annotation (Shakya et al. 2019). Once annotations are completed, tools like MinPath (Ye and Doak 2009) or iPath (Yamada et al. 2011) may be used to map enzymatic functions to known metabolic pathways.

Besides providing taxonomic and functional description of the microbial community metatranscriptome studies can tell us about the genes that are being expressed at a particular time point under differing conditions and environmental parameters. Several bioinformatics tools that were originally developed to analyze single genomes can be used to perform metatranscriptomic differential gene expression studies. A number of R packages such as EdgeR (Robinson et al. 2010), DeSeq2 (Love et al. 2014), and limma (Ritchie et al. 2015) can also be used to identify genes which are significantly differentially expressed among a number of samples (conditions/time points). Pathways enriched in one condition over another can be determined with the aid of tools such as Gene-Set/Pathway Analysis (GAGE) (Luo et al. 2009).

Bioinformatics analyses discussed above can be done by using some workflow packages that aim to streamline the complex analysis by linking various individual tools into a workflow that can deal with raw sequencing reads, perform taxonomic assignments, functional annotations, as well as differential gene expression analysis (Shakya et al. 2019). Examples of such packages include MetaTrans (Martinez et al. 2016), COMAN (Ni et al. 2016), FMAP (Kim et al. 2016b), SAMSA2 (Westreich et al. 2018), HUMAnN2 (Franzosa et al. 2018), SqueezeMeta (Tamames and

Puente-Sánchez 2019), IMP (Narayanasamy et al. 2016), and MOSCA (Sequeira et al. 2019) (reviewed in Shakya et al. 2019).

24.13 Metaproteomics

Metaproteomics is the characterization of all the proteins expressed at a given time within an ecosystem (Wilmes and Bond 2004) and is considered as an important tool for determination of microbial functionality. The technique has been successfully employed to understand microbial function in diverse environments (Benndorf et al. 2007; Rudney et al. 2010; Williams et al. 2010; Jehmlich et al. 2010; Bruneel et al. 2011; Habicht et al. 2011; Rooijers et al. 2011; Burnum et al. 2011; Wang et al. 2011; Lauro et al. 2011; Sowell et al. 2011).

The metaproteomic approaches typically involve the following basic steps: Collection of sample from the target environment, recovery of the desired fraction of the sample, extraction of protein, separation and/or fractionation of the extracted protein, mass spectrometric analysis of the peptide fractions, searching the peptide sequences against databases, and interpretation of the data. The expressed proteins and pathways identified are then used to obtain information about how the microbes function under a certain environment (Wilmes and Bond 2006; VerBerkmoes et al. 2009). The extreme diversity of sampling environments renders it difficult to have a standard protocol for protein extraction, purification, quantification, and processing, therefore optimization of the whole procedure for every environmental sample is crucial to get proper results of the metaproteomic study (Russo et al. 2019).

After sample collection and recovery of the desired fraction of the sample, proteins are extracted from the recovered fraction using a standard, reproducible, and mass spectrometry (MS)-compatible protocol that gives high yield and sufficient purity (Leary et al. 2012). Protocols for cell disruption may differ depending on the presence of cell types in the sample. Both physical and chemical methods are used for protein extraction. Physical methods (e.g., bead beating) are frequently used because of its high efficiency and free from chemicals that may interfere with the downstream workflow. Extraction buffers are used to maintain pH in combination with protease inhibitors (to protect the protein part) and nucleases (to degrade the DNA and RNA). The extracted protein mixture is then cleaned through precipitation with trichloroacetic acid, acetone, or ethanol to remove any compounds that may interfere with the enzymatic digestion, fractionation, and/or MS analysis. The clean protein extracts are then subjected to either gel electrophoresis separation [2-D polyacrylamide gel electrophoresis (2-D PAGE)] or in-solution digestion (Russo et al. 2019). Due to the laborious nature and artifact (e.g., co-migration of proteins) associated with 2-D PAGE (Schneider and Riedel 2010) in-solution digestion followed by multidimensional LC-MS has been routinely used (Motoyama and Yates 2008). In-solution digestion is performed to reduce disulfide bonds, alkylation, and enzymatic digestion (mostly trypsin) of the sample protein present in the solution. The peptide mixture is then fractionated and subjected to MS analysis (Russo et al. 2019).

To resolve the individual peptide from the peptide mixture, multidimensional protein identification technology (MudPIT) has been utilized (Motoyama and Yates 2008) which separates complex peptide mixtures by employing two- or multidimensional chromatography. An usual two-dimensional approach fractionated the peptide mixture first through offline liquid chromatography (LC) and then the fractions are further separated by online LC connected to MS (Bereszczak and Brancia 2009). Once the peptides are fractionated using LC, the peptides are ionized and analyzed by MS (most commonly through time of flight (TOF) and ion trap. The most frequently used ionization techniques are matrix assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). However, tandem MS is recommended if high mass accuracy and high resolution are required (Yates et al. 2009). The MS provides the results as spectra and to interpret the MS spectra a number of bioinformatics tools are used which use algorithms for protein identification (Muth et al. 2013). The most common algorithms used to interpret MS spectra are available in commercial platforms such as MASCOT (Perkins et al. 1999); however, a number of free and open-source tools are also available for the same purpose (Craig and Beavis 2004). But without an appropriate database and search parameters these algorithms cannot produce meaningful results. Thus selecting the appropriate database and search parameters is highly important. Once the appropriate databases and search parameters are fixed, the algorithms give the search output a list of proteins and taxonomic assignments. The search results in the form of a list of proteins and taxonomic assignments need to be validated statistically and quantified using platforms such as MASCOT or Trans-Proteomic Pipeline. Finally, proteins and taxonomic assignments are functionally annotated using a combination of publicly available databases, viz., UniProt knowledgebase (The UniProt Consortium 2019), Cluster of Orthologous Groups database (Tatusov et al. 2003), the Gene Ontology project (Ashburner et al. 2000), the Kyoto Encyclopedia of Genes and Genomes (Kanehisa and Goto 2000), etc. The data obtained from the annotations are then further studied to understand the biochemical composition and significance of the target environment (Russo et al. 2019).

24.14 Conclusions

During the recent decades, rapid strides have been made in isolating and characterizing number of prokaryotic from different ecological niches including freshwater ecosystem. However, current estimates indicate that $<1\%$ of one million species of prokaryotes has been identified. It is estimated that an approximately 250,000 cubic kilometers of freshwater on this planet resides in the forms of rivers, lakes, and streams which potentially harbor diverse microbial communities. Microbes are major contributors to the transformation of complex organic compounds and minerals in freshwater ecosystem. The freshwater ecosystem that acts as sinks for various organic pollutants, the microbes present in such ecosystems act as natural scavengers. The microbes are a warehouse of beneficial resources and also a potential source of products of industrial importance and novel enzymes. As

such microbial communities and their functional activity are pivotal to the environment and its functioning. Given their importance, study of microbial diversity and their functioning has been a subject of much interest. The classical methods of isolation and characterization of microbes were limited in their methodology by age-old techniques. However, the recent improvements in molecular methods and techniques have helped expand our knowledge of microbial world not only from phylogenetic and taxonomic perspectives but also from an ecological aspect. The advent of high-throughput DNA and protein sequencing technologies has had a profound effect on the approaches adopted for microbiological studies. The NGS techniques have helped elucidate not only the diverse microbial community along with deciphering their function alone or as a community in an ecological niche but also provided a facet of the evolutionary and ecological relationships among diverse species. The advent of new tools is also aiding in identification of novel genes and biosynthetic pathways and may provide opportunities to develop useful products from the microbes that remain uncultivable.

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References

Abia ALK, Alisoltani A, Keshri J, Ubomba-Jaswa E (2018) Metagenomic analysis of the bacterial communities and their functional profiles in water and sediments of the Apies River, South Africa, as a function of land use. Sci Total Environ 616–617:326–334. https://doi.org/10.1016/j.scitotenv.2017.10.322

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci 105:11512–11519. https://doi.org/10.1073/PNAS.0801925105

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169. https://doi.org/10.1128/mmbr.59.1.143-169.1995

Amato P, Besaury L, Joly M et al (2019) Metatranscriptomic exploration of microbial functioning in clouds. Sci Rep 9:1–12. https://doi.org/10.1038/s41598-019-41032-4

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. ScienceOpen, Boston

Ashburner M, Ball CA, Blake JA et al (2000) Gene ontology: tool for the unification of biology. Nat Genet 25:25–29

Aziz RK, Bartels D, Best AA et al (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75

Bai Y, Liang J, Liu R et al (2014) Metagenomic analysis reveals microbial diversity and function in the rhizosphere soil of a constructed wetland. Environ Technol 35:2521–2527. https://doi.org/10.1080/09593330.2014.911361

Benndorf D, Balcke GU, Harms H, Von Bergen M (2007) Functional metaproteome analysis of protein extracts from contaminated soil and groundwater. ISME J 1:224–234. https://doi.org/10.1038/ismej.2007.39

Bereszczak JZ, Brancia FL (2009) Offline and online liquid chromatography mass spectrometry in quantitative proteomics. Comb Chem High Throughput Screen 12:185–193

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Breton-deval L, Sanchez-reyes A, Sanchez-flores A, Ju K (2020) Functional analysis of a polluted river microbiome reveals a metabolic potential for bioremediation. Microorganisms 8(4):554

Bruneel O, Volant A, Gallien S et al (2011) Characterization of the active bacterial community involved in natural attenuation processes in arsenic-rich creek sediments. Microb Ecol 61:793–810. https://doi.org/10.1007/s00248-011-9808-9

Buchfink B, Xie C, Huson DH (2014) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60

Burnum KE, Callister SJ, Nicora CD et al (2011) Proteome insights into the symbiotic relationship between a captive colony of Nasutitermes corniger and its hindgut microbiome. ISME J 5:161–164. https://doi.org/10.1038/ismej.2010.97

Buyer JS, Sasser M (2012) High throughput phospholipid fatty acid analysis of soils. Appl Soil Ecol 61:127–130. https://doi.org/10.1016/j.apsoil.2012.06.005

Caporaso JG, Kuczynski J, Stombaugh J et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Carlson CA (2002) Production and removal processes. In: Dennis AH, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Academic Press, Cambridge, MA, pp 91–151

Celaj A, Markle J, Danska J, Parkinson J (2014) Comparison of assembly algorithms for improving rate of metatranscriptomic functional annotation. Microbiome 2:39. https://doi.org/10.1186/2049-2618-2-39

Chaya A, Kurosawa N, Kawamata A et al (2019) Community structures of bacteria, archaea, and eukaryotic microbes in the freshwater Glacier Lake Yukidori-Ike in Langhovde, East Antarctica. Diversity 11:105. https://doi.org/10.3390/d11070105

Chen S, Zhou Y, Chen Y, Gu J (2018) Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Chin WC, Orellana MV, Verdugo P (1998a) Spontaneous assembly of marine dissolved organic matter into polymer gels. Nature 391:568–572. https://doi.org/10.1038/35345

Chin Y-P, Traina SJ, Swank CR, Backhus D (1998b) Abundance and properties of dissolved organic matter in pore waters of a freshwater wetland. Limnol Oceanogr 43:1287–1296. https://doi.org/10.4319/lo.1998.43.6.1287

Chopyk J, Allard S, Nasko DJ et al (2018) Agricultural freshwater pond supports diverse and dynamic bacterial and viral populations. Front Microbiol 9:1–14. https://doi.org/10.3389/fmicb.2018.00792

Chowdhury N, Goswami G, Hazarika S et al (2018) Microbial dynamics and nutritional status of Namsing: a traditional fermented fish product of Mishing Community of Assam. Proc Natl Acad Sci India Sect B Biol Sci 89(3):1027–1038. https://doi.org/10.1007/s40011-018-1022-9

Colwell RR (1970) Polyphasic taxonomy of the genus vibrio: numerical taxonomy of Vibrio cholerae, Vibrio parahaemolyticus, and related Vibrio species. J Bacteriol 104:410–433. https://doi.org/10.1128/jb.104.1.410-433.1970

Costanza R, D'Arge R, De Groot R et al (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260. https://doi.org/10.1038/387253a0

Cotner JB, Biddanda BA (2002) Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5:105–121

Cotter PD, Gahan CGM, Hill C (2000) Analysis of the role of the Listeria monocytogenes F0F1-ATPase operon in the acid tolerance response. Int J Food Microbiol 60:137–146. https://doi.org/10.1016/S0168-1605(00)00305-6

Craig R, Beavis RC (2004) TANDEM: matching proteins with tandem mass spectra. Bioinformatics 20:1466–1467. https://doi.org/10.1093/bioinformatics/bth092

Crump BC, Adams HE, Hobbie JE, Kling GW (2007) Biogeography of bacterioplankton in lakes and streams of an arctic tundra catchment. Ecology 88:1365–1378. https://doi.org/10.1890/06-0387

Daniel R (2005) The metagenomics of soil. Nat Rev Microbiol 3:470–478. https://doi.org/10.1038/nrmicro1160

Danilova OV, Belova SE, Gagarinova IV, Dedysh SN (2016) Microbial community composition and methanotroph diversity of a subarctic wetland in Russia. Microbiology 85:545–554. https://doi.org/10.1134/S0026261716050039

Das S, Bora SS, Yadav RNS, Barooah M (2017) A metagenomic approach to decipher the indigenous microbial communities of arsenic contaminated groundwater of Assam. Genom Data 12:89–96. https://doi.org/10.1016/j.gdata.2017.03.013

Deka P, Goswami G, Das P et al (2019) Bacterial exopolysaccharide promotes acid tolerance in Bacillus amyloliquefaciens and improves soil aggregation. Mol Biol Rep 46:1079–1091. https://doi.org/10.1007/s11033-018-4566-0

Deng Y, Cui X, Hernández M, Dumont MG (2014) Microbial diversity in hummock and hollow soils of three wetlands on the Qinghai-Tibetan plateau revealed by 16S rRNA pyrosequencing. PLoS One 9:e103115. https://doi.org/10.1371/journal.pone.0103115

Dopheide A, Lear G, He Z et al (2015) Functional gene composition, diversity and redundancy in microbial stream biofilm communities. PLoS One 10:e0123179. https://doi.org/10.1371/journal.pone.0123179

Durack PJ (2015) Ocean salinity and the global water cycle. Oceanography 28:20–31

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998. https://doi.org/10.1038/nmeth.2604

Eiler A, Bertilsson S (2004) Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environ Microbiol 6:1228–1243. https://doi.org/10.1111/j.1462-2920.2004.00657.x

Eiler A, Bertilsson S (2007) Flavobacteria blooms in four eutrophic lakes: linking population dynamics of freshwater bacterioplankton to resource availability. Appl Environ Microbiol 73:3511–3518. https://doi.org/10.1128/AEM.02534-06

Fatima J, Goswami G, Bora S et al (2018) " Doi ", a naturally fermented milk product of Assam contains indigenous lactobacillus spp. with probiotic attributes. Res J Biotechnol 13:1–15

Fechner LC, Vincent-Hubert F, Gaubert P et al (2010) Combined eukaryotic and bacterial community fingerprinting of natural freshwater biofilms using automated ribosomal intergenic spacer analysis. FEMS Microbiol Ecol 74:542–553. https://doi.org/10.1111/j.1574-6941.2010.00968.x

Fenchel TM, Jørgensen BB (1977) Detritus food chains of aquatic ecosystems: the role of bacteria. In: Alexander M (ed) Advances in microbial ecology, vol 1. Springer, Boston, MA, pp 1–58

Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl Environ Microbiol 71:4117–4120. https://doi.org/10.1128/AEM.71.7.4117-4120.2005

Fierer N, Leff JW, Adams BJ et al (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc Natl Acad Sci 109:21390–21395. https://doi.org/10.1073/PNAS.1215210110

Finley SJ, Lorenco N, Mulle J et al (2016) Assessment of microbial DNA extraction methods of cadaver soil samples for criminal investigations. Aust J Forensic Sci 48:265–272. https://doi.org/10.1080/00450618.2015.1063690

Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl Environ Microbiol 65:4630–4636. https://doi.org/10.1128/aem.65.10.4630-4636.1999

Franzosa EA, McIver LJ, Rahnavard G et al (2018) Species-level functional profiling of metagenomes and metatranscriptomes. Nat Methods 15:962–968. https://doi.org/10.1038/s41592-018-0176-y

Freitas TAK, Li PE, Scholz MB, Chain PSG (2015) Accurate read-based metagenome characterization using a hierarchical suite of unique signatures. Nucleic Acids Res 43:1–14. https://doi.org/10.1093/nar/gkv180

Garrido-Cardenas JA, Garcia-Maroto F, Alvarez-Bermejo JA, Manzano-Agugliaro F (2017) DNA sequencing sensors: an overview. Sensors (Basel) 17:588. https://doi.org/10.3390/s17030588

Ghosh A, Mehta A, Khan AM (2019) Metagenomic analysis and its applications. In: Ranganathan S, Gribskov M, Nakai K et al (eds) Encyclopedia of bioinformatics and computational biology. Academic Press, Oxford, pp 184–193

Gilbert JA, Field D, Huang Y et al (2008) Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities. PLoS One 3:e3042. https://doi.org/10.1371/journal.pone.0003042

Giovannoni SJ, Stingl U (2005) Molecular diversity and ecology of microbial plankton. Nature 437:343–348. https://doi.org/10.1038/nature04158

Goswami G, Bora S, Parveen A et al (2017a) Identification and functional properties of dominant lactic acid bacteria isolated from Kahudi, a traditional rapeseed fermented food product of Assam, India. J Ethn Foods 4:187–197. https://doi.org/10.1016/j.jef.2017.08.008

Goswami G, Deka P, Das P et al (2017b) Diversity and functional properties of acid-tolerant bacteria isolated from tea plantation soil of Assam. 3 Biotech 7:229. https://doi.org/10.1007/s13205-017-0864-9

Grabherr MG, Haas BJ, Yassour M et al (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29:644–652. https://doi.org/10.1038/nbt.1883

Gude GV (2015) A new perspective on microbiome and resource management in wastewater systems. J Biotechnol Biomater 5:1–7. https://doi.org/10.4172/2155-952x.1000184

Gupta P, Manjula A, Rajendhran J et al (2017) Comparison of metagenomic DNA extraction methods for soil sediments of high elevation Puga hot spring in Ladakh, India to explore bacterial diversity. Geomicrobiol J 34:289–299. https://doi.org/10.1080/01490451.2015.1128995

Habicht KS, Miller M, Cox RP et al (2011) Comparative proteomics and activity of a green sulfur bacterium through the water column of Lake Cadagno, Switzerland. Environ Microbiol 13:203–215. https://doi.org/10.1111/j.1462-2920.2010.02321.x

Handelsman J, Rondon MR, Brady SF et al (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5:R245–R249. https://doi.org/10.1016/S1074-5521(98)90108-9

Harpole WS (2010) Neutral theory of species diversity. Nat Educ Knowl 1(8):31

Hartman WH, Richardson CJ, Vilgalys R, Bruland GL (2008) Environmental and anthropogenic controls over bacterial communities in wetland soils. Proc Natl Acad Sci U S A 105:17842–17847. https://doi.org/10.1073/pnas.0808254105

Hayden HL, Savin KW, Wadeson J et al (2018) Comparative metatranscriptomics of wheat Rhizosphere microbiomes in disease suppressive and non-suppressive soils for Rhizoctonia solani AG8. Front Microbiol 9:859. https://doi.org/10.3389/fmicb.2018.00859

Hazarika DJ, Goswami G, Gautom T et al (2019) Lipopeptide mediated biocontrol activity of endophytic Bacillus subtilis against fungal phytopathogens. BMC Microbiol 19:71. https://doi.org/10.1186/s12866-019-1440-8

Hazarika DJ, Gautom T, Parveen A et al (2020) Mechanism of interaction of an endofungal bacterium Serratia marcescens D1 with its host and non-host fungi. PLoS One 15:1–19. https://doi.org/10.1371/journal.pone.0224051

He S, Malfatti SA, McFarland JW et al (2015) Patterns in wetland microbial community composition and functional gene repertoire associated with methane emissions. MBio 6:1–15. https://doi.org/10.1128/mBio.00066-15

Hu H-W, Han X-M, Shi X-Z et al (2016) Temporal changes of antibiotic-resistance genes and bacterial communities in two contrasting soils treated with cattle manure. FEMS Microbiol Ecol 92:fiv169

Hugenholtz P (2002) Exploring prokaryotic diversity in the genomic era. Genome Biol 3:1–8

Ivanov V, Tay STL, Tay JH (2003) Monitoring of microbial diversity by fluorescence in situ hybridization and fluorescence spectrometry. Water Sci Technol 47(5):133–138

Ivanova AA, Wegner C-E, Kim Y et al (2016) Identification of microbial populations driving biopolymer degradation in acidic peatlands by metatranscriptomic analysis. Mol Ecol 25:4818–4835. https://doi.org/10.1111/mec.13806

Jehmlich N, Kleinsteuber S, Vogt C et al (2010) Phylogenetic and proteomic analysis of an anaerobic toluene-degrading community. J Appl Microbiol 109:1937–1945. https://doi.org/10.1111/j.1365-2672.2010.04823.x

Jones JG, Rheinheimer G (1986) Aquatic microbiology. J Ecol 74:911. https://doi.org/10.2307/2260413

Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30

Kerner M, Hohenberg H, Ertl S et al (2003) Self-organization of dissolved organic matter to micelle-like micropadicles in river water. Nature 422:150–154. https://doi.org/10.1038/nature01469

Kim H, Yu SM (2020a) Chryseobacterium salivictor sp. nov., a plant-growth-promoting bacterium isolated from freshwater. Antonie van Leeuwenhoek. Int J Gen Mol Microbiol 113:989–995. https://doi.org/10.1007/s10482-020-01411-8

Kim H, Yu SM (2020b) Complete genome sequence of Chryseobacterium sp. strain NBC 122, a plant growth-promoting bacterium isolated from freshwater. Microbiol Resour Announc 9: e01028-19. https://doi.org/10.1128/mra.01028-19

Kim D, Song L, Breitwieser FP, Salzberg SL (2016a) Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Res 26:1721–1729. https://doi.org/10.1101/gr.210641.116

Kim J, Kim MS, Koh AY et al (2016b) FMAP: functional mapping and analysis pipeline for metagenomics and metatranscriptomics studies. BMC Bioinformatics 17:420. https://doi.org/10.1186/s12859-016-1278-0

Kimura N (2018) Novel biological resources screened from uncultured bacteria by a metagenomic method. In: Metagenomics: perspectives, methods, and applications. Elsevier, Amsterdam, pp 273–288

Kirchman DL (2003) The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic material in aquatic ecosystems. In: Aquatic ecosystems. Academic Press, Cambridge, MA, pp 217–241. https://doi.org/10.1016/B978-012256371-3/50010-X

Kolmakova OV, Gladyshev MI, Rozanov AS et al (2014) Spatial biodiversity of bacteria along the largest Arctic river determined by next-generation sequencing. FEMS Microbiol Ecol 89:442–450. https://doi.org/10.1111/1574-6941.12355

Kolmeder CA, Ritari J, Verdam FJ et al (2015) Colonic metaproteomic signatures of active bacteria and the host in obesity. Proteomics 15:3544–3552. https://doi.org/10.1002/pmic.201500049

Kopylova E, Noé L, Touzet H (2012) SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28:3211–3217. https://doi.org/10.1093/bioinformatics/bts611

Korlach J, Bjornson KP, Chaudhuri BP et al (2010) Real-time DNA sequencing from single polymerase molecules. Methods Enzymol 472:431–455. https://doi.org/10.1016/S0076-6879(10)72001-2

Kumar R, Bisht S, Sharma RC, Rawat S (2018) Physico–chemical attributes and bacterial diversity of river water at Rudraprayag, Garhwal Himalaya. MOJ Ecol Environ Sci 3:277–282. https://doi.org/10.15406/mojes.2018.03.00100

Kurilkina MI, Zakharova YR, Galachyants YP et al (2016) Bacterial community composition in the water column of the deepest freshwater Lake Baikal as determined by next-generation sequencing. FEMS Microbiol Ecol 92:1–13. https://doi.org/10.1093/femsec/fiw094

Lagacé L, Pitre M, Jacqeus M, Roy D (2004) Identification of the bacterial community of Maple sap by using amplified ribosomal DNA (rDNA) restriction analysis and rDNA sequencing. Appl Environ Microbiol 70:2052–2060. https://doi.org/10.1128/AEM.70.4.2052-2060.2004

Langille MGI, Zaneveld J, Caporaso JG et al (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814–821. https://doi.org/10.1038/nbt.2676

Laureto LMO, Cianciaruso MV, Samia DSM (2015) Functional diversity: an overview of its history and applicability. Nat Conserv 13:112–116

Lauro FM, Demaere MZ, Yau S et al (2011) An integrative study of a meromictic lake ecosystem in Antarctica. ISME J 5:879–895. https://doi.org/10.1038/ismej.2010.185

Leary DH, Hervey WJ, Li RW et al (2012) Method development for metaproteomic analyses of marine biofilms. Anal Chem 84:4006–4013. https://doi.org/10.1021/ac203315n

Leung HCM, Yiu SM, Parkinson J, Chin FYL (2013) IDBA-MT: De novo assembler for metatranscriptomic data generated from next-generation sequencing technology. J Comput Biol 20:540–550. https://doi.org/10.1089/cmb.2013.0042

Leung HCM, Yiu SM, Chin FYL (2014) IDBA-MTP: a hybrid metatranscriptomic assembler based on protein information. In: Lecture notes in computer science (including subseries lecture notes in artificial intelligence and lecture notes in bioinformatics). Springer, Cham, pp 160–172

Levy-Booth DJ, Prescott CE, Grayston SJ (2014) Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol Biochem 75:11–25. https://doi.org/10.1016/j.soilbio.2014.03.021

Li D, Liu CM, Luo R et al (2015) MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31:1674–1676. https://doi.org/10.1093/bioinformatics/btv033

Li PE, Lo CC, Anderson JJ et al (2017) Enabling the democratization of the genomics revolution with a fully integrated web-based bioinformatics platform. Nucleic Acids Res 45:67–80. https://doi.org/10.1093/nar/gkw1027

Li F, Hitch TCA, Chen Y et al (2019) Comparative metagenomic and metatranscriptomic analyses reveal the breed effect on the rumen microbiome and its associations with feed efficiency in beef cattle 06 biological sciences 0604 genetics 06 biological sciences 0605 microbiology. Microbiome 7:6. https://doi.org/10.1186/s40168-019-0618-5

Lim NYN, Roco CA, Frostegård Å (2016) Transparent DNA/RNA co-extraction workflow protocol suitable for inhibitor-rich environmental samples that focuses on complete DNA removal for transcriptomic analyses. Front Microbiol 7:1588. https://doi.org/10.3389/fmicb.2016.01588

Lindström ES, Kamst-Van Agterveld MP, Zwart G (2005) Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. Appl Environ Microbiol 71:8201–8206. https://doi.org/10.1128/AEM.71.12.8201-8206.2005

Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522. https://doi.org/10.1128/aem.63.11.4516-4522.1997

Llirós M, Inceoğlu Ö, García-Armisen T et al (2014) Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status. PLoS One 9:e116145. https://doi.org/10.1371/journal.pone.0116145

Lo CC, Chain PSG (2014) Rapid evaluation and quality control of next generation sequencing data with FaQCs. BMC Bioinform 15:366. https://doi.org/10.1186/s12859-014-0366-2

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550. https://doi.org/10.1186/s13059-014-0550-8

Lozupone CA, Knight R (2007) Global patterns in bacterial diversity. Proc Natl Acad Sci U S A 104:11436–11440. https://doi.org/10.1073/pnas.0611525104

Luo W, Friedman MS, Shedden K et al (2009) GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinform 10:161. https://doi.org/10.1186/1471-2105-10-161

Madhavan A, Sindhu R, Parameswaran B et al (2017) Metagenome analysis: a powerful tool for enzyme bioprospecting. Appl Biochem Biotechnol 183:636–651. https://doi.org/10.1007/s12010-017-2568-3

Magee J (1993) Whole-organism fingerprinting. In: Goodfellow M, O'Donnell AG (eds) Handbook of new bacterial systematics. Academic Press, London, pp 383–427

Mardis ER (2008) Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet 9:387–402. https://doi.org/10.1146/annurev.genom.9.081307.164359

Martinez X, Pozuelo M, Pascal V et al (2016) MetaTrans: an open-source pipeline for metatranscriptomics. Sci Rep 6:1–12. https://doi.org/10.1038/srep26447

Martins PD, Frank J, Mitchell H et al (2019) Wetland sediments host diverse microbial taxa capable of cycling alcohols. Appl Environ Microbiol 85:e00189-19. https://doi.org/10.1128/AEM.00189-19

Maxam AM, Gilbert W (1977) A new method for sequencing DNA. Proc Natl Acad Sci U S A 74:560–564. https://doi.org/10.1073/pnas.74.2.560

Meinicke P (2015) UProC: tools for ultra-fast protein domain classification. Bioinformatics 31:1382–1388. https://doi.org/10.1093/bioinformatics/btu843

Metzker ML (2010) Sequencing technologies — the next generation. Nat Rev Genet 11:31–46. https://doi.org/10.1038/nrg2626

Meyer F, Paarmann D, D'Souza M et al (2008) The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinform 9:1–8. https://doi.org/10.1186/1471-2105-9-386

Mitsch WJ, Bernal B, Nahlik AM et al (2013) Wetlands, carbon, and climate change. Landsc Ecol 28:583–597. https://doi.org/10.1007/s10980-012-9758-8

Motoyama A, Yates JR (2008) Multidimensional LC separations in shotgun proteomics. Anal Chem 80:7187–7193

Mrozik A, Nowak A, Piotrowska-Seget Z (2014) Microbial diversity in waters, sediments and microbial mats evaluated using fatty acid-based methods. Int J Environ Sci Technol 11 (5):1487–1496

Muth T, Benndorf D, Reichl U et al (2013) Searching for a needle in a stack of needles: challenges in metaproteomics data analysis. Mol BioSyst 9:578–585

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Najar IN, Sherpa MT, Das S et al (2020) Diversity analysis and metagenomic insights into the antibiotic resistance and metal resistances among Himalayan hot spring Bacteriobiome-insinuating inherent environmental baseline levels of antibiotic and metal tolerance. J Glob Antimicrob Resist 21:342–352. https://doi.org/10.1016/j.jgar.2020.03.026

Namiki T, Hachiya T, Tanaka H, Sakakibara Y (2012) MetaVelvet: an extension of velvet assembler to de novo metagenome assembly from short sequence reads. Nucleic Acids Res 40:e155. https://doi.org/10.1093/nar/gks678

Narayanasamy S, Jarosz Y, Muller EEL et al (2016) IMP: a pipeline for reproducible reference-independent integrated metagenomic and metatranscriptomic analyses. Genome Biol 17:1–21. https://doi.org/10.1186/s13059-016-1116-8

Narrowe AB, Borton MA, Hoyt DW et al (2019) Uncovering the diversity and activity of methylotrophic methanogens in freshwater wetland soils. mSystems 4:e00320-19. https://doi.org/10.1128/msystems.00320-19

Neves ALA, Li F, Ghoshal B et al (2017) Enhancing the resolution of rumen microbial classification from metatranscriptomic data using Kraken and Mothur. Front Microbiol 8:2445. https://doi.org/10.3389/fmicb.2017.02445

Newton RJ, Jones SE, Eiler A et al (2011) A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:14–49. https://doi.org/10.1128/mmbr.00028-10

Ni Y, Li J, Panagiotou G (2016) COMAN: a web server for comprehensive metatranscriptomics analysis. BMC Genomics 17:622. https://doi.org/10.1186/s12864-016-2964-z

Niu S-Y, Yang J, McDermaid A et al (2018) Bioinformatics tools for quantitative and functional metagenome and metatranscriptome data analysis in microbes. Brief Bioinform 19:1415–1429. https://doi.org/10.1093/BIB/BBX051

Noble RT, Lee CSC, Kim M et al (2016) The microbiota of recreational freshwaters and the implications for environmental and public health. Front Microbiol 7:1–15. https://doi.org/10.3389/fmicb.2016.01826

Nurk S, Meleshko D, Korobeynikov A, Pevzner PA (2017) MetaSPAdes: a new versatile metagenomic assembler. Genome Res 27:824–834. https://doi.org/10.1101/gr.213959.116

O'Leary NA, Wright MW, Brister JR et al (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745. https://doi.org/10.1093/nar/gkv1189

Ohl M (2015) Principles of taxonomy and classification: current procedures for naming and classifying organisms. In: Henke W, Tattersall I (eds) Handbook of paleoanthropology. Springer, Berlin, pp 213–236

Osunmakinde CO, Selvarajan R, Mamba BB, Msagati TAM (2019) Profiling bacterial diversity and potential pathogens in wastewater treatment plants using high-throughput sequencing analysis. Microorganisms 7:506. https://doi.org/10.3390/microorganisms7110506

Ounit R, Wanamaker S, Close TJ, Lonardi S (2015) CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. BMC Genomics 16:1–13. https://doi.org/10.1186/s12864-015-1419-2

Pace ML, Reche I, Cole JJ et al (2012) pH change induces shifts in the size and light absorption of dissolved organic matter. Biogeochemistry 108:109–118. https://doi.org/10.1007/s10533-011-9576-0

Parveen A, Deka A, Goswami G et al (2016) Cellulase producing thermophilic bacteria from hot spring of Assam. Int J Microbiol Res 8:776–780

Payne JT, Millar JJ, Jackson CR, Ochs CA (2017) Patterns of variation in diversity of the Mississippi river microbiome over 1,300 kilometers. PLoS One 12:1–18. https://doi.org/10.1371/journal.pone.0174890

Peimbert M, Alcaraz LD (2016) A Hitchhiker's guide to metatranscriptomics. In: Field guidelines for genetic experimental designs in high-throughput sequencing. Springer, Cham, pp 313–342

Peng Y, Leung HCM, Yiu SM, Chin FYL (2012) IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28:1420–1428. https://doi.org/10.1093/bioinformatics/bts174

Penton CR, Johnson TA, Quensen JF et al (2013) Functional genes to assess nitrogen cycling and aromatic hydrocarbon degradation: primers and processing matter. Front Microbiol 4:279. https://doi.org/10.3389/fmicb.2013.00279

Perkins D, Pappin D, Creasy D, Cottrell J (1999) Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis 20 (18):3551–3567. https://doi.org/10.1002/(SICI)1522-2683(19991201)20:18<3551::AID-ELPS3551>3.0.CO;2-2

Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. Ecol Lett 9:741–758

Philipson C, Davenport K, Voegtly L et al (2017) Brief protocol for EDGE bioinformatics: analyzing microbial and metagenomic NGS data. Bio-protocol 7:e2622. https://doi.org/10.21769/BioProtoc.2622

Pollard MO, Gurdasani D, Mentzer AJ et al (2018) Long reads: their purpose and place. Hum Mol Genet 27:R234–R241. https://doi.org/10.1093/hmg/ddy177

Prakash O, Verma M, Sharma P et al (2007) Polyphasic approach of bacterial classification - an overview of recent advances. Indian J Microbiol 47:98–108. https://doi.org/10.1007/s12088-007-0022-x

Ranjan R, Rani A, Metwally A et al (2016) Analysis of the microbiome: advantages of whole genome shotgun versus 16S amplicon sequencing. Biochem Biophys Res Commun 469:967–977. https://doi.org/10.1016/j.bbrc.2015.12.083

Rappé MS, Giovannoni SJ (2003) The uncultured microbial majority. Annu Rev Microbiol 57:369–394. https://doi.org/10.1146/annurev.micro.57.030502.090759

Rathour R, Gupta J, Kumar M et al (2017) Metagenomic sequencing of microbial communities from brackish water of Pangong lake of the Northwest Indian Himalayas. Genome Announc 5: e01029-17. https://doi.org/10.1128/genomeA.01029-17

Read DS, Gweon HS, Bowes MJ et al (2015) Catchment-scale biogeography of riverine bacterioplankton. ISME J 9:516–526. https://doi.org/10.1038/ismej.2014.166

Rechenberger J, Samaras P, Jarzab A et al (2019) Challenges in clinical metaproteomics highlighted by the analysis of acute leukemia patients with gut colonization by multidrug-resistant enterobacteriaceae. Proteomes 7:2. https://doi.org/10.3390/proteomes7010002

Rho M, Tang H, Ye Y (2010) FragGeneScan: predicting genes in short and error-prone reads. Nucleic Acids Res 38:1–12. https://doi.org/10.1093/nar/gkq747

Ritchie ME, Phipson B, Wu D et al (2015) Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43:e47. https://doi.org/10.1093/nar/gkv007

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. https://doi.org/10.1093/bioinformatics/btp616

Rooijers K, Kolmeder C, Juste C et al (2011) An iterative workflow for mining the human intestinal metaproteome. BMC Genomics 12:1–11. https://doi.org/10.1186/1471-2164-12-6

Rudney JD, Xie H, Rhodus NL et al (2010) A metaproteomic analysis of the human salivary microbiota by three-dimensional peptide fractionation and tandem mass spectrometry. Mol Oral Microbiol 25:38–49. https://doi.org/10.1111/j.2041-1014.2009.00558.x

Russo DA, Couto N, Beckerman AP, Pandhal J (2019) Metaproteomics of freshwater microbial communities. In: Evans C, Wright P, Noirel J (eds) Mass spectrometry of proteins. Methods in molecular biology. Humana Press, New York, NY, pp 3–15

Salazar G, Paoli L, Alberti A et al (2019) Gene expression changes and community turnover differentially shape the global ocean metatranscriptome. Cell 179:1068–1083.e21. https://doi.org/10.1016/j.cell.2019.10.014

Salkar K, Gadgil V, Dubey SK et al (2018) Largest freshwater lake "Loktak" in Manipur needs urgent conservation. Curr Sci 114:2436–2437

Samson R, Shah M, Yadav R et al (2019) Metagenomic insights to understand transient influence of Yamuna River on taxonomic and functional aspects of bacterial and archaeal communities of river Ganges. Sci Total Environ 674:288–299. https://doi.org/10.1016/j.scitotenv.2019.04.166

Sanger F, Coulson AR (1975) A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol 94:441–448. https://doi.org/10.1016/0022-2836(75)90213-2

Savio D, Sinclair L, Ijaz UZ et al (2015a) Bacterial diversity along a 2600km river continuum. Environ Microbiol 17:4994–5007. https://doi.org/10.1111/1462-2920.12886

Savio D, Sinclair L, Ijaz UZ et al (2015b) Bacterial diversity along a 2600km river continuum. Environ Microbiol 17:4994–5007. https://doi.org/10.1111/1462-2920.12886

Schadt EE, Turner S, Kasarskis A (2010) A window into third-generation sequencing. Hum Mol Genet 19:R227–R240. https://doi.org/10.1093/hmg/ddq416

Schloss PD, Westcott SL, Ryabin T et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/AEM.01541-09

Schneider T, Riedel K (2010) Environmental proteomics: analysis of structure and function of microbial communities. Proteomics 10:785–798

Schulz MH, Zerbino DR, Vingron M, Birney E (2012) Oases: robust de novo RNA-seq assembly across the dynamic range of expression levels. Bioinformatics 28:1086–1092. https://doi.org/10.1093/bioinformatics/bts094

Schwieger F, Tebbe CC (1998) A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. Appl Environ Microbiol 64:4870–4876. https://doi.org/10.1128/AEM.64.12.4870-4876.1998

Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153
Sekar R, Pernthaler A, Pernthaler J et al (2003) An improved protocol for quantification of freshwater Actinobacteria by fluorescence in situ hybridization. Appl Environ Microbiol 69:2928–2935. https://doi.org/10.1128/AEM.69.5.2928-2935.2003
Sequeira JC, Rocha M, Madalena Alves M, Salvador AF (2019) MOSCA: an automated pipeline for integrated metagenomics and metatranscriptomics data analysis. In: Advances in intelligent systems and computing. Springer, Berlin, pp 183–191
Shafi S, Kamili AN, Shah MA et al (2017) Aquatic bacterial diversity: magnitude, dynamics, and controlling factors. Microb Pathog 104:39–47
Shakya M, Lo CC, Chain PSG (2019) Advances and challenges in metatranscriptomic analysis. Front Genet 10:1–10
Sharma P, Slathia PS, Raina N, Bhagat D (2019) Microbial diversity in freshwater ecosystems and its industrial potential. Elsevier, Amsterdam
Sharpton TJ (2014) An introduction to the analysis of shotgun metagenomic data. Front Plant Sci 5:209. https://doi.org/10.3389/fpls.2014.00209
Shen M, Li Q, Ren M et al (2019) Trophic status is associated with community structure and metabolic potential of planktonic microbiota in plateau lakes. Front Microbiol 10:1–15. https://doi.org/10.3389/fmicb.2019.02560
Shi Y, Tyson GW, Delong EF (2009) Metatranscriptomics reveals unique microbial small RNAs in the oceans water column. Nature 459:266–269. https://doi.org/10.1038/nature08055
Shuikan A, Ali Alharbi S, Hussien M, Alkhalifah D, Hozzein WN (2020) High-throughput sequencing and metagenomic data analysis. In: Metagenomics - basics, methods and applications. IntechOpen, London
Silva GGZ, Cuevas DA, Dutilh BE, Edwards RA (2014) FOCUS: an alignment-free model to identify organisms in metagenomes using non-negative least squares. PeerJ 2014:e425. https://doi.org/10.7717/peerj.425
Silva GGZ, Green KT, Dutilh BE, Edwards RA (2016) SUPER-FOCUS: a tool for agile functional analysis of shotgun metagenomic data. Bioinformatics 32:354–361. https://doi.org/10.1093/bioinformatics/btv584
Simon C, Daniel R (2017) Construction of small-insert and large-insert metagenomic libraries. Methods Mol Biol 1539:1–12. https://doi.org/10.1007/978-1-4939-6691-2_1
Singh T, Singh DK (2018) Assessing the bacterial community structure in the rhizoplane of wetland plants. Bull Environ Contam Toxicol 101:521–526. https://doi.org/10.1007/s00128-018-2426-1
Sogin ML, Morrison HG, Huber JA et al (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci 103:12115–12120. https://doi.org/10.1073/PNAS.0605127103
Sowell SM, Abraham PE, Shah M et al (2011) Environmental proteomics of microbial plankton in a highly productive coastal upwelling system. ISME J 5:856–865. https://doi.org/10.1038/ismej.2010.168
Srivastava N, Gupta B, Gupta S et al (2019) Analyzing functional microbial diversity. In: Microbial diversity in the genomic era. Elsevier, Amsterdam, pp 79–102
Staley C, Unno T, Gould TJ et al (2013) Application of Illumina next-generation sequencing to characterize the bacterial community of the Upper Mississippi River. J Appl Microbiol 115:1147–1158. https://doi.org/10.1111/jam.12323
Stanish LF, Hull NM, Robertson CE et al (2016) Factors influencing bacterial diversity and community composition in municipal drinking waters in the Ohio River basin, USA. PLoS One 11:1–21. https://doi.org/10.1371/journal.pone.0157966
Stocker R (2012) Marine microbes see a sea of gradients. Science 338:628–633
Stockner JG, Porter KG (1988) Microbial food webs in freshwater planktonic ecosystems. In: Carpenter SR (ed) Complex interactions in lake communities. Springer, New York, pp 69–83

Štursová M, Žifčáková L, Leigh MB et al (2012) Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiol Ecol 80:735–746. https://doi.org/10.1111/j.1574-6941.2012.01343.x

Sujun L, Tang H, Ye Y (2019) A meta-proteogenomic approach to peptide identification incorporating assembly uncertainty and genomic variation. Mol Cell Proteomics 18:S183–S192. https://doi.org/10.1074/mcp.TIR118.001233

Tabacchioni S, Chiarini L, Bevivino A et al (2000) Bias caused by using different isolation media for assessing the genetic diversity of a natural microbial population. Microb Ecol 40:169–176. https://doi.org/10.1007/s002480000015

Tamames J, Puente-Sánchez F (2019) SqueezeMeta, a highly portable, fully automatic metagenomic analysis pipeline. Front Microbiol 10:3349. https://doi.org/10.3389/fmicb.2018.03349

Tatusov RL, Fedorova ND, Jackson JD et al (2003) The COG database: an updated vesion includes eukaryotes. BMC Bioinform 4:41. https://doi.org/10.1186/1471-2105-4-41

The UniProt Consortium (2019) UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47:D506–D515. https://doi.org/10.1093/nar/gky1049

Trevors JT (1998) Bacterial biodiversity in soil with an emphasis on chemically-contaminated soils. Water Air Soil Pollut 101:45–67

Truong DT, Franzosa EA, Tickle TL et al (2015) MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods 12:902–903. https://doi.org/10.1038/nmeth.3589

Uchiyama K, Suzuki T, Tatsumi H et al (2002) Amplified 16S ribosomal DNA restriction analysis of microbial community structure during rapid degradation of a biopolymer, PHA, by composting. In: Microbiology of composting. Springer, Berlin, pp 83–98

Ugarte A, Vicedomini R, Bernardes J, Carbone A (2018) A multi-source domain annotation pipeline for quantitative metagenomic and metatranscriptomic functional profiling. Microbiome 6:149. https://doi.org/10.1186/s40168-018-0532-2

van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C (2014) Ten years of next-generation sequencing technology. Trends Genet 30:418–426

Vandamme P, Pot B, Gillis M et al (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60:407–438

Varshney RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends Biotechnol 27:522–530. https://doi.org/10.1016/J.TIBTECH.2009.05.006

VerBerkmoes NC, Denef VJ, Hettich RL, Banfield JF (2009) Systems biology: functional analysis of natural microbial consortia using community proteomics. Nat Rev Microbiol 7:196–205

Vieites JM, Guazzaroni ME, Beloqui A et al (2009) Metagenomics approaches in systems microbiology. FEMS Microbiol Rev 33:236–255

Vincent AT, Derome N, Boyle B et al (2017) Next-generation sequencing (NGS) in the microbiological world: how to make the most of your money. J Microbiol Methods 138:60–71

Wagner M, Smidt H, Loy A, Zhou J (2007) Unravelling microbial communities with DNA-microarrays: challenges and future directions. Ecology 53:498–506. https://doi.org/10.1007/s00248-006-9197-7

Wang HB, Zhang ZX, Li H et al (2011) Characterization of metaproteomics in crop rhizospheric soil. J Proteome Res 10:932–940. https://doi.org/10.1021/pr100981r

Wang Y, Zhou Y, Xiao X et al (2020) Metaproteomics: a strategy to study the taxonomy and functionality of the gut microbiota. J Proteome 219:103737

Westreich ST, Treiber ML, Mills DA et al (2018) SAMSA2: A standalone metatranscriptome analysis pipeline. BMC Bioinform 19:1–11. https://doi.org/10.1186/s12859-018-2189-z

Wilke A, Bischof J, Gerlach W et al (2016) The MG-RAST metagenomics database and portal in 2015. Nucleic Acids Res 44:D590–D594. https://doi.org/10.1093/nar/gkv1322

Williams MA, Taylor EB, Mula HP (2010) Metaproteomic characterization of a soil microbial community following carbon amendment. Soil Biol Biochem 42:1148–1156. https://doi.org/10.1016/j.soilbio.2010.03.021

Wilmes P, Bond PL (2004) The application of two-dimensional polyacrylamide gel electrophoresis and downstream analyses to a mixed community of prokaryotic microorganisms. Environ Microbiol 6:911–920. https://doi.org/10.1111/j.1462-2920.2004.00687.x

Wilmes P, Bond PL (2006) Metaproteomics: studying functional gene expression in microbial ecosystems. Trends Microbiol 14:92–97. https://doi.org/10.1016/j.tim.2005.12.006

Wood DE, Salzberg SL (2014) Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:1–12. https://doi.org/10.1186/gb-2014-15-3-r46

Wood DE, Lu J, Langmead B (2019) Improved metagenomic analysis with Kraken 2. Genome Biol 20:257. https://doi.org/10.1186/s13059-019-1891-0

Yamada T, Letunic I, Okuda S et al (2011) iPath2.0: interactive pathway explorer. Nucleic Acids Res 39:W412–W415. https://doi.org/10.1093/nar/gkr313

Yan Y-W, Jiang Q-Y, Wang J-G et al (2018) Microbial communities and diversities in mudflat sediments analyzed using a modified metatranscriptomic method. Front Microbiol 9:93. https://doi.org/10.3389/fmicb.2018.00093

Yates JR, Ruse CI, Nakorchevsky A (2009) Proteomics by mass spectrometry: approaches, advances, and applications. Annu Rev Biomed Eng 11:49–79. https://doi.org/10.1146/annurev-bioeng-061008-124934

Ye Y, Doak TG (2009) A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. PLoS Comput Biol 5:e1000465. https://doi.org/10.1371/journal.pcbi.1000465

Ye Y, Tang H (2016) Utilizing de Bruijn graph of metagenome assembly for metatranscriptome analysis. Bioinformatics 32:1001–1008. https://doi.org/10.1093/bioinformatics/btv510

Young OR, Steffen W (2009) The earth system: sustaining planetary life-support systems. In: principles of ecosystem stewardship: resilience-based natural resource Management in a Changing World. Springer, New York, pp 295–315

Young JM, Rawlence NJ, Weyrich LS, Cooper A (2014) Limitations and recommendations for successful DNA extraction from forensic soil samples: a review. Sci Justice 54:238–244

Zhao D, Huang R, Zeng J et al (2012) Diversity analysis of bacterial community compositions in sediments of urban lakes by terminal restriction fragment length polymorphism (T-RFLP). World J Microbiol Biotechnol 28:3159–3170. https://doi.org/10.1007/s11274-012-1126-y

Zhong C, Edlund A, Yang Y et al (2016) Metagenome and metatranscriptome analyses using protein family profiles. PLoS Comput Biol 12:e1004991. https://doi.org/10.1371/journal.pcbi.1004991

Zoccarato L, Grossart HP (2019) Relationship between lifestyle and structure of bacterial communities and their functionality in aquatic systems. In: Lectures on quantum statistics. Springer, Cham, pp 13–52

Zwart G, Crump B, Kamst-van Agterveld M et al (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 28:141–155. https://doi.org/10.3354/ame028141

Functional Defense Signals in Plants

25

Tabish Qidwai, Tejal Shreeya, Sudipta Saha, and Monica Sharma

Abstract

Plants live in a complex environment where they interact with a number of microbial pathogens with varying lifestyles and infection strategies. Numerous morphological, biochemical, and molecular mechanisms exist to cope with the effects of pathogen infection. Some mechanisms are preexisting and others induced upon infection of pathogens or attack of herbivores. Phytohormones have been shown to play key role in plant defense, and they mediate defense signaling cascades in plants. Phytohormones such as salicylic acid, jasmonic acid, and ethylene have been shown to play crucial role in the regulation of defense signaling. Understanding of function of complex defense signaling network is important. The present chapter is aimed to study about the role of phytohormones in induction of defense mechanism in plants. Moreover, this study covers the defense mechanisms (existing/induced) in the plants against the phytopathogens.

Keywords

Phytohormones · Phytopathogen · Defense signaling and induced resistance

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25.1 Introduction

Every living organism has a self-defense mechanism against any kind of alarming condition. This defense response can be readily seen in animal system as compared to plants. Plants are immovable and have developed complex defense mechanisms those different from animals. Plants encounter the effect of external signals such as light, temperature, minerals, water, atmospheric gases and wounding, etc. Moreover, they are exposed with several internal signals such as signals from growth regulators, sugars, peptides, and cell wall fragments (Swamy 1999). Moreover, plants are also exposed with many pathogenic agents. Because of mobile nature of animals, they are able to escape themselves from predators, while plants are fixed in soil and without difficulty attacked by pathogens. In order to protect themselves from the attack of microbial pathogens and herbivorous insects, plants are armed with various defense mechanisms. These defense mechanisms may be preexisting or may be activated upon pathogen or insect invasion. Fitness cost is involved in induced defense responses. Plants have regulatory mechanisms that control activation of attacker-specific defenses so that the optimal resistance is maintained and fitness cost is minimized (Pieterse and Dicke 2007). Defense signaling in plants is the major focus of research so as to explore the mechanism of action by which alters their responses to various attackers and to examine how plants confront multiple consistent interactions with attackers (Koornneef and Corne 2008).

For survival, plants have to sense attacks by pathogenic organisms and respond quickly by stimulating suitable defense mechanisms. Primary immune response recognizes some common features of the attacking organism and to convert this recognition into a defense response against encountered attacker (Jones and Dangl 2006). Induced resistance acts systemically and is effective against a wide range of attackers (Walters et al. 2007). Depending on the attacking organism, plants can activate various induced resistance.

Phytohormones play an important role in regulating these induced defenses, and their roles are well studied. Salicylic acid, jasmonic acid, and ethylene are documented as crucial factor in regulation of signaling pathways of plant self-defenses (Howe 2004; Pozo et al. 2004; Lorenzo and Solano 2005; Grant and Lamb 2006; Van Loon et al. 2006; Von Dahl and Baldwin 2007). Further, the role of other plant hormones such as abscisic acid, brassinosteroids, and auxin has also been implicated in plant defense, but they are not well explored. In this chapter, an attempt has been made to analyze various defense mechanisms in plants and the role of phytohormones in regulation of signaling pathways involved in plant self-defenses.

25.2 Functional Defense Mechanisms in Plants

In plants, disease may be caused by biotic/abiotic factors. Biotic factors include living agents such as virus, bacteria, and fungi. Abiotic factors include, for instance, nutrient deficiency, drought, dearth of oxygen, high temperature, and pollution

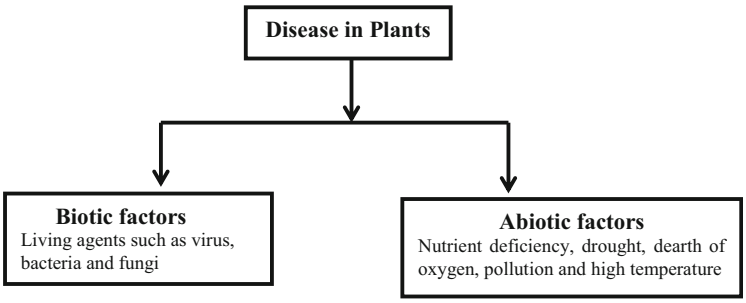

Fig. 25.1 Contribution of biotic and abiotic factors in disease

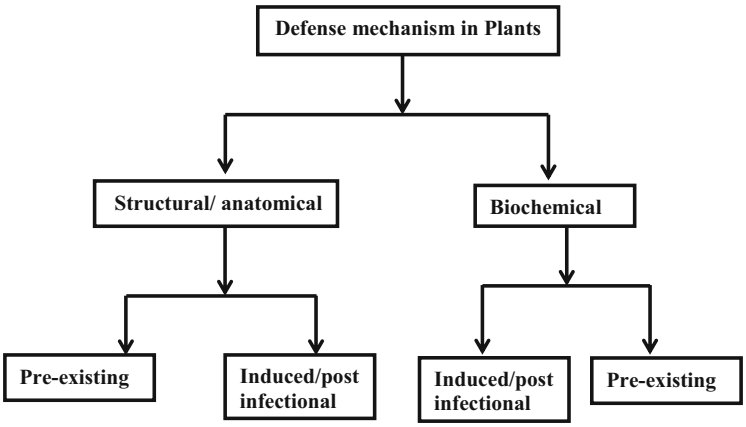

Fig. 25.2 Defense mechanism in plants

(Fig. 25.1). In plant cell walls, waxy epidermal cuticle and bark are constitutive defenses. Besides barriers, all living plant cells have capacity to identify attacking pathogens and tendency to give response with inducible defenses encompassing making of toxic chemicals, pathogen-degrading enzymes, and cell suicide. Basal resistance could be activated when plant cells identify microbe-associated molecular patterns (MAMPs) comprising particular lipopolysaccharides, proteins, and cell wall present in pathogens.

In order to protect themselves from different attackers, plants have developed advanced tactics to recognize the attack and to convert this perception into an efficacious immune response (Gimenez-Ibanez and Solano 2013). Defense mechanism may be structural/anatomical and biochemical (Fig. 25.2). Structural mechanisms include preexisting and post-inflectional or induced structural. Biochemical mechanisms include preexisting biochemical and post-inflectional or induced biochemical mechanisms.

25.2.1 Structural Mechanisms

Preexisting structural mechanisms include various events such as quantity and quality of wax and cuticle, shapes, size, and sites of natural openings (stomata and lenticels). Existence of thick cell wall in tissues of plant hinders development of pathogen. Induced structural includes cellular defense structure and hyphal sheathing. Preexisting biochemical comprises inhibitors, released by plants and phenolic compounds, tannins, glucanases, and chitinase. Postinfection response includes hypersensitivity response (HR) and release of phytoalexins.

25.2.2 Biochemical Defense Mechanism

It includes preexisting biochemical defense mechanisms and postinfection or induced biochemical defense mechanisms. This mechanism consists of inhibitors released by the plants in the environments and phenolics such as tannins, glucanases, and chitinase. Hypersensitivity response and production of antimicrobial response such as phytoalexins and plantibodies are the part of induced chemical defense. Plants have primary and secondary metabolite. Secondary metabolite includes terpenoids, phenolics, and alkaloids (Fig. 25.3).

25.2.2.1 Proteins and Enzymes

Several plants and seeds comprise proteins that inhibit pathogen and pest enzymes through the formation of complexes resulting in either blockage of active sites or alteration of enzyme conformations. As a result, the normal function of enzyme is reduced.

25.2.2.2 Defensins

Small cysteine-rich proteins named as defensins display antimicrobial activities. Defensins have been first isolated from endosperm of barley and wheat.

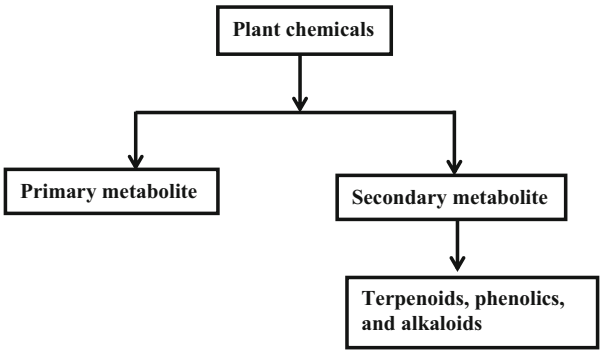

Fig. 25.3 Secondary metabolite such as terpenoids, phenolics, and alkaloids in plant defense

25.2.2.3 Protease Inhibitors

Protease inhibitors inhibit the protease activity of phytopathogens, and they are characteristically expressed in response to herbivore and phytopathogen attack. They are found to inhibit digestive enzymes including trypsin and chymotrypsin. Recently, it has been suggested that protease inhibitors reduce nutrient availability, which diminishes pathogen growth and may lead to the death of the pathogen (Rodríguez-Sifuentes et al. 2020).

25.2.2.4 Hypersensitive Response (HR)

Hypersensitive response when a pathogen has capability of defeating basal defense, plants might reply with alternative line of defense called as hypersensitive response. This response is characterized by intentional plant cell suicide at the site of infection. In addition to hypersensitive response, plants have an array of mechanisms comprising RNA silencing to protect themselves against attackers such as viruses.

25.3 Functional Defense Signals

Plants encounter infinite number of phytopathogens with distinct modes of attack, during their entire life span. Plants have another line of self-defense known as induced resistance. Systemic acquired resistance is an example of induced resistance, activated by microbial pathogens that cause limited infection such as hypersensitive necrosis (Durrant and Dong 2004). Colonization of roots by selected nonpathogenic rhizobacteria activates rhizobacteria-induced systemic resistance (Van Loon et al. 1998) and wound-induced resistance which is activated upon tissue damage (due to insect feeding) (Kessler and Baldwin 2002; Howe 2004).

25.3.1 Phytohormones in Regulation of Defense Network

Phytohormones govern the plant defense network that converts pathogen-induced early signaling actions into the stimulation of specific defense reactions (Pieterse et al. 2012). The phytohormones are small signaling molecules that are present in low concentrations and are required for regulating a number of processes like growth, reproduction, and survival of plants under various biotic and abiotic stresses (Robert-Seilaniantz et al. 2011). Upon pathogen attack, level, composition, and timing of the phytohormone released by plant differ within plant species. Mostly, it is governed by lifestyle and infection strategy of attacking agents (De Vos et al. 2005a, b).

Important phytohormones include auxins, cytokinins (CKs), abscisic acid (ABA), ethylene (ET), and gibberellins (GAs); however, brassinosteroids (BRs), jasmonates (JAs), and salicylic acid (SA) are also considered as phytohormones (Pieterse et al. 2012). Plants have established advanced tactics to recognize the attack and to convert this observation into productive immune response (Gimenez-Ibanez and Solano 2013). Salicylic acid, jasmonic acid, and ethylene are the important players

that regulate the signaling pathways. Phytohormones, abscisic acid (Mauch-Mani and Mauch 2005), brassinosteroids (Nakashita et al. 2003), and auxin (Navarro et al. 2006) have also shown their role in plant defense, but their importance is less understood.

25.3.2 Phytohormones in Signaling

A number of studies suggest that plant hormones like jasmonates, ethylene, and salicylic acid are indulged in a complex signaling network in which various pathways affect each other via positive and negative regulations (Kunkel and Brooks 2002).

Salicylic acid plays a key role in plant defense against microbial pathogens. Upon pathogen infection, salicylic acid (SA) level increases, and exogenous application of SA provides resistance against broad range of pathogens. Combination of positive and negative regulation assures regulation of SA synthesis and plant defense response. On insect or pathogen invasion, plants produce some alarm signals such as JA, SA, and ethylene of varying composition and quantity. It is believed that the signal is specific to induced defense response of plants (De Vos et al. 2006). The signaling pathways, triggered by these signals, are activated which regulate defense responses that have been found effective against different classes of attackers. Genomic and molecular tools are being used to reveal the complexity of the signaling mechanism involved in defense (Pieterse and Dicke 2007). Interaction between JA and SA response pathway is the best known example of defense related crosstalk (Bostock 2005; Beckers and Spoel 2006).

Microbe-associated molecular pattern (MAMP)-triggered immunity is activated when highly conserved microbe-associated molecular patterns (MAMPs) is recognized by transmembrane protein of host cell which acts as pattern recognition receptors (Jones and Dangl 2006). The defense system activated is sufficient enough to confront nonpathogenic microbes and some other pathogens as well. In order to overcome these lines of defenses, the microbial pathogens have developed capability to present virulence effector proteins in plant cells to stimulate susceptibility of plants (Jones and Dangl 2006).

Other branch identifies microbial effectors in plant cell via nucleotide binding site leucine-rich repeat (NB-LRR) resistance (R) proteins. It activates effector triggered immunity (ETI) and has shown association with programmed cell death. This is termed as hypersensitive response. Hypersensitive response is a type of defense that inhibits microbial spread by killing infected cells. The result relies upon counterbalance between the pathogen capacity to suppress plant immune system and tendency of the plants to recognize the pathogen and to trigger specific defense mechanisms against the pathogen.

25.4 Pathogen Defense Response Pathways

Immunity of plant depends on two hormones, i.e., JA and SA, which are antagonistic to each other (Glazebrook 2005). JA and SA control various types of microbes, and they coordinate in such a complex transcriptional programming leading to resistance of plants. Receptors for these hormones have been identified recently which are helpful in understanding their role in plant immunity. Depending upon the encountered pathogen, the crosstalk among defense signaling pathway assists in determining which defense approach is to be followed. Attackers manipulate plants for their own advantage by either inhibiting defense mechanisms or by modifying them (Pieterse and Dicke 2007). For example, herbivorous nymphs of silver leaf whitefly (*Bemisia tabaci*) activate SA signaling pathway as a deceiving tactic since JA-dependent defense pathways are inhibited that give rise to increased performance of insect (Zarate et al. 2007). Egg-derived elicitors from *Pieris rapae* and huge cabbage white *Pieris brassicae* destroy JA-dependent defenses through SA/JA crosstalk to provide advantage to hatching larvae (Little et al. 2007). Pathogens manipulate plant's signaling mechanism either by producing phytohormones or by functionally mimicking them so as to make plant to activate inappropriate defenses (Robert-Seilaniantz et al. 2007). For example, virulent bacteria *Pseudomonas syringae* give rise coronatine that mimics the action of jasmonic acid- isoleucine (JA-Ile) signaling (Nomura et al. 2005).

25.4.1 Nonexpressor of Pathogenesis-Related Genes1 (NPR1)-Dependent SA Signaling

Nonexpressor of pathogenesis-related genes1 (NPR1) is a master regulator, regulating multiple immune responses including systemic acquired resistance. It is the main node in signaling downstream from SA (Dong 2004; Durrant and Dong 2004). SA is sensed by two types of receptors, namely, NPR1 and NPR3/4; nevertheless they have opposite actions in transcriptional regulation of defense-related genes. NPR1 activates SA-induced defense gene expression and pathogen resistance (Ana Radojicic et al. 2018).

NPR1 consists of an ankyrin repeat motif and a broad complex, tramtrack, and bric a brac/poxvirus and zinc finger (BTB/POZ) domain (Maier et al. 2011). The NPR1 gene's promoter region consists of W-box sequences that act as interaction sites of WRKY family protein. With the absence of microbial pathogen challenge or SA, NPR1 is present in cytoplasm as an oligomer. On induction, NPR1 monomer is released that enters nucleus and activates transcription of defense gene (Mao et al. 2007). SA influences NPR1 action at two stages:

1. Triggers NPR1 gene expression
2. Stimulates translocation of NPR1 into nucleus

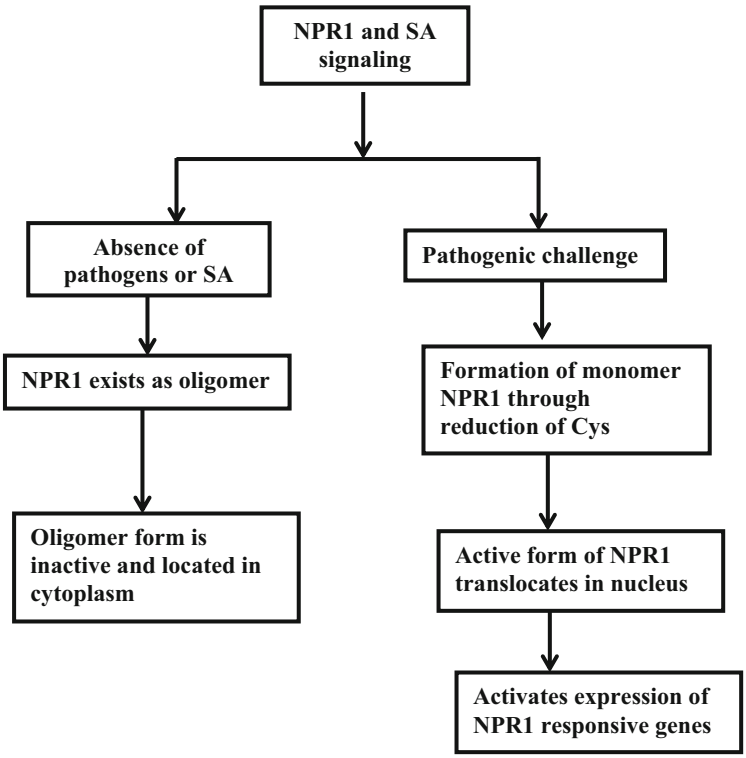

Fig. 25.4 NPR1-dependent SA signaling (Gonzalez-Bosch 2018)

Salicylic acid-mediated immune defense is moved to nucleus upon endoplasmic reticulum (ER) stress-induced reduction of the cytosolic redox potential, which is usually induced by SA (Ya-Shiuan Lai et al. 2018). SA-induced alteration in cellular redox state causes reduction of two cysteine residues (Cys82 and Cys216) by TRX-H5 and/or TRX-H3 (Mao et al. 2007). Variations in cellular redox state can be detected by NPR1 in plants. Oligomeric form of NPR1 which remains inactive is transformed to active monomer through redox modification of Cys residues which is catalyzed by thioredoxins. This NPR1 moves in nucleus and triggers NPR1-associated genes (Fig. 25.4). Binding of SA makes NPR1 stimulation and controls nuclear levels of NPR1 by proteasome-mediated degradation. NO-mediated S-nitrosylation supports formation of NPR1 oligomers (Gonzalez-Bosch 2018).

Hormone signaling pathway starts with the interaction between the hormone ligand and the respective receptor which leads to amplification of the signal, thereby leading to some alterations in expression of genes in nucleus (Lumba et al. 2010). Phytohormones such as ABA, SA, CK, and ET start signaling in cytoplasm and then propagate from cytoplasm to nucleus (Santner and Estelle 2009; Fu et al. 2012; Pieterse et al. 2012). Plant nuclear receptors do not belong to transcriptional factors, but they work on directly or just upstream of transcriptional regulators (Chini et al.

2009; Fonseca et al. 2009). This pathway is a direct regulator of gene expression, directly responsive to ligand concentration leading to speedy stimulation of defense-associated genes that decide the nature and efficacy of immune response activated by pathogen.

25.4.2 NPR1-Independent SA Signaling

Few characteristics of defense are governed by SA-dependent, NPR1-independent signaling pathway(s) (Shah et al. 2001; Murray et al. 2002). Study suggested that NPR1-independent pathways can too trigger PR expression and disease resistance (Kachroo et al. 2001). Involvement of NPR1-independent SA signaling in plant defense has been suggested (Takahashi et al. 2002).

25.4.3 Jasmonic Acid-Dependent Defenses

Jasmonic acid, a fatty-acid-derived molecule, has role in pollen and seed development, and it also provides defense against wounding, insect pests, ozone, and microbial pathogens. Jasmonate originated from alpha-linolenic acid in membrane of plastid (Schaller and Stintzi 2009). JA has a crucial role in harmonizing various physiological processes. It has played role in activation of immune responses to most insect herbivores and necrotrophic microorganisms (Glazebrook 2005). The active form of hormone found in nature is (+)-7-iso-JA-L-Ile.

25.4.4 Antagonistic Crosstalk Between Jasmonic Acid and Salicylic Acid

Plant defense activation denotes allocation and ecological costs. For instance, allotment of defense assets against pathogens may suppress competence of plant to respond to different invaders (Pieterse et al. 2012). So there is an antagonistic relationship between SA and JA which optimize immune response against particular attackers. Biotrophic pathogens frequently need SA signaling, while necrotrophic pathogens mostly trigger jasmonate/ethylene (JA/ET)-dependent pathway. Crosstalk between these two independent signaling may cause synergistic or antagonistic behavior (Silvia Proietti et al. 2013). SA inducing biotrophic pathogen-infected plants suppresses JA-dependent defense (Spoel et al. 2007).

Jasmonic acid and salicylic acid are important as they act as primary signals in regulating plant immune response (Robert-Seilaniantz et al. 2011; Pieterse et al. 2012). Increased resistance against necrotrophs is associated with elevated predisposition to biotrophs and vice versa (Grant and Lamb 2006). Auxins, ABA, BRs, CKs, ET, GAs, and oxylipins work as modulators of immune signaling network and calibrate hormonal balances so as to become resistant to attacker that invades plant (Robert-Seilaniantz et al. 2011). Cooperative involvement of the hormones

throughout plant and microbial pathogen interactions is vital for accomplishment of interaction.

25.4.5 Ethylene-Dependent Responses

Ethylene gives resistance in some interactions but stimulates disease production in others. Ethylene works with JA to regulate defense against necrotrophic pathogens. The phytohormone ethylene is recognized by many membrane-located receptor proteins named as ETR1 (ethylene response 1), ETR2 (ethylene response 2), ERS1 (ethylene response sensor 1), ERS2 (ethylene response sensor 2), and EIN4 (ethylene insensitive 4) (Hua and Meyerowitz 1998).

25.4.6 Auxin, Abscisic Acid, and Gibberellic Acid

Phytohormone auxin has been shown to affect almost all aspects of plant growth and development. It has been suggested that numerous plant pathogens can either form auxin themselves or alter the biosynthesis of host to hinder normal development processes of host (Chen et al. 2006; Robert-Seilaniantz et al. 2007). Abscisic acid has been shown to play important role in the adaptation to abiotic stress. Its role in biotic stress responses is not recognized very well. ABA showed negative regulatory effect in disease resistance (Bari and Jones 2009; Ton et al. 2009). It has been shown that exogenous application of ABA blocks SA accumulation and suppresses resistance to *P. syringae* in *Arabidopsis* (Mohr and Cahill 2003). The role of gibberellic acid, a growth-promoting phytohormone, in defense response is less explored. Rice dwarf virus inhibits production of *ent*-kaurene oxidase, a GA biosynthetic enzyme. This leads to decrease of GA levels and a dwarf phenotype which is alike to GA-deficient symptoms (Zhu et al. 2005).

25.5 Reactive Oxygen Species Are Key Signals That Mediate Defense Gene Activation

The generation of reactive oxygen species (ROS) via oxygen consumption is the initial cellular response on pathogen recognition. Apoplastic production of superoxide (O_2^-), or hydrogen peroxide (H_2O_2), has been recorded on recognition of various microbial pathogens (Auh and Murphy 1995). In plants, ROS strengthens host cell wall through glycoprotein cross-linking (Lamb and Dixon 1997) or lipid peroxidation and membrane damage. ROS are key signals that mediate defense gene activation. ROS plays a regulatory function in defense in conjugation with SA and nitric oxide. Various enzymes are involved in apoplastic ROS generation on pathogen recognition. The NADPH oxidase, also named as respiratory burst oxidase, has been

primarily explored in mammalian neutrophils as a multicomponent complex enabling microbial killing (Lambeth 2004).

25.6 Transcription Factors in Defense Against Pathogens

Transcription factors play various roles in plants like defense against pathogens. Five TF families play important role in plant defense mechanisms: WRKY, APETALA2/ethylene responsive factor (AP2/ERF), basic helix-loop-helix (bHLH), basic-domain leucine-zipper (bZIP), and NAM/ATAF/CUC (NAC) (Choi 2015). Transcription factor expression is induced or suppressed upon pathogen attack, and expression is monitored using transcriptome analysis. Suppression and activation of plant defense genes are regulated by WRKY family transcription factors. Suppression of the multiple defense signaling mechanisms by CaWRKY70 promotes susceptibility in chickpea under *Fusarium oxysporum* stress condition (Chakraborty et al. 2020).

25.7 Conclusion

Various morphological, biochemical, and molecular mechanisms exist in plants to protect them against pathogens and herbivores. Induced resistance mechanism is found effective against the disease causing agents. Phytohormones regulate induced defenses and mediate defense signaling cascades. This study highlighted the understanding of complex defense signaling in plants. It would be helpful to uncover mechanism of regulation of induced defense in plants.

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References

Auh CK, Murphy TM (1995) Plasma membrane redox enzyme is involved in the synthesis of O_2^- and H_2O_2 by Phytophthora elicitor-stimulated rose cells. Plant-microbe and plant-insect interactions. Plant Physiol 107(4):1241–1247

Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant Mol Biol 69:473–488

Beckers GJ, Spoel SH (2006) Fine-tuning plant defence signalling: salicylate versus Jasmonate. Plant Biol (Stuttg) 8(1):1–10

Bostock RM (2005) Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu Rev Phytopathol 43:545–580

Chakraborty J, Sen S, Ghosh P, Jain A, Das S (2020) Inhibition of multiple defense responsive pathways by CaWRKY70 transcription factor promotes susceptibility in chickpea under Fusarium oxysporum stress condition. BMC Plant Biol 20(1):319

Chen Z, Zhang H, Jablonowski D, Zhou X, Ren X, Hong X, Schaffrath R, Zhu JK, Gong Z (2006) Mutation in ABO1/ELO2, a subunit of Holo-Elongator, increase abscisic acid sensitivity and drought tolerance in Arabidopsis thaliana. Mol Cell Biol 26:6902–6912

Chini A, Fonseca S, Chico JM et al (2009) The ZIM domain mediates homo- and heteromeric interactions between Arabidopsis JAZ proteins. Plant J 59:77–87
Choi ESDC (2015) Functional studies of transcription factors involved in plant defenses in the genomics era. Brief Funct Genomics 14(4):260–267
De Vos AS, Strydom H, Fouché CB, Delport CSL (2005a) Research at grass roots: for the social science professionals, 3rd edn. Van Schaik, Pretoria
De Vos M, Van Oosten VR, Van Poecke RM et al (2005b) Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. Mol Plant-Microbe Interact 18 (9):923–937
De Vos M, Van Zaanen W, Koornneef A et al (2006) Herbivore-induced resistance against microbial pathogens in Arabidopsis. Plant Physiol 142:352–363
Dong X (2004) NPR1, all things considered. Curr Opin Plant Biol 7:547–552
Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209
Fonseca S, Chico JM, Solano R (2009) The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr Opin Plant Biol 12:539–547
Fu MS, De Sordi L, Muhlschlegel FA (2012) Functional characterization of the small heat shock protein Hsp12p from Candida albicans. PLoS One 7(8):e42894
Gimenez-Ibanez S, Solano R (2013) Nuclear jasmonate and salicylate signaling and crosstalk in defense against pathogens. Front Plant Sci 4:72
Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227
Gonzalez-Bosch C (2018) Priming plant resistance by activation of redox-sensitive genes. Free Radical Biol and Med 122:171–180
Grant M, Lamb C (2006) Systemic immunity. Curr Opin Plant Biol 9:414–420
Howe GA (2004) Jasmonates as signals in the wound response. J Plant Growth Regul 23:223–237
Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell 94(2):261–271
Jones JD, Dangl JL (2006) The plant immune system. Nature 444(7117):323–329
Kachroo P, Shanklin J, Shah J, Whittle EJ, Klessig DF (2001) A fatty acid desaturease modulates the activation of defense signaling pathways in plants. Proc Natl Acad Sci U S A 98:9448–9453
Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol 53:299–328
Koornneef A, Corne MJ (2008) Pieterse cross talk in defense signaling. Plant Physiol 146 (3):839–844
Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 5(4):325–331
Lai Y-S, Renna L, Yarema J et al (2018) Salicylic acid-independent role of NPR1 is required for protection from proteotoxic stress in the plant endoplasmic reticulum. PNAS 115(22):E5203–E5212
Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol 48:251–275
Lambeth J (2004) NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4:181–189
Little D, Gouhier-Darimont C, Bruessow F, Reymond P (2007) Oviposition by pierid butterflies triggers defense responses in Arabidopsis. Plant Physiol 143:784–800
Lorenzo O, Solano R (2005) Molecular players regulating the jasmonate signalling network. Curr Opin Plant Biol 8:532–540
Lumba S, Cutler S, McCourt P (2010) Plant nuclear hormone receptors: a role for small molecules in protein-protein interactions. Annu Rev Cell Dev Biol 26:445–469
Maier F, Zwicker S, Huckelhoven A et al (2011) Nonexpressor of pathogenesis-related proteins1 (NPR1) and some NPR1-related proteins are sensitive to salicylic acid. Mol Plant Pathol 12 (1):73–91

Mao P, Duan M, Wei C, Li Y (2007) WRKY62 transcription factor acts downstream of cytosolic NPR1 and negatively regulates jasmonate-responsive gene expression. Plant Cell Physiol 48:833–842

Mauch-Mani B, Mauch F (2005) The role of abscisic acid in plant-pathogen interactions. Curr Opin Plant Biol 8:409–414

Mohr PG, Cahill DM (2003) Abscisic acid influences the susceptibility of *Arabidopsis thaliana* to pseudomonas syringae pv. tomato and Peronospora parasitica. Funct Plant Biol 30:461–469

Murray SL, Thomson C, Chini A, Read ND, Loake GJ (2002) Characterization of a novel, defense-related Arabidopsis mutant, cir1, isolated by luciferase imaging. Mol Plant-Microbe Ineract 15:557–566

Nakashita H, Yasuda M, Nitta T, Asami T, Fujioka S, Arai Y, Sekimata K, Takatsuto S, Yamaguchi I, Yoshida S (2003) Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. Plant J 33:887–898

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science 312:436–439

Nomura K, Melotto M, He SY (2005) Suppression of host defense in compatible plant-pseudomonas syringae interactions. Curr Opin Plant Biol 8(4):361–368

Pieterse CMJ, Dicke M (2007) Plant interactions with microbes and insects: from molecular mechanisms to ecology. Trends Plant Sci 12:564–569

Pieterse CM, Van der Does D, Zamioudis C et al (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521

Pozo MJ, Van Loon LC, Pieterse CMJ (2004) Jasmonates-signals in plant-microbe interactions. J Plant Growth Regul 23:211–222

Proietti S, Bertini L, Timperio AM et al (2013) Crosstalk between salicylic acid and jasmonate in Arabidopsis investigated by an integrated proteomic and transcriptomic approach. Mol BioSyst 9:1169–1187

Radojicic A, Li X, Zhang Y (2018) Salicylic acid: a double-edged sword for programed cell death in plants. Front Plant Sci 9:1133

Robert-Seilaniantz A, Navarro L, Bari R, Jones JDG (2007) Pathological hormone imbalances. Curr Opin Plant Biol 10:372–379

Robert-Seilaniantz A, Grant M, Jones JD (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 49:317–343

Rodríguez-Sifuentes L, Marszalek JE, Chuck-Hernández C, Serna-Saldívar SO (2020) Legumes protease inhibitors as biopesticides and their defense mechanisms against biotic factors. Int J Mol Sci 21(9):3322

Santner A, Estelle M (2009) Recent advances and emerging trends in plant hormone signalling. Nature 459(7250):1071–1078

Schaller A, Stintzi A (2009) Enzymes in jasmonate biosynthesis - structure, function, regulation. Phytochemistry 70:1532–1538

Shah J, Kachroo P, Nandi A, Klessig DF (2001) A recessive mutation in the Arabidopsis SSI2 gene confers SA- and NPR1-independent expression of PR genes and resistance against bacterial and oomycete pathogens. Plant J 25:563–574

Spoel SH, Johnson JS, Dong X (2007) Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc Natl Acad Sci U S A 104:18842–18847

Swamy GS (1999) Drought signaling in plants. Resonance 4(6):34–44

Takahashi H, Miller J, Nozaki Y et al (2002) RCY1, an *Arabidopsis thaliana* RPP8/HRT family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. Plant J 32:655–667

Ton J, Flors V, Mauch-Mani B (2009) The multifaceted role of ABA in disease resistance. Trends Plant Sci 14:310–317

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Van Loon LC, Geraats BPJ, Linthorst HJM (2006) Ethylene as a modulator of disease resistance in plants. Trends Plant Sci 11:184–191

Von Dahl CC, Baldwin IT (2007) Deciphering the role of ethylene in plant-herbivore interactions. J Plant Growth Regul 26:201–209

Walters D, Newton A, Lyon G (2007) Induced resistance for plant defence: a sustainable approach to crop protection. Blackwell, Oxford

Zarate SI, Kempema LA, Walling LL (2007) Silver leaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol 143(2):866–875

Zhu S, Cao X, Cao X et al (2005) The rice dwarf virus P2 protein interacts with ent-kaurene oxidases in vivo, leading to reduced biosynthesis of gibberellins and rice dwarf symptoms. Plant Physiol 139:1935–1945

Metatranscriptomics: A Recent Advancement to Explore and Understand Rhizosphere

26

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Abstract

In this terrestrial ecosystem, plant is the major creator. With the help of composite root system, they use soil resources. Here the role of rhizosphere comes into consideration which keeps intact the varied microbial communities which eventually affects biogeochemical cycling, plant health and nutrition. But the minutes of mechanisms of plant–microbe interaction is still not explored properly. Thus, it is required to advance new experimental approach adapted to these microorganisms to unveil functional diversity of microbes and the actions they perform in situ in the soil because of various ecological limitations. One of the recent approaches developed for microorganisms is metatranscriptomics. It helps in characterization of genome in community and also explores gene expression patterns. This approach is thus helpful to develop another comprehension on the components that administer plant–organism communications in the rhizosphere. This chapter comprises review on different metatranscriptomics approaches to explore microbial community transcriptomes.

Keywords

Rhizosphere · Sequencing · Microbe community · Plant–microbe interaction

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26.1 Introduction

The chief manufacturer in this earthly planet is plants which utilize their intricate root structures to retrieve mineral and nutrition from the soils. The communities of microbes are nourished by rhizosphere which eventually influences nutrient availability and, thus, the plant strength. However, due to lack of proper methods which help in complete profiling of actions done by several groups of microbes present in rhizosphere, different mechanisms governing plant–microbe relation between microorganism and plant host are not well stated (de Weert et al. 2006; Simons et al. 1997; Walker et al. 2003). Metatranscriptomics enable characterization of gene expression patterns at community level. This effective technology assures to clarify the mechanisms governing relations between plant and microorganism. The emphasis of metatranscriptomics is on RNA which explains about specific genes transcribed from living group. As only a small percentage of the genetic diversity existing in earth is available in the database which is publicly accessible, the study of metatranscriptomics combined with metagenomics is much more efficient. During metatranscriptomics study, consideration of experiment design is crucial to explore molecular signals linked with plant–microbe interactions within data. A disadvantage of this method is that it is tough to know which among different studied variables correlated with transcriptional patterns is really affecting the transcription. Another way is to take control experiments along with different treatments which could help in documentation of differentially expressed genes and various pathways. Time (Carvalhais et al. 2013).

RNA sequencing (RNASeq) offers nearer view of living members in a community by documenting transcripts which are expressed in a microbiome at a particular time period exposed to a group of environmental circumstances. RNASeq could be helpful to less expressed genes as it gives huge data which comprises the whole metatranscriptome that consists non-coding RNAs. These non-coding RNAs could be spotted, explained and plotted to various metabolic pathways. Next-generation sequencing technology has potential to estimate identified targets of transcript. Directly from the data of sequences, NGS also unveils new transcripts and transcript variants which were previously unidentified. Study of microbial communities using metatranscriptomics has multiplied appreciably in a short span since it was initially introduced. Regarding capacities, the procedure has been utilized to describe life forms in a network (Bashiardes et al. 2016), find new microbial collaborations (Bikel et al. 2015), identify governing antisense RNA (Bao et al. 2015) and detect gene expressions and also distinguish the relationship among pathogen and their host (Moniruzzaman et al. 2017). Thus, in this chapter, the role of metatranscriptomics to explore and to understand the minutes of the rhizosphere is focused, and its present and future aspects are also emphasized.

26.2 Basic Procedure of Metatranscriptomics Analysis

Elementary method includes sampling of soil is done, i.e. procuring rhizospheric soil from definite area which is followed by isolation of RNA from the soil sample and preparation of cDNA with reverse transcription followed by high-throughput sequencing. In isolated RNA, 95–99% is rRNA due to presence of consortium. In every cellular organism, such molecule is available and utilized widely as phylogenetic markers. These markers have specific conserved and variable regions which help in the study of diverse range of similarity. In prokaryotes and eukaryotes, the commonly used genetic markers are 16S rRNA and 18S rRNA genes, respectively. Sequencing has led to knowing the existing taxa of organism, and also in exploring the new one via comparing with known database of rRNA to isolate RNA from soil, there are several approaches with few benefits and some disadvantages. The main focus is to eliminate lignin and humic acid which could interfere with further molecular processes (Turner et al. 2013). Different sequencing technique is used like processing directly by Illumina technology. For the analysis of soil sample which include different communities, read lengths approximately 120–160 base pair along with huge sequencing depth are required. Next to sequencing, the major issue is analysis of data which requires complete computational power for data storing and processing. Next to it is the role of bioinformatic to decipher valuable outcome from nucleotide sequences (Yilmaz et al. 2011; de Bruijn 2011). Examples of such bioinformatic tools are the basic local alignment search tool (BLAST), HMMER, USEARCH and MEGAN (Turner et al. 2013) (Fig. 26.1 and Table 26.1).

26.3 Role of Metatranscriptomics in Rhizosphere

26.3.1 Understanding the Functional Diversity Existing in Rhizosphere

Soil is a hotspot for diverse group of microbial entity forming a very complex environment. It is encompassing thousands of different species of bacteria in 1 g of soil sample, where a larger portion of these population are unknown and uncultivable on standard microbiological media (Rappe and Giovannoni 2003). Hence, to analyse the functional diversity existing in soil microorganisms, metatranscriptomic assay is used. For example, from a forest soil, cDNA library is constructed using extracted polyadenylated mRNA. This cDNA library consists of diverse expressed genes present in different organisms of the soil microbiome, which serve as its metatranscriptome. Furthermore, to evaluate diversity of the organisms conferred to the library, sequencing of a portion of 18S rDNA gene is done, which is either amplified from soil DNA or reverse transcribed from extracted polyadenylated mRNA. The output sequencing shows that most of the sequences are contributed by fungi and unicellular eukaryotes (protists), i.e. about more than 70%, and the rest 30% mostly constitute metazoa. In the soil samples, more than 180 species could be found as per calculation of richness estimators. There is no homology found in the

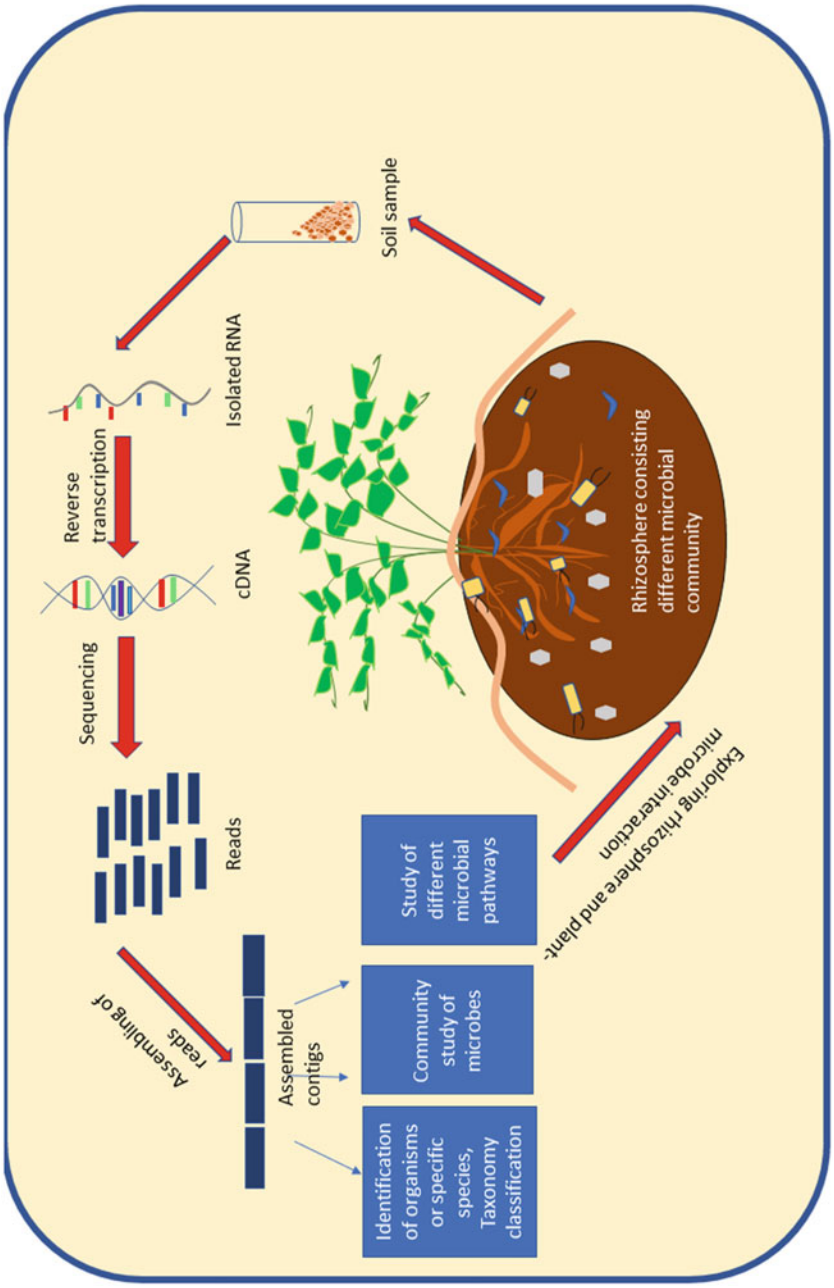

Fig. 26.1 Metatranscriptomic analysis in rhizosphere

Table 26.1 Software and tools utilized in metatranscriptomics

Software name	Function	Reference
SortMeRNA	It is a core algorithm tool used for filtering and mapping of reads	https://bioinfo.lifl.fr/RNA/sortmerna/
BLASTN	It is a core algorithm used for searching an unknown nucleotide sequence in NCBI database	http://nebc.nox.ac.uk/bioinformatics/docs/blastn.html
KAAS	With help of BLAST, it gives functional annotation of genes through comparing it with KEGG GENES database	https://www.genome.jp/kegg/kaas/
MG-RAST	This analyses the quality of sequence and with nominal input automatically annotation is done by comparing with respect to various reference databases	Keegan et al. (2016)
COMAN	This tool provides functional annotation, study of co-expression network relative statistical study	Ni et al. (2016)
MLST	This provide analysis of genetic diversity	https://pubmlst.org/general.shtml
Diamond	*It is a software which provides* crystal structure and assembles the functions	https://www.crystalimpact.com/diamond/
MEGAN	This gives analysis of huge sequencing data and provides taxonomical and functional information	https://omictools.com/megan-tool
HMMER	This tool is used in the alignment of sequence and preparation of homologs of sequence	http://hmmer.org/
Bowtie2	It is a quick and effective tool for alignment of sequence reads to lengthy reference sequences	http://bowtiebio.sourceforge.net/bowtie2/index.shtml

databases (32%) as well as for genes coding proteins needed for different cellular and biochemical processes, when sequencing of 119 cDNA from identified genes was done. With a marked under-representation of the protists, there is on overlapping observed between the taxonomic distribution of the18S rDNA genes and the cDNA. From such an environmental cDNA library, a specific gene could be isolated by using *Saccharomyces cerevisiae*, a heterologous microbial host. This is explained by the functional complementation of histidine auxotrophic yeast mutant by two cDNA derived probably from a basidiomycete and an ascomycete fungal group. To reveal the adaptations to local environmental conditions by the whole microbial communities, metatranscriptome studies are potentially used, which open an access to abundant genes source of biotechnological interest (Bailly et al. 2007). Furthermore, for kingdom level changes in the rhizosphere microbiome of plants, comparative metatranscriptomics studies are more useful. To maintain the plant health and productivity and biogeochemical cycling, rhizospheric plant–microbe interactions playing important roles, this is not yet understood properly. From soil and rhizospheres of different crops, the global active microbiomes could be analysed using RNA-based metatranscriptomics. For example, wheat, oat, pea and an oat mutant (sad1) deficient in production of antifungal avenacins were used in an

experiment and studied. According to the plant species used and bulk soil taken, the rhizosphere microbiomes were differed. Therefore, a dramatically different rhizosphere community was observed as wheat and oat (cereals) had a much weaker effect on the rhizosphere than pea (a legume). The pea rhizosphere was tremendously enriched in fungi, whereas all other rhizospheres were more enriched with nematodes and bacterivorous protozoa. H2 oxidation (pea), cellulose degradation (cereals) and methylotrophy (all plants) were also included, when selection of metabolic capabilities of rhizosphere colonization was done. Anacins have a broader role than protecting from the plant fungal pathogens, as in the sad1 mutant the eukaryotic community was greatly altered, whereas in oat, it had a little effect on the prokaryotic community. To avoid biasness of polymerase chain reaction, metatranscriptomics profiling of microbial communities allows comparison of relative abundance across all domains of life and from multiple samples. Between plants, particularly at the kingdom level, profound differences in the rhizosphere microbiome can be revealed by metatranscriptomics studies (Turner et al. 2013).

26.3.2 Plant-Derived Compounds in the Rhizosphere

Carbon-containing substances liberated from plant roots are called rhizodeposits. It also includes root exudates, volatile compounds and sloughed off cells/tissues. A large proportion of non-volatile rhizodeposits is contributed by root exudates released from at root apices (Dennis et al. 2010), i.e. composed of organic acids, amino acids, carbohydrates and secondary metabolites which help in attracting beneficial motile bacteria towards roots (Shi et al. 2011). This is also done by released border cells of roots, which later compete with attracted foreign pathogenic microbes for nutrients, space and energy, thereby preventing the disease to occur. Modification in the bacterial attachment and nematodes immobilization also ensue due to some compound released from active border cells (Hawes et al. 2000; Vicre et al. 2005). It is also reported with strong evidence that rhizosphere microbial communities are being influenced by carbon-containing components present in root exudates and in other pools of rhizodeposits (Dennis et al. 2010). Root exudate profiles are also influenced by variety of factors such as developmental stage of crop (Gransee and Wittenmayer 2000), crop species (Lesuffleur et al. 2007), nutrient status of soil (Carvalhais et al. 2011) and soil type (Berg and Smalla 2009). However, in some instance, the substances involved in plant–microbe interactions may be released from the microorganisms themselves (Dennis et al. 2010). Therefore, it is uncertain how much extent the plant is controlling the rhizosphere microbial communities, while the mechanisms of interspecies relationships are of even greater concern, in terms of knowledge gap. To address this problem, metatranscriptomics could be used as a powerful approach to elucidate the level of microbial gene expression profiles being influenced by the exudates. For assessing a particular trait, mutants of plant can be used, which may be helpful in improving plant health and productivity. In *Arabidopsis thaliana*, a single mutation on the ABC transporter gene *abcg30* may alter the chemical profile of root exudates, thereby

affecting the soil microbial community profiles (Badri et al. 2009). So correlation can be made between changes in root exudate profiles and abundances of microbial transcripts. This would be helpful in highlighting the components of rhizodeposits that probably influence the structure and activity of specific rhizosphere microbial populations. After validating the correlation, rhizodeposition patterns could be changed accordingly to increase (e.g. PGPR) or decrease (e.g. pathogens) the abundance of specific targeted rhizosphere population (Carvalhais et al. 2013).

26.3.3 Exploring Climate Change Impact on Microbiomes of Rhizosphere

On plant growth, the effect of elevated level of atmospheric CO_2 is well established, while its consequences on the activity and structure of below-ground biota remain ambiguous. It can be explored with the help of transcriptomic. In an experiment which was carried out in grassland microbiomes for 2 years, i.e.in 2015 and 2017, where effects of elevated CO2 on its composition and activity were well observed in 2015, i.e. in elevated atmospheric eCO2 plots, the amount of eukaryotic mRNA and rRNA isolated from rhizospheric soil was reduced in comparison to bacteria. No such effects were found in 2017 as temperature of summer in 2017 was quite long. Increased production of plant secondary metabolites was also recorded by functional analysis of root mRNA (Bei et al. 2019).

26.4 Present Status of Rhizospheric Metatranscriptomics and Its Forthcoming Prospects

In recent past, research based on rhizosphere metatranscriptomics are less, but now its count is increasing. The reason behind its lacking may be its procedural challenges especially when it comes to studying huge population of soil microbes and also the nature of soil. Wide array of queries related to novel investigation could be established through utilizing metatranscriptomics tools, i.e. by comprehensive functional profiling of rhizospheric microbial communities. Some of the areas related to rhizosphere which has been explored and benefitted with metatranscriptomics are study of host ISR (induced systemic resistance), stimulated rhizospheric microorganism, evaluation of rhizospheric gene expression profiles of bacteria and its interaction with signalling molecules like salicylic acid, jasmonic acid, etc. (Carvalhais et al. 2013). Moreover, initiation of next-generation sequencing has led to an upheaval, thus enhancing metatranscriptomic projects in which maximum are study of differential gene expression studies that aims to gain a complete vision of microbiome like total microbes, functions of genes and their pathways. But this aim is still restricted due to absence of sufficient reference genomes that causes poor-quality reads by datasets; thus, there is huge need to

make efforts to gather metatranscriptomic data along with metagenomic data. Submission of metatranscriptomic information into public repositories permits large analysis in our upcoming future and unearthing significant genes, diverse organisms, their interactions and pathways. To exploit complete potential of metatranscriptomic data, metadata is also submitted along with it. Submission of satisfactory metadata should establish as a major concern of forthcoming days. For including adequate and thorough metadata along with metatranscriptomics, several crucial approaches have to be taken by scientific groups like MIxS (Minimum Information about any Sequence) (Yilmaz et al. 2011). Metatranscriptomics needs massive numbers of reads as there is abundance of microbiome membership. Thus, this field is dominated by high-throughput short-read technologies, whereas with improvement of throughput, the growth of long-read technologies holds great future ahead. Every aspects of analysis like functional analysis or determination of taxonomy will be analysed with the help of longer reads. Moreover, it will also deliver dissimilar genes with high likeness, better transcript isoform's resolution and polycistronic operons. The present researches are basically achieved via a solitary short-read technology like Illumina, and a huge number of investigative apparatuses are available to explore every aspect of data (Shakya et al. 2019).

26.5 Conclusion

Metatranscriptomics utilization in exploring microbiome of rhizosphere assists in reframing and raising familiarity with the known and known microbes, knowledge about their communities and interactions within them. It also provides the molecular explanation about restricting pathogenic attack by rhizospheric microbes on the host and advancement of metabolic pathways productive of managing with ecological contaminant (Kothari et al. 2017). Further, metatranscriptomics data can easily be linked coupled with numerous acknowledged microbial metabolic pathways, particularly for gene sequences that encode enzymes, regardless of their taxonomic assembly. Additionally, numerous metagenome scale models could be built using genome-scale models of completely sequenced microorganisms which would delineate biochemical processes in an extensive way and may perhaps permit to measure biochemical exercises of microbial network. Thus we observed that metatranscriptomics encourages all-encompassing evaluation of community-level reactions of multi-territory communications by expression of genes under trial circumstances (Carvalhais et al. 2013). This technology could be likewise extended to understand symbiotic nature of microbes developing novel consideration towards the intricate rhizosphere and making easy for further studies on plant–microbe interactions (Kothari et al. 2017) and thus to develop new tactics to use rhizospheric microbes in different fields especially in agriculture.

References

Badri DV, Quintana N, El Kassis EG, Kim HK, Choi YH, Sugiyama A, Verpoorte R, Martinoia E, Manter DK, Vivanco JM (2009) An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. Plant Physiol 151:2006–2017

Bailly J, Fraissinet-Tachet L, Verner MC, Debaud JC, Lemaire M, Wésolowski-Louvel M, Marmeisse R (2007) Soil eukaryotic functional diversity, a mettranscriptomic approach. ISME J 1(7):632–642

Bao G, Wang M, Doak TG, Ye Y (2015) Strand-specific community RNA-seq reveals prevalent and dynamic antisense transcription in human gut microbiota. Front Microbiol 6:896

Bashiardes S, Zilberman-Schapira G, Elinav E (2016) Use of metatranscriptomics in microbiome research. Bioinform Biol Insights 10:19–25

Bei Q, Moser G, Wu X, Müller C, Liesack W (2019) Metatranscriptomics reveals climate change effects on the rhizosphere microbiomes in European grassland. Soil Biol Biochem 138:107604

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Bikel S, Valdez-Lara A, Cornejo-Granados F, Rico K, Canizales-Quinteros S, Soberón X, Del Pozo-Yauner L, Ochoa-Leyva A (2015) Combining metagenomics, metatranscriptomics and viromics to explore novel microbial interactions: towards a systems-level understanding of human microbiome. Comput Struct Biotechnol J 13:390–401

Carvalhais LC, Dennis PG, Fedoseyenko D, Hajirezaei MR, Borriss R, von Wiren N (2011) Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. J Plant Nutr Soil Sci 174:3–11

Carvalhais LC, Dennis PG, Tyson GW, Schenk PM (2013) Rhizosphere metatranscriptomics: challenges and opportunities. Mol Microb Ecol Rhizosphere 2:1137–1144

de Bruijn FJ (2011) Handbook of molecular microbial ecology I. Metagenomics and complementary approaches. Wiley-Blackwell, Hoboken, pp 1–761

De Weert S, Dekkers LC, Bitter W, Tuinman S, Wijfjes AH, Van Boxtel R, Lugtenberg BJ (2006) The two-component colR/S system of Pseudomonas fluorescens WCS365 plays a role in rhizosphere competence through maintaining the structure and function of the outer membrane. FEMS Microbiol Ecol 58(2):205–213

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72:313–327

Gransee A, Wittenmayer L (2000) Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. J Plant Nutr Soil Sci 163:381–385

Hawes MC, Gunawardena U, Miyasaka S, Zhao XW (2000) The role of root border cells in plant defense. Trends Plant Sci 5:128–133

http://bowtiebio.sourceforge.net/bowtie2/index.shtml

http://hmmer.org/

http://nebc.nox.ac.uk/bioinformatics/docs/blastn.html

https://bioinfo.lifl.fr/RNA/sortmerna/

https://omictools.com/megan-tool

https://pubmlst.org/general.shtml

https://www.crystalimpact.com/diamond/

https://www.genome.jp/kegg/kaas/

Keegan KP, Glass EM, Meyer F (2016) MG-RAST, a metagenomics service for analysis of microbial community structure and function. Methods Mol Biol 1399:207–233

Kothari V, Kothari C, Rank J, Joshi A, Singh RP, Kothari R (2017) Metatranscriptomic studies of the plant Rhizosphere for finding biological agents. In: Understanding host-microbiome interactions-an Omics approach. Springer, Singapore, pp 267–275

Lesuffleur F, Paynel F, Bataille MP, Le Deunff E, Cliquet JB (2007) Root amino acid exudation: measurement of high efflux rates of glycine and serine from six different plant species. Plant Soil 294:235–246

Moniruzzaman M, Wurch LL, Alexander H, Dyhrman ST, Gobler CJ, Wilhelm SW (2017) Virus-host relationships of marine single-celled eukaryotes resolved from metatranscriptomics. Nat Commun 8:16054

Ni Y, Li J, Panagiotou G (2016) COMAN: a web server for comprehensive metatranscriptomics analysis. BMC Genomics 17(1):622

Rappe MS, Giovannoni SJ (2003) The uncultured microbial majority. Ann Rev Microbiol 57:369–394

Shakya M, Lo CC, Chain PS (2019) Advances and challenges in metatranscriptomic analysis. Front Genet 10:904

Shi YM, Tyson GW, Eppley JM, DeLong EF (2011) Integrated metatranscriptomic and metagenomic analyses of stratified microbial assemblages in the open ocean. ISME J 5:999–1013

Simons M, Permentier HP, de Weger LA, Wijffelman CA, Lugtenberg BJJ (1997) Amino acid synthesis is necessary for tomato root colonization by *Pseudomonas fluorescens* strain WCS365. Mol Plant Microb Interact 10:102–106

Turner TR, Ramakrishnan K, Walshaw J, Heavens D, Alston M, Swarbreck D, Osbourn A, Grant A, Poole PS (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME J 7(12):2248–2258

Vicre M, Santaella C, Blanchet S, Gateau A, Driouich A (2005) Root border-like cells of *Arabidopsis*. Microscopical characterization and role in the interaction with rhizobacteria. Plant Physiol 138:998–1008

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. Plant Physiol 132:44–51

Yilmaz P, Gilbert JA, Knight R, Amaral-Zettler L, Karsch Mizrachi I, Cochrane G, Nakamura Y, Sansone SA, Glöckner FO, Field D (2011) The genomic standards consortium: bringing standards to life for microbial ecology. ISME J 5:1565–1567

Advances in Biotechnological Tools and Techniques for Metatranscriptomics

27

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Abstract

To discover the novel functions and pathways in microorganisms for various reactions, a metatranscriptomics is a suitable tool. The use of culture-based techniques leads to the limited information of microbial communities regarding their composition and utilities. Identification of uncultured organisms can be done by molecular-based techniques in a culture-free manner. Metatranscriptomics is the study of the function and activity of the complete set of transcripts (RNA sequences) of microbiome from environmental samples. The functional study of microbial communities can be done by next-generation sequencing (NGS) techniques. This will provide the whole metatranscriptomics data including biogeochemical cycles, pathogenic processes, metabolism, and development. The advancement of high-performance bioinformatics tools helps to improve our understanding regarding microbial communities. In this present study, a review is given on microbial community transcriptomes using computational metatranscriptomics approaches. The different available bioinformatics tools will also be discussed here for computational analysis of the data to study the evolutionary processes in a specified pool of microorganisms.

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Keywords

Metatranscriptomics · Bioinformatics tools · Computational analysis · NGS

27.1 Introduction

Over the past few decades, explosive growth in the biological information generated had been noted by the scientific community. Modern technologies have pushed the study of plant biology to a higher level than before as its significant role not only for humans but also for other living organisms. Rapid technological development helps in the analysis of biomolecules. The advent of next-generation sequencing methods enabled the analysis of complex transcriptome, proteome, and metabolome data (Baldrian and López-Mondéjar 2014). At the level of transcription, metatranscriptomics means the cataloguing of all species of transcripts and their annotation. It also analyzes the transcriptional structure of genes, splicing patterns, and other maturation processes along with quantifying the change in expression level of each transcript under environmental variations. They depend on technical advancement in DNA fragment arraying as well as next-generation sequencing for RNA transcriptomics or "RNA-seq." It is an extremely powerful tool to unravel genetic expression of complete genomes. For this, suitable bioinformatics software and statistical tools were developed to analyze huge quantity of raw new type of data. Using RNA sequencing, the expressed transcripts can be documented within a microbiome. In certain environmental conditions, it will provide a closer gaze of active genes. Current advancements of mass spectrometry for proteomics also offer information of proteins which are actively expressed under such condition. Relatively low expressed genes and their entire metatranscriptome can be detected by RNA sequencing. Further, it can be annotated as well as mapped to different metabolic pathways.

Microarray technologies were frequently explored to analyze the expression levels of known transcripts before the development of high-throughput sequencing (Parro et al. 2007). The advancement of next-generation sequencing technologies for RNA makes it possible to analyze known transcript targets. This technique can also discover the transcripts which were previously unknown. Several platforms like Illumina, ion semiconductor, and nanopore sequencing are available now, but all utilize nanotechnology. Every system has its own strengths as well as weaknesses, such as different sequence read lengths and error rates (Loman et al. 2012). But each one has been put a step to investigate the gene workings of the microbial genome. These will bring manifold improvements in microbiome analysis techniques (Bokulich and Mills 2012).

The current sequencing tools directly depend on the analysis of DNA. First of all the marker-genes are amplified from mixed genomic DNA, and amplicon is directly sequenced. Similar process can also be useful in case of RNA (reverse transcribed to cDNA) to profile the actively transcribing genes. The amplicon sequencing provides the taxonomic information of lower resolution as compared to metagenome

sequencing. The current technology discussed whose results are more satisfactory for more exploration of miocrobial genome.

Metagenome sequencing is the tool, also known as shotgun metagenome sequencing, in which a pure DNA was taken from an entire microbial community subjected to develop a sequencing library for complete sequencing. This will result in reconstruction of individual genome fragments for analysis and comparison. Metagenomics sequencing provides the functional potential of microbiome. Additionally metagenomic analysis has also been utilized for identifying novel microorganisms and their enzymatic functions with related genes that may be useful for bioremediation (Russell et al. 2011; Lovley 2003). It is also helpful in identifying the host-pathogens interactions (Vazquez-Castellanos et al. 2014) and exploration of new therapeutic strategies for various diseases in human being (Suez et al. 2014). However, metagenomics approaches have limited role in identifying microbial activity as compared to gene expression.

The shotgun method of RNA sequencing for metatranscriptomic gives the access to the metatranscriptome of the microbial genome. This will allow the profiling of complete genome of the microbiome under different conditions. Further, the metatranscriptome sequencing will aid to identify the RNA-based regulation and expressions of human microbiome (He et al. 2010). The result obtained is a mixed expression profile of a given sample. This characterizes the expression behavior entire communities under variable conditions. Metatranscriptomics deals with those sets of genes which transcribe and exhibit activity to a given environment at a given time (Moran 2009; Chao-Rong and Zhang 2011). Hence, the functional metatranscriptomic is a powerful approach that characterized the genes expressed by diverse microorganisms. This method has a significant application in biotechnology to explore new genes of interest for bioremediation and other bio-industries. This technique can play a significant role in the degradation of organic matter and also make it convenient to characterize the novel genes adapted under various stress conditions. Functional analysis of metatranscriptomics can be utilized for the analysis of mRNA. That can provide regulation and expression profiles information of the entire microbiome. The cDNA of these mRNAs can be cloned in appropriate expression vectors and permit expression of the cloned genes particularly in eukaryotes (Yadav et al. 2014). In this chapter, both novel findings and shortcomings are discussed. Several available tools and workflows (Fig. 27.1) specifically designed to analyze metatranscriptomic datasets are given below.

27.2 Process of Metatranscripomtic Analysis

The metatranscriptomic analysis involves the isolation of the total RNA including mRNA, microRNA, and lincRNA, from the microbiome which is to be sequenced. The RNA is then fragmented to smaller pieces and subjected to cDNA synthesis using random hexamers or oligo (dT) primers and reverse transcriptase enzyme. Thus a metagenomic library was constructed from that. The 5′ and/or 3′ ends of the cDNA are repaired, and adapters are ligated, followed by library cleanup,

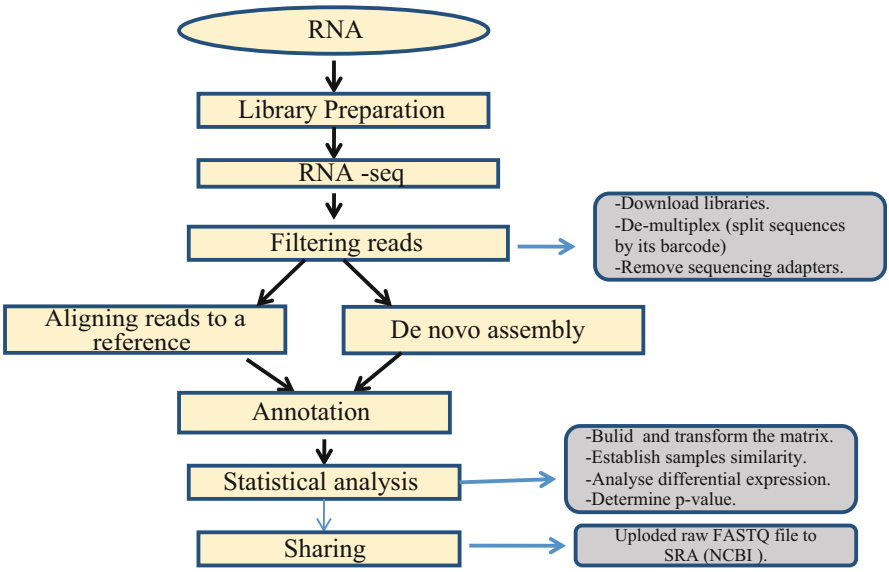

Fig. 27.1 Workflow of metatranscriptomic sequencing with bioinformatics

amplification, and quantification. Finally the library is sequenced. The produced cDNA provide the biases for transcripts quantification (Liu and Graber 2006). Semi-direct method of RNA sequencing has been created and studied without the synthesis of cDNA (Ozsolak and Milos 2011; Ozsolak et al. 2009: Hickman et al. 2013). Still the large-scale applications of metatranscriptomics have some technical issues:

1. Collection as well as preservation methods of the RNA of the given sample.
2. Sufficient quantity of high-quality RNA isolation is one of the major limitations.
3. Average shelf life of mRNA is one of the major limitations in the analysis of quick responses for limited time toward to environmental variables.
4. The insufficient transcriptome database is another limitation.
5. Currently available rRNA purification methods are not efficient to remove host RNA contamination.
6. The poly-A RNA selection kits are not feasible to capture the mRNA population in prokaryotes.

27.2.1 Role of Bioinformatics in Metatranscriptomics

The application of bioinformatic tools in metatranscriptomics analysis facilitated the visualization of host-microbiome interactions, with the focus on primary metabolites (Kurtz et al. 2016; Purroy and Wu 2018). NGS coupled with numerous bioinformatics tools generates a spectacular technological progress in metagenomics and metatranscriptomics. These techniques are giving insight into taxonomic profiles

and genomic components of microbial communities. KEGG orthology and enzyme codes on the iPath 3 platform are used to derive identifiers for the visualization of the shared enzymatic modules (Darzi et al. 2018). At this platform, EC and KO identify overlapping metabolic functions of host and microbiome using metabolic maps of general metabolic pathways, secondary metabolism, and bacterial metabolism. Metatranscriptomic studies shows that microorganisms are able to develop complex tropic networks for communication through chemical signals known as quorum sensing (Estrada-Pena et al. 2016; Ezenwa et al. 2012). However, this process is not only shown by microorganisms, but other organisms also exhibit such signaling (Killian et al. 2016; Valle-Gough et al. 2018; Frias-López et al. 2010).

The workflow designed for metatranscriptomic analysis has these steps:

1. Preprocess
2. Extract and analyze the community structure (taxonomic information)
3. Extract and analyze the community functions (functional information)
4. Combine taxonomic and functional information to offer insights into taxonomic contribution to a function or functions expressed by a particular taxonomy

27.2.1.1 Preprocess

The data obtained and analyzed using typical bioinformatics software for metatranscriptomic experiment is almost similar to that in metagenomics. There are two strategies for this: (1) mapping sequences in reference to genes and genomes (2) de novo assembly of new transcriptomes. Mapping sequence strategy facilitated the RNA sequences of diverse genomes, or pathways make it easy to identify the taxonomical classification of expressed genes of microbiome, and their function such as mapping of metatranscriptomic sequences to KEGG database (Kanehisa and Goto 2000), the pathways of the expressed genes during healthy, and diseased conditions are obtained in the microbiome (Jorth et al. 2014). Bioinformatic programs used in metagenomics are ABySS (Birol et al. 2009), SOAPdenovo (Li et al. 2009), and Velvet-Oases (Schulz et al. 2012). These are effectively applied for the metatranscriptome assembly of microbiomes (Shi et al. 2011; Robertson et al. 2010; Garg et al. 2010; Ness et al. 2011). However, the Trinity program is efficient in recovering full-length transcripts and their isoforms especially for de novo transcriptome assembly. Nowadays, this bioinformatics tool is frequently used for de novo transcriptome analysis (Grabherr et al. 2011; Ghaffari et al. 2014; Luria et al. 2014).

27.2.1.2 Transcript Taxonomy

Taxonomic profiling coupled with metagenomic data will lead to the use of similar tools to understand actively expressing RNA in organisms. Read-based taxonomy classification tools include KRAKEN (Wood and Salzberg 2014), MetaPhlan2 (Truong et al. 2015), GOTTCHA (Freitas et al. 2015), etc. These all are successfully utilized and explored in the metatranscriptomics study (Neves et al. 2017). All these tools work on nucleotide matches of short reads. But their application is restricted to closely related members of microbiomes in existing sequence databases.

27.2.1.3 Functional Annotation

The main goal of metatranscriptomics is to assess the functional activity of a microbiome. The characterization of expressed transcripts explains the function of transcripts which is a proxy of actual phenotype. Functional annotation of genes can be conducted using contigs or reads, whereas functional profilers based on reads are UProC (Meinicke 2015), HMM-GRASPx (Zhong et al. 2016), and MetaCLADE (Ugarte et al. 2018). They require very specific databases and accepted predicted open reading frames as input, obtained from FragGeneScan (Rho et al. 2010). Annotation is similar for assembled transcripts of genomes and metagenomes. Prodigal (Hyatt et al. 2010) and FragGeneScan (Rho et al. 2010) can be used to identify genes followed by functional analysis. For this, similarity searching tools DIAMOND (Buchfink et al. 2015) can be used which help to search functional databases such as KEGG (Kanehisa and Goto 2000), NCBI RefSeq (O'leary et al. 2016), UniProt (Uniprot 2019), etc. Other bioinformatics software for gene finding and annotation are Prokka (Seemann 2014), MG-RAST (Wilke et al. 2016), and EDGE Bioinformatics (Li et al. 2017). They all can combine a similarity search against different databases and can perform couple assembly, gene calling, and annotation.

27.2.1.4 Differential Expression Analyses

Downstream analysis can solve numerous questions, viz., the detection of differentially expressed genes, splice isoforms, and identification of up- and downregulated pathways or single nucleotide variant (SNV) enrichments. Differential gene expression analysis tools work as per gene read counts from different RNA sequence samples. A number of different tools were developed like EdgeR (Robinson et al. 2010), DeSeq2 (Love et al. 2014), and limma (Ritchie et al. 2015) that are frequently used for differential gene expression studies of metatranscriptomics. These tools are very informative and can identify genes which are differentially expressed statistically in various samples. Similar tools such as Generally Applicable Gene-Set/Pathway Analysis (GAGE) can also be utilized to reveal various pathways (Luo et al. 2009).

27.3 Workflow Pipelines for Metatranscriptomic Analysis

Today, metagenomic analysis will provide the access of microbial community profiling. The analysis of metatranscriptomic can predict the profiling of gene expression and regulatory mechanisms that will contribute extensively in drug discovery for human fitness (Bashiardes et al. 2016). In the past few years, several efficient Web servers have been developed for metatranscriptomic analysis (Meyer et al. 2008; Martinez et al. 2016; Westreich et al. 2018; Abubucker et al. 2012; Leimena et al. 2013).

Bioinformatic workflows can couple together multiple individual tools which take raw sequencing reads. The process output results can be used for characterizing

functional genes or differentially expressed transcripts. There are four bioinformatics tools, i.e., MetaTrans, HUMAnN2, Leimena-2013, and SAMSA.

27.3.1 MetaTrans

It is developed to analyze the RNA sequence for taxonomic and gene expression with quality assessment. It can also be helpful in sorting of RNA into mRNA/non-mRNA. It is also associated with database of differential gene expression (Martinez et al. 2016).

27.3.2 HUMAnN2

This pipeline is equipped with functional profile of community and can be used for mapping of microbial pathways profiling. It is developed to study the metagenomics along with metatranscriptomics. The databases like MetaPhlAn2, ChocoPhl Anpangenome database, and DIAMOND can accelerate the functional profiling and translated searches (Buchfink et al. 2015).

27.3.3 Leimena-2013

This tool is developed for functional annotation and mapping based on RNA sequence data in reference to human small intestine macrobiotic data. Leimena-2013 uses SortMeRNA and BLASTN for removal and alignment of reads in tRNA, whereas it uses MegaBLAST for reads in mRNA for assignment as well as to predict the phylogenetic origin (Leimena et al. 2013).

27.3.4 Annotation of Metatranscriptomes by Sequence Analysis (SAMSA)

SAMSA is a comprehensive pipeline for metatranscriptomics analysis. It includes four phases for gut microbiome data analysis, i.e., preprocessing phase, annotation phase, aggregation phase, and analysis phase (Westreich et al. 2018). When SAMSA works with MG-RAST together, it will help in analyzing the expression activity within microbial communities.

27.4 Metaservers for Metagenomic and Metatranscriptomics Analysis

The bioinformatics tools discussed here can be complicated to start up any bioinformatic analysis. Therefore, other open-source options can be used such as the metaservers to analyze the data in a graphical pattern. Metaservers include a series of programs and applications of Web service providers (Table 27.1). Mostly used metaservers are TRUFA, Galaxy, and MG-RAST (Komobis et al. 2015; Keegan et al. 2016; Afgan et al. 2018).

Galaxy The Galaxy project is an advanced bioinformatics tool which is easily accessible without prior training. Galaxy makes the data-intensive research more accessible, transparent, and reproducible. The user can do computational analyses and track all the details for reuse (Afgan et al. 2018). This is a mutual approach that provides a number of bioinformatic tools and software. Some of their examples are sequence editors, FASTQC sequences, sequence mapping tool (Bowtie), data grouping and assembly (Trinity), metagenomic analysis programs (Kraken), and transcript quantification. Galaxy itself contains the series of servers which offers different programs for prediction of functional metagenome by PICRUST (Huttentowe and Langille Lab) and for functional annotation of transcriptomes (ANASTASIA) (Grüning et al. 2017).

CHIPSTER It is a high-throughput customer-friendly data analysis software (contains more than 360 tools). Its graphical interpretation enables biologists to access a powerful collection of data analysis to visualize data interactively. Users

Table 27.1 Some websites and resources of metaservers

S. no	Name	Web address
1	Galaxy	https://usegalaxy.org
2	Galaxy-RNA-Workbench	https://github.com/bgruening/galaxy-rna-workbench
3	FastQC	http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
4	Bowtie2	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
5	Salmon	https://combine-lab.github.io/salmon/
6	Kallisto	https://pachterlab.github.io/kallisto/
7	Kraken package	https://ccb.jhu.edu/software/kraken/
8	Chipster	http://chipster.csc.fi/
9	Cutadapt	https://github.com/marcelm/cutadapt
10	SILVA	https://www.arb-silva.de/
11	Leimena-2013	https://www.ncbi.nlm.nih.gov/pubmed/23915218
12	MetaTrans	http://www.metatrans.org/
13	SAMSA	https://github.com/transcript/SAMSA
14	HUMAnN2	http://huttenhower.sph.harvard.edu/humann2

can collaborate by sharing analysis and workflows for better results (Kallio et al. 2011).

TRUFA TRUFA is an easy transcriptome analysis program developed by the Institute of Physics, Cantabria (Komobis et al. 2015). It contains several sets of programs solely for metatranscriptomic analysis. The range of programs are assembly of sequences (Trinity), quality control (FASTQC and PRINSEQ), sequences edit (CutAdapt), transcripts quantification (RSEM and eXpress), and functional annotation (BLAST2GO and HMMER).

KNIME Konstanz Information Miner enables easy visual assembly and interactive execution of a data pipeline. It is designed to easily teach, research, and create collaborative platform. The property of this tool enables simple integration of new algorithms and tools, as well as data manipulation or visualization methods in the form of new modules or nodes (De la Garza et al. 2016).

MG-RAST Metagenomic Rapid Annotation based on Subsystems Technology is an open platform which can analyze sequences obtained from next-generation sequencing systems (Keegan et al. 2016). MG-RAST works on the basis of quality control of the sequences, transcript isoform detection, and functional assignment. The results of the function such as KEEG, SEED, COG, NOG, and taxonomy including ITS, RDP, SILVA, and Greengenes can be analyzed by this server database. The MG-RAST tools can export data in the form of table, in FASTA format, or in BIOM-type matrix.

BLAST2GO It is a sequence annotator and works on BLAST algorithms. It performs its all searches through NCBI open assess network. It can accelerate the annotation by using taxonomic filters. It allows searches of inter protein domains (InterProScan), classification of proteins based on gene orthology database, function enrichment analysis (Fisher's exact test), analysis of the metabolic modules (KEGG), etc. CLOUD-BLAST of BLAST2GO PRO version can perform several annotations at the same time. It can also perform differential expression of transcripts (Conesa et al. 2005).

27.5 Conclusion

High-throughput transcriptomics techniques have demonstrated their impressive analytical potential for gene expression studies. Recently, there is the need of more evidences to fully understand the mechanisms behind microbial communities. More sophisticated analyses are required which include whole metabolite and metaproteomic data analysis. This will help to understand the various biological processes involved in the microbiome. The present chapter has the brief explanation of various metatranscriptomics approaches and methods were discussed which can help to study functional genomics of microbes. Various bioinformatics tools are used

to study functional diversity and identification of novel genes which are involved in various biological processes of the microbiome.

References

Abubucker S, Segata N, Gol J, Schubert AM, Izard J, Cantarel BL, Rodriguez-Mueller B, Zucker J, Thiagarajan M, Henrissat B, White O (2012) Metabolic reconstruction for metagenomic data and its application to the human microbiome. PLoS Comput Biol 8:e1002358

Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Čech M (2018) The galaxy platform for accessible, reproducible and collaborative biomedical analyses. Nucleic Acids Res 46(1):537–W544

Baldrian P, López-Mondéjar R (2014) Microbial genomics, transcriptomics and proteomics: new discoveries in decomposition research using complementary methods. Appl Microbiol Biotechnol 98:1531–1537

Bashiardes S, Zilberman-Schapira G, Elinav E (2016) Use of metatranscriptomics in microbiome research. Bioinform Biol Insights 10:19–25

Birol I, Jackman SD, Nielsen CB, Qian JQ, Varhol R, Stazyk G (2009) De novo transcriptome assembly with ABySS. Bioinformatics 25(21):2872–2877

Bokulich NA, Mills DA (2012) Next-generation approaches to the microbial ecology of food fermentations. BMB Rep 45:377–389

Buchfink B, Xie C, Huson D (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60

Chao-Rong GE, Zhang QC (2011) Microbial community structure and enzyme activities in a sequence of copper-polluted soils. Pedosphere 21:164–169

Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21(18):3674–3676

Darzi Y, Letunic I, Bork P, Yamada T (2018) iPath3.0: interactive pathways explorer v3. Nucleic Acids Res 46(1):510–513

De la Garza L, Veit J, Szolek A, Röttig M (2016) From the desktop to the grid: scalable bioinformatics via workflow conversion. BMC Bioinform 17:127

Estrada-Pena A, Sprong H, Cabezas-Cruz A, De La Fuente J, Ramo A, Coipan EC (2016) Nested coevolutionary networks shape the ecological relationships of ticks, hosts, and the Lyme disease bacteria of the Borreliaburgdorferi (s.l.) complex. Parasites Vectors 9(517):1–15

Ezenwa VO, Gerardo NM, Inouye DW, Medina M, Xavier JB (2012) Animal behavior and microbiome. Science 338(6104):198–199

Freitas TAK, Li P-E, Scholz MB, Chain PSG (2015) Accurate read-based metagenome characterization using a hierarchical suite of unique signatures. Nucleic Acids Res 43:e69

Frias-López J, Shi Y, Tyson GW, Coleman ML, Schuster SC, Crisholm SW (2010) Microbial community gene expression in ocean surface waters. Proc Natl Acad Sci U S A 105 (10):3805–3810

Garg R, Patel RK, Tyagi AK, Jain M (2010) De novo assembly of chickpea transcriptome using short reads for gene discovery and marker identification. DNA Res 18(1):53–63

Ghaffari N, Sanchez-Flores A, Doan R, Garcia-Orozco KD, Chen PL, Ochoa-Leyva A (2014) Novel transcriptome assembly and improved annotation of the whiteleg shrimp (Litopenaeusvannamei), adominant crustacean in global seafood mariculture. Sci Rep 4:7081

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29 (7):644–652

Grüning BA, Fallmann J, Yusuf D, Will S (2017) The RNA workbench: best practices for RNA and high-throughput sequencing bioinformatics in galaxy. Nucleic Acids Res 45(W1):W560–W566

He S, Kunin V, Haynes M, Martin HG, Ivanova N, Rohwer F (2010) Metatranscriptomic array analysis of 'CandidatusAccumulibacterphosphatis'—enriched enhanced biological phosphorus removal sludge. Environ Microbiol 12(5):1205–1217

Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang LC, Means TK (2013) The microglial sensome revealed by direct RNA sequencing. Nat Neurosci 16(12):1896–1905

Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinform 11:119

Jorth P, Ciulla DM, Huang K, Haas BJ, Izard J, Levin JZ (2014) Metatranscriptomics of the human oral microbiome during health and disease. MBio 5(2):e01012–e01014

Kallio MA, Tuimala JT, Hupponen T, Klemelä P (2011) Chipster: user-friendly analysis software for microarray and other high-throughput data. BMC Genom 12:507

Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30

Keegan KP, Glass EM, Meyer F (2016) MG-RAST, a metagenomics service for analysis of microbial community structure and function. Methods Mol Biol 1399:207–233

Killian M, Chapple ILC, Hanning M, Marsh PD, Meuric V, Pedersen AML (2016) The oral microbiome- an update for oral healthcare professionals. Br Dent J 221(10):657–666

Komobis E, Cabellos L, Aguilar F, Frias-López C, Rpzas J, Marco ZR (2015) TRUFA: a user-friendly web server for de novo RNA-seq analysis using cluster computing. Evol Bioinform 11:97–104

Kurtz J, Schulenburg H, Reusch TBH (2016) Host–parasite coevolution—rapid reciprocal adaptation and its genetic basis. Zoology 119:241–243

Leimena MM, Ramiro-Garcia J, Davids M, van den Bogert B, Smidt H, Smid EJ, Boekhorst J, Zoetendal EG, Schaap PJ, Kleerebezem M (2013) A comprehensive metatranscriptome analysis pipeline and its validation using human small intestine microbiota datasets. BMC Genom 14:530

Li R, Yu C, Li Y, Lam TW, Yiu SM, Kristiansen K (2009) SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics 25(15):1966–1967

Li P-E, Lo C-C, Anderson JJ, Davenport KW, Bishop-Lilly KA, Xu Y (2017) Enabling the democratization of the genomics revolution with a fully integrated web-based bioinformatics platform. Nucleic Acids Res 45:67–80

Liu D, Graber JH (2006) Quantitative comparison of EST libraries requires compensation for systematic biases in cDNA generation. BMC Bioinform 7:77

Loman NJ, Misra RV, Dallman TJ (2012) Performance comparison of benchtop high-throughput sequencing platforms. Nat Biotechnol 30:434–439

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550

Lovley DR (2003) Cleaning up with genomics: applying molecular biology to bioremediation. Nat Rev Microbiol 1(1):35–44

Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ (2009) GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinform 10:161

Luria N, Sela N, Yaari M, Feygenberg O, Kobiler I, Lers A (2014) De-novo assembly of mango fruit peel transcriptome reveals mechanisms of mango response to hot water treatment. BMC Genom 15:957

Martinez X, Pozuelo M, Pascal V, Campos D, Gut I, Gut M (2016) MetaTrans: an open-source pipeline for metatranscriptomics. Sci Rep 6:26447

Meinicke P (2015) UProC: tools for ultra-fast protein domain classification. Bioinformatics 31:1382–1388

Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A, Wilkening J, Edwards RA (2008) The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinform 9:386. https://doi.org/10.1186/1471-2105-9-386

Moran MA (2009) Metatranscriptomics: eavesdropping on complex microbial communities-large-scale sequencing of mRNAs retrieved from natural communities provides insights into microbial activities and how they are regulated. Microbe 4:329
Ness RW, Siol M, Barrett SC (2011) De novo sequence assembly and characterization of the floral transcriptome in cross- and self-fertilizing plants. BMC Genom 12:298
Neves ALA, Li F, Ghoshal B, Mcallister T, Guan LL (2017) Enhancing the resolution of rumen microbial classification from metatranscriptomic data using Kraken and Mothur. Front Microbiol 8:2445
O'leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, Mcveigh R (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745
Ozsolak F, Milos PM (2011) Single-molecule direct RNA sequencing without cDNA synthesis. Wiley Interdiscip Rev RNA 2(4):565–570
Ozsolak F, Platt AR, Jones DR, Reifenberger JG, Sass LE, McInerney P (2009) Direct RNA sequencing. Nature 461(7265):814–818
Parro V, Moreno-Paz M, González-Toril E (2007) Analysis of environmental transcriptomes by DNA microarrays. Environ Microbiol 9:453–464
Purroy N, Wu CJ (2018) Co-evolution of leukemia and host immune cells in chronic lymphocytic leukemia (CLL). Cold Spring Harb Perspect Med 7(4):1–28
Rho M, Tang H, Ye Y (2010) FragGeneScan: predicting genes in short and error-prone reads. Nucleic Acids Res 38:e191
Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W (2015) Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43:e47
Robertson G, Schein J, Chiu R, Corbett R, Field M, Jackman SD (2010) De novo assembly and analysis of RNA-seq data. Nat Methods 7(11):909–912
Robinson MD, Mccarthy DJ, Smyth GK (2010) edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140
Russell JR, Huang J, Anand P, Kucera K, Sandoval AG, Dantzler KW (2011) Biodegradation of polyester polyurethane by endophytic fungi. Appl Environ Microbiol 77(17):6076–6084
Schulz MH, Zerbino DR, Vingron M, Birney E (2012) Oases: robust de novo RNA-seq assembly across the dynamic range of expression levels. Bioinformatics 28(8):1086–1092
Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069
Shi CY, Yang H, Wei CL, Yu O, Zhang ZZ, Jiang CJ (2011) Deep sequencing of the Camellia sinensistranscriptome revealed candidate genes for major metabolic pathways of tea-specific compounds. BMC Genom 12:131
Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O (2014) Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature 514(7521):181–186
Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E (2015) MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods 12:902–903
Ugarte A, Vicedomini R, Bernardes J, Carbone A (2018) A multi-source domain annotation pipeline for quantitative metagenomic and metatranscriptomic functional profiling. Microbiome 6:149
Uniprot C (2019) UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47:D506–D515
Valle-Gough RE, Apodaca-Hernández JE, Rodriguez-Dorantes M, Arena-Ortiz ML (2018) Metatrancriptomic analysis from the Hepatopancreas of adult white leg shrimp (Litopenaeusvannamei). Symbiosis 76(1):51–62
Vazquez-Castellanos JF, Garcia-Lopez R, Perez-Brocal V, Pignatelli M, Moya A (2014) Comparison of different assembly and annotation tools on analysis of simulated viral metagenomic communities in the gut. BMC Genom 15:37
Westreich ST, Treiber ML, Mills DA, Korf I, Lemay DG (2018) SAMSA2: a standalone metatranscriptome analysis pipeline. BMC Bioinform 19:175

Wilke A, Bischof J, Gerlach W, Glass E, Harrison T, Keegan KP (2016) The MGRAST metagenomics database and portal in 2015. Nucleic Acids Res 44:D590–D594

Wood DE, Salzberg SL (2014) Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:R46

Yadav RK, Barbi F, Ziller A, Luis P, Marmeisse R, Reddy MS, Fraissinet-Tachet L (2014) Construction of sized eukaryotic cDNA libraries using low input of total environmental metatranscriptomic RNA. BMC Biotechnol 14:80

Zhong C, Edlund A, Yang Y, Mclean JS, Yooseph S (2016) Metagenome and metatranscriptome analyses using protein family profiles. PLoS Comput Biol 12:e1004991

Microbes and Soil Health for Sustainable Crop Production

28

Nikita Nehal, Utkarsh Singh Rathore, and Nitish Sharma

Abstract

In the last few decades increased population and climatic changes are the most severe challenge to our farmers that demands more crop productivity. To meet this challenge, they are using limitless inorganic fertilizers and chemicals in their field to enhance their crop production and stress management that caused a big threat to soil degradation and also puts our fertile soils and lives of humans in danger as these chemicals are very harmful to soil and animal health. Recently, researchers have found plant growth-promoting bacteria (PGPB) as one of the most promising ways to meet the needs of increasing population in an effective manner with increased crop growth and productivity with no harmful impact on soil, plants, and animals. Rhizospheric microbes not only help in increased crop production but it also enhances soil fertility as well as helps the plant in mitigating the various biotic and abiotic stresses. Thereby, exploring the beneficial properties of these microorganisms we may improve crop growth and productivity in a sustainable way.

Keywords

PGPR · Trichoderma · Consortia · Soil health · Sustainability · Stress · Plant–microbe interaction

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28.1 Introduction

Soil is the most significant constituent for maintaining ecosystem equilibrium on the earth. It is a crucial non-renewable reserve and is formed by chemical and biological weathering of underlying rocks. The growing population demands advanced food production, for which the use of chemical inputs has become mandatory. Haphazard and non-judicious application of chemicals is harmful to animals as well as soil health. The use of environment-friendly and potentially cost-effective microbial bio-fertilizer could be an improved solution (Rathore et al. 2018a, b). The relation of soil fertility and microorganisms for expression of better crop health, production, and excellence is well known. Therefore, soil health and its protection in agricultural production is important. According to Doran et al. (1994), soil quality (health) is the capability of a soil to task within ecosystem and land-use boundaries, to maximize biological productivity, maintain environmental quality, and promote plant and animal well-being. In agriculture, preservation of ecosystem equilibrium is based upon the dynamics of microorganisms. In soil, these microorganisms live in rhizosphere and comprise a multifaceted organization of endophytes, saprophytes, and actinomycetes, both harmful and beneficial ones. In agricultural ecosystem, connections between plant and microbes are important areas of attention and form the foundation for all ecosystems (Bélanger and Avis 2002). In natural system, soil is the home of abundant microbes, comprising of beneficial and harmful ones. Microflora present in soil (especially, in rhizosphere) proved their potential in the control of soil-borne diseases by the procedure of biocontrol and microbes engaged in this technique are referred as bioagents or biocontrol agents. Rhizobacteria also play a crucial role to improve soil configuration and in the production of phosphatase, â-gluconase, dehydrogenase and antibiotics, solubilization of mineral phosphates and additional nutrients, as well as stabilization of soil aggregates (Miller and Jastrow 2000). This actuality being known since long (Mitchell 1973) has been incorporated with novel technologies and management systems of various pests as "integrated pest management" (Antoun and Prévost 2005).

28.2 Soil and Crop Growth

A plant cannot complete its life cycle in the absence of certain mineral elements which are called as essential mineral elements (Arnon and Stout 1939), and soil acts as a source for these essential mineral elements needed by plants for their growth and development. These essential mineral elements are classified on the basis of amount of requirement by the plants as presented in Table 28.1. Plants get carbon, hydrogen, and oxygen from atmosphere and take up rest all mineral elements from soil. Nitrogen (N), phosphorus (P), and potassium (K) together they can be called as trio as NPK along with this they also require calcium, magnesium, and sulfur in large quantities and they all are called as macronutrients or major nutrients. Plants also require some minerals in minute quantities and they are called as micronutrients such as iron, manganese, zinc, copper, etc. (Table 28.1). However, the source of these

Table 28.1 Classification of essential plant elements/nutrients

Essential plant elements/ nutrients	Classified essential plant elements/nutrients				Symbol	Primary form
	Non-mineral elements/nutrient			Carbon	C	CO_2 (g)
				Hydrogen	H	H_2O (l), H^+
				Oxygen	O	H_2O (l), O_2(g), CO_2 (g)
	Mineral elements/ nutrients	Macroelements/ macronutrients	Primary elements/ macronutrients	Nitrogen	N	NH_4^+, NO_3^-
				Phosphorus	P	HPO_4^{2-}, $H_2PO_4^-$
				Potassium	K	K^+
			Secondary elements/ macronutrients	Calcium	Ca	Ca^{2+}
				Magnesium	Mg	Mg^{2+}
				Sulfur	S	SO_4^{2-}
		Microelements/macronutrients		Iron	Fe	Fe^{3+}, Fe^{2+}
				Manganese	Mn	Mn^{2+}
				Zinc	Zn	Zn^{2+}
				Copper	Cu	Cu^+, Cu^{2+}
				Boron	B	$B(OH)_3/H_3BO_3$
				Molybdenum	Mo	MoO_4^{2-}
				Chlorine	Cl	Cl^-
				Nickel	Ni	Ni^{2+}

essential mineral elements is soil and availability of these mineral elements in soil is influenced by the microbiota present in the rhizospheric region. Therefore, to enhance crop growth and productivity it is of prime importance to study about rhizospheric microbiota.

28.3 Rhizosphere and Its Microbiota

Rhizosphere and its microbiota have been a striking area of research, wherein rhizospheric microbes exist in the surrounding region of the plant roots of a variety of crops has been center of importance. The gained curiosity towards these microbes is not only for its latent to mineralize nutrients for plants, other than this they also produce plant growth hormones and augment the soil with nitrogen content, thus are being recommended as biofertilizers. Beneficial effects on growth, yield, and biomass of plants have been reported for several bacterial and fungal species and strains isolated from rhizosphere and phyllosphere. Endophytic bacteria capable of colonizing tissues have also been detected for promotion of growth of host plant. *Rhizobium, Azotobacter, Acetobacter, Bacillus, Azospirillum, Enterobacter, Gluconacetobacter, Paenibacillus*, *Pseudomonas,* and various blue-green algae are representatives of plant growth-promoting rhizobacteria (PGPR). As these bacterial isolates are from plants' natural habitat, thus application of such PGPRs is desirable alternative to chemical fertilizers that ultimately impede the environment from unwanted chemical moieties resulting in healthy soil with abundant beneficial biomass.

Besides growth promotion certain microbes are probable biocontrol agents skilled at antagonizing several phytopathogens and inducing systemic resistance in crop plants (Amaresan et al. 2020). Biocontrol through microbial agents is generally the determined exploitation of resident living organisms or introduced microbial formulations. Quite a few strains of bacteria and fungal genera counting *Pseudomonas*, *Bacillus*, *Streptomyces*, *Agrobacterium*, *Beauveria Serratia*, *Trichoderma*, *Metarrhizium,* and non-pathogenic *Fusarium*, respectively, have proven their potential as biocontrol agents. Maintaining soil health must be a principal aim to attain, as it acts as the first line of defense. Various activities take place in the rhizosphere and its surroundings; unboxing and understanding these mechanisms can provide a clear picture that how these interactions affect the plant and the pathogens.

Single strain of growth-promoting bacteria or fungi may not have a desirable outcome at field level, merging different growth-promoting strains of potential rhizobacteria bacteria with antagonistic properties might have a noteworthy change in the preceding scenario.

However, the effectiveness of the action of plant growth-promoting bacteria and other advantageous microbes depends on plant species, circumstances of their expansion, and other factors. Clear understandings of the nature of microbes in the formulation might reduce labor inputs in selection of bacterial species, development of effective technologies and their application in combinations with several microbial genera, species, and strains.

28.4 Soil Microorganisms and Their Types

Microflora (bacteria, fungi, actinomycetes, etc.) comprise up to 75–90% of the soil-living biomass and are the primary decomposers of organic matter. Two major components of microbial biota in soil are as follows:

1. *Disease-inducing microbes:* These fungi or bacteria can cause disease to plants or disgrace the soil quality by interfering with favorable microorganism(s), thereby upsetting plant health. These soil-borne pathogens can survive in soil for countless years. Detection and diagnosis of soil-borne diseases or pathogens are quite complex due to miscellaneous forms of microbes present in soil environment. Fungal pathogens like *Fusarium*, *Rhizoctonia*, *Verticillium*, *Phytophthora*, *Pythium,* and *Sclerotinia* and bacterial pathogens like *Ralstonia solanacearum* (wilt), *Erwinia* sp. (soft rot), and *Streptomyces scabies* (potato scab) cause a great extent of damage to crops.
2. *Biocontrol agents inhabiting in soil:* Soil residing microorganisms (bacteria/fungi) are used productively for controlling diseases. Disease control and improved crop health can be attained through a mixture of activities performed by these soil microbes like siderophores production, hydrocyanic acid (HCN) production, nitrogen assimilation, antibiotic production, hydrolytic enzyme (lipase, chitinase, etc.) production, induced systemic resistance, and systemic acquired resistance. *Bacillus* spp. and *Pseudomonas* spp. serve as exceptional examples of biocontrol agents having important PGPR (plant growth-promoting rhizobacteria) behavior and disease lessening ability.

However, we can also classify the soil microorganisms on the basis of their microbial function which is as follows:

1. *Decomposers:* Those soil microorganisms that breaks down dead or decaying organisms, carry out decomposition, are considered to be the decomposers and can function in the following two ways:
   (a) *Microbial putrefaction (harmful fermentation):* It is the procedure by which facultative heterotrophic microorganisms decay proteins anaerobically, leading to some extent oxidized metabolites with a terrible odor (e.g., mercaptans, indole, and ammonia). These metabolites are usually toxic to plants and animals. For example, *Clostridium* sp.
   (b) *Microbial fermentation:* It is an anaerobic progression by which facultative microorganisms transform complex organic molecules (e.g., carbohydrates) into simple organic compounds that frequently can be absorbed directly by plants. For example, *Saccharomyces* sp.
2. Fixers: Those microorganisms which have the ability of "fixing" atmospheric nitrogen and/or carbon dioxide in which biosynthetic potential of a number of microorganisms is exploited to obtain metabolic energy are considered in this group. For example, *Azotobacter.*

Classification of Soils

1. **On the basis of microbial activity occurring in them**

Since all these activities of soil microbes take place in the soil, soils can also be classified on the basis of the microbial activity occurring in them which are as follows:

(a) *Disease-inducing soil:* These soils are disease causing soil which means that in this soil pathogenic microbes consist of 5%–20% of whole soil microflora. When fresh organic matter is applied in this sort of soil, incomplete oxidized harvest is released, which is hazardous to plants and in turn is with no nuisance attacked by pathogens or insects. Such soils can be amended into diseases suppressive soils by accumulation of inoculum of efficient microorganisms (Parr et al. 1994).

(b) *Disease suppressive soil:* In this type, soil population encompasses microbes that suppress the activity or growth of phytopathogens devoid of any chemical usage (Timmusk 2003). This skill is naturally borne by the soil, which is specific functional position (antagonistic activity) for favorable microbes (Weller et al. 2002). Antagonistic microbes like *Trichoderma*, *Penicillium*, actinomycetes, etc. are the inhabitants of such soils generating sufficient amount of antibiotics, which confine soil-borne pathogens like *Fusarium*, *Pythium*, etc. Plants cultivated in such soils are healthy and seldom infected with diseases or attacked by insects. According to Baker and Cook (1974), the suppressive soils are those "soils in which disease severity or occurrence remains low, in spite of the occurrence of a pathogen and a susceptible host plant".

2. On the Basis of Abundance of Processes that Occur in It

Soils can also be classified as zymogenic or synthetic soil based on the abundance of processes that occur in it.

(a) *Zymogenic soils:* These are the soils in which fermentation/zymosis like process takes place (breakdown of complex substances into simpler ones). Microbes in such soil come up from organic materials like crop residues, animal manures, green manures, and community wastes as well as composts.

(b) *Synthetic soils*: These soils comprise nitrogen and carbon fixers so that they can adapt complex organic matter and change them into carbohydrate, proteins, and amino acid. Photosynthetic bacteria, phycomycetes, and blue-green algae are distinctive examples of such soil microflora.

Soil Microbes and Their Importance

Microorganisms that exist in rhizosphere of soil and participate in active plant growth by inducing root exudation, enhancing the accessibility of nutrients to plant, and releasing growth regulators and assist in soil-borne infection control are referred as rhizospheric microbes. Beneficial rhizospheric microorganisms are broadly classified into two groups (on the basis of their major effects):

(a) *Biocontrol agents*: They circuitously assist with plant productivity all the way by inhibiting the plant pathogen activity. For example, *Trichoderma* spp., *Pseudomonas* spp.

(b) *Plant growth-promoting microorganisms (PGPM)*: They apply direct effect on plant growth promotion, e.g., *Rhizobium* and *Glomus* spp. Bacteria which have the propensity to colonize roots vigorously (Schroth and Hancock 1982) are called as PGPR. In order to enhance microbial population in soil of these capable microbes, they are applied as inoculants which brought to the forefront a new promising technology in the formulation of biocontrol agents. Soil strength and crop form the foundation for the population of rhizobacteria in soil and it fluctuates from species to species (Tilak et al. 2005). For soil-borne pathogens or disease management, rhizospheric microbes come out as a biological weapon that triggers the mechanism of disease reduction through systemic acquired resistance (SAR) and induced systemic resistance (ISR). Favorable microbes or disease suppressive soil microbes are those which are constructive for plant growth and advancement by improving the soil health and quality and providing necessary nutrients and minerals from soils which are normally not available to the plant. For example, *Bacillus*, *Trichoderma*, *Pseudomonas*, *Rhizobium*, etc. Plant growth promotion engrosses siderophore production, antibiosis, phytohormones like indole acetic acid (IAA), solubilization of phosphate, inhibition of plant ethylene synthesis, production of volatile compounds such as HCN, and induction of plant systemic resistance to pathogens (Richardson et al. 2009).

1. *Importance of disease suppressive soil in plant health*: A soil is considered disease suppressive, when in spite of existence of favorable conditions for disease, a pathogen either cannot become established or even if it establishes is unable to produce any disease symptoms or establishes and produces disease for a short time and then declines. The methods by which disease organisms are concealed in these soils including: induced resistance, direct parasitism (one organism consuming another), nutrient competition, and direct inhibition by beneficial organisms.
   (a) Certain suppressive soils while pasteurized (by wet heat at 60 °C for 30 min) drop their suppressiveness. Similarly, additional harsh antimicrobial treatments (gamma radiation or autoclaving) have the same consequence (Stutz et al. 1986).
   (b) An inoculum of 0.1%–10% of a suppressive soil introduced into a conducive soil can establish disease suppression. Incompatible results about transferability have also been reported where the compassion to antimicrobial treatments and transferability tip out that disease control results from the behavior of soil microorganisms that act as antagonists against pathogen (Weller et al. 2002).
   (c) When the pH of a *Fusarium* wilt suppressive soil was lowered from 8 to 6 by the addition of sulfuric acid, carnations were less protected from wilting (Scher and Baker 1980). This hammering of suppressive was caused by a

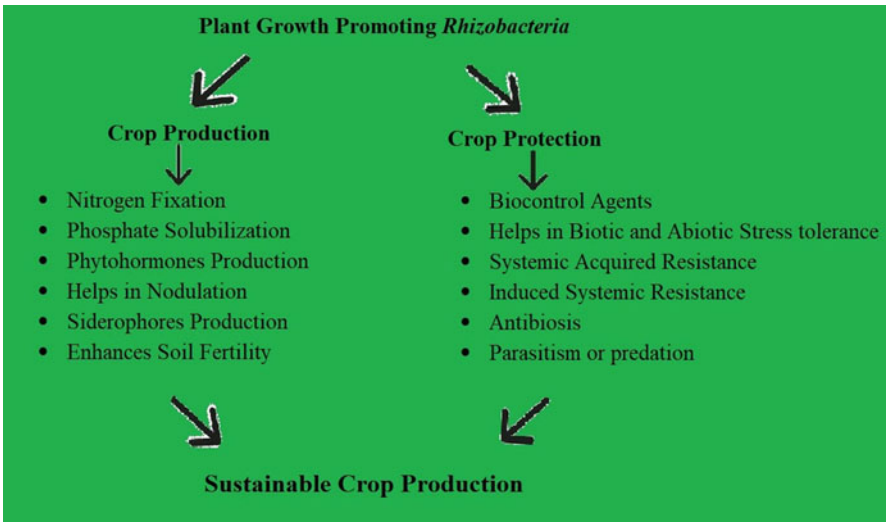

Fig. 28.1 Flow chart representation of role of plant growth-promoting *Rhizobacteria* in sustainable crop production

simple pH change, illustrating the significance of soil environment in disease expansion and control.

(d) Several years of monoculture can persuade disease suppression in some soils. The best studied instance takes all decline of wheat (*Gaeumannomyces graminis*) which has been experiential in soils in the Northwestern United States, the Netherlands, and Australia (Weller et al. 2002).

2. *Characteristic features of soil-inhabiting PGPR*: As depicted in Fig. 28.1 about the role of PGPR in sustainable crop production here are some of the characteristic features of soil-inhabiting PGPR which influence crop growth and production in normal as well as in stressed conditions:

(a) *Siderophore production*:

Pathogen suppression through siderophore is possible through various reasons:

(i) Pathogens are not able to produce their own siderophores.

(ii) Siderophores produced by the antagonists or by other microorganisms are not utilized by pathogens in their immediate environment.

(iii) They construct few siderophores than PGPR or the latter create siderophore that has a higher resemblance for iron than those produced by fungal pathogens.

(iv) They are incapable to utilize antagonist's siderophore, but their siderophores can be used by the antagonist (Bashan and De-Bashan 2005).

(b) *Antibiotic production*: Rhizobacteria contributes to disease control with antibiotic production. There are six classes of antibiotic compounds that are associated with the biocontrol of root diseases, viz. pyrrolnitrin, phenazines,

pyoluteorin, phloroglucinols, cyclic lipopeptides (all of which are diffusible), and HCN (Haas and Défago 2005).

(c) *Hormone production*: Phytohormones which play a very crucial role in plant growth were also produced by PGPR. The most common phytohormone produced by PGPR is indole-3-acetic acid and gibberellins.

(d) *Phosphate solubilization*: Phosphorus holds second significant role after nitrogen in a variety of necessary processes of plant growth and development including cell division, photosynthesis, breakdown of sugar, energy, and nutrient transfer in crop plant. Plants employ phosphate ion in the form of phosphate anions, but phosphate anions are tremendously reactive and get powerless through precipitation with cations present in soil such as Ca^{2+}, Mg^{2+}, Fe^{3+}, and Al^{3+}. Rhizobacteria assist in decomposition of organic compounds and build phosphorus accessible by the action of minerals and acids released by soil bacteria. Phosphorus mineralization is really affected by microbial community, and phosphate solubilizing bacteria such as species of *Bacillus* and *Paenibacillus* have been applied to soils to especially boost the phosphorus status of plants. *Pseudomonas, Bacillus,* and *Rhizobium* are the most influential phosphate solubilizers in cropping system (Rodriguez and Fraga 1999).

(e) *Nitrogen fixation*: Soil often has low nitrogen content although it is a vital nutrient for crop growth and expansion. Soil microorganisms are proficient in nitrogen fixing and help plants to get adequate nitrogen by converting atmospheric essential dinitrogen (N_2) into ammonia (Shiferaw 2004).

(f) *Induced systemic resistance*: Induced systemic resistance (ISR) was clarified and reported incarnation plant in which *Pseudomonas* strain was found efficient against *F. oxysporum* sp. *dianthi* (Van Peer et al. 1991). Induced resistance is the ability of plants to develop and enhance defensive ability when appropriately stimulated (Van Loon 1997). Some pathogenesis-related proteins (PRs) like 1,3-glucanases and chitinases are proficient in hydrolyzing fungal cell walls and insects (Singh et al. 2015a, b). *Pseudomonas* and *Bacillus* spp. are the most accepted rhizobacteria surrounding ISR (Van Wees et al. 2008).

(g) *Root colonization*: The main characteristic of biocontrol is the colonization of rhizosphere soil or external/internal root region by microbes, particularly bacteria (Bahme and Schroth 1987). When it is there or set up in soil as inoculums, it gets disseminated in natural soil, propagates, and stays alive for several days (Scher et al. 1984). In biocontrol method, root colonization is completed in two phases: Firstly, bacteria get attached to rhizosphere and are then transported on the elongating root tip. Secondly, bacteria extend locally, propagate to the limitations of niche by opposing other native microorganisms, and survive. Although root colonization is essential for rhizobacterial activity, sometimes inadequate colonization leads to decreased plant growth-promoting activities (Schippers et al. 1987). It is noteworthy for baseline of capable biocontrol strategies that root colonization engrosses recognition of pathogens by potential antagonists (Barak and Chet 1990).

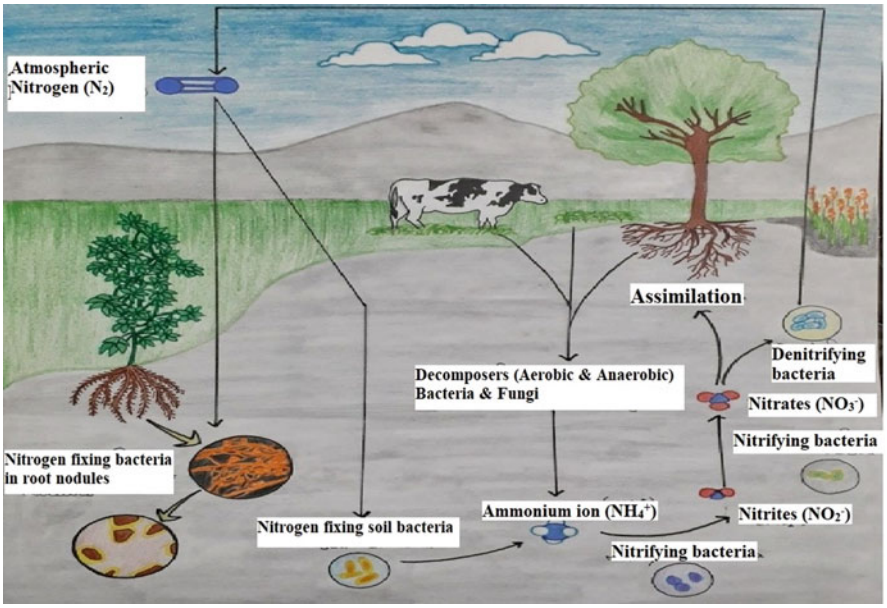

Fig. 28.2 Diagrammatic representation of nitrogen fixation

3. *Sustainable crop production through rhizospheric soil microbes*
   As we discussed above that soil microbes play an important role in maintaining soil health and provide adequate amount of mineral nutrients to the plants which enhances crop growth and productivity and also helps the crop plants to survive under stressful conditions, thereby enhancing crop growth and productivity. Some important functions of soil microbes are as follows:
   (a) *Soil microbes and nitrogen fixation:* For most advantageous pulse's productivity, N is a crucial plant nutrient (Dudeja et al. 2011). Since it is broadly consumed by the majority of plants, the majority of the soils are deficient in it. Moreover, soil N is also vanished due to leakage and volatilization (Brahmaprakash and Sahu 2012). Although air contains 78.09% N, plants cannot make use of it as such. As represented in Fig. 28.2 microorganisms play an important role in nitrogen fixation. The N-fixing bacteria synonymously called diazotrophs are a special type of microorganisms which can reduce atmospheric N into ammonia in the presence of nitrogenase enzyme. Microorganisms and plants assimilate N in their body parts in ammonical form for growth and development. On the basis of their mode of N-fixation, these bacteria are classified into three physiological groups, i.e., symbiotic, associative symbiotic, and free living. In most of the agricultural systems, N is often the most limiting nutrient that dictates crop production. Despite its occurrence in huge quantities in the atmosphere, plants cannot exploit N since it is in an inert form (Brahmaprakash and Sahu 2012). N is made available in the form of fertilizers which are chemical fixation of atmospheric

N through the Haber–Bosch process (Motsara et al. 1995). Dinitrogen is the most stable diatomic molecule known, and two atoms are joined by a very stable triple bond. Very high amount of energy (945 kJ) is required to break this triple bond and therein rests one of the major challenges of dinitrogen fixation (Herridge et al. 2008). The magnitude of BNF in the biosphere is not easy to determine, but approximately it amounts to ~107 Mt/year compared to ~160 Mt/year of man-made N-fixation which is 1.5 times higher than the natural fixation (Galloway et al. 2008). BNF supplies 65% of N consumption in agriculture (Burris and Roberts 1993). All the bacteria fixing atmospheric N catalyze the reaction through nitrogenase enzyme. The nitrogenase enzyme has two components: Mo-Fe protein, called dinitrogenase, and Fe protein, called dinitrogenase reductase. First Mo-Fe protein takes part in reducing dinitrogen to ammonia, and second Fe protein assists Mo-Fe protein by providing electrons for reduction of dinitrogen. The mechanism of N-fixation is the same in all N-fixing bacteria; the reduction of one molecule of dinitrogen requires 16 ATP in in vitro condition and 20–30 ATP under field conditions, as it is less efficient symbiotic nitrogen fixation. Legume–rhizobium symbiosis is an important aspect of symbiotic nitrogen fixation (SNF) which is optimally exploited to benefit agriculture for sustainability. Over a century ago, German scientists, Hellriegel and Wilfarth, experimentally demonstrated the N-fixation in legume nodule by nodule-inducing ferment (Rhizobium): the stage was set for the popularity of the rhizobium inoculation technology world over. In this symbiosis, macro-symbiont is the legume plant, and micro-symbiont is the prokaryotic bacteria (rhizobium).

(b) *Beneficial microbes for pulse production:* Rhizosphere, the narrow zone of soil surrounding plant roots, contains ~1011 microbial cells per gram of root and >30,000 prokaryotic species that in general improve plant growth and productivity (Egamberdieva et al. 2008; Mendes et al. 2013). The collective genome of rhizosphere microbial community is larger as compared to that of plants and is referred to as microbiome (Bulgarelli et al. 2013) whose interactions determine crop health in natural agroecosystem through numerous services being provided to crop plants, viz. nutrient acquisition, OM decomposition, nutrient recycling, water absorption, and pest control (Berg et al. 2013). Rhizosphere microbial communities as an option for synthetic fertilizers have become a subject of importance in sustainable agriculture and biosafety program. The agriculturally helpful microbial populations include plant growth-promoting rhizobacteria (PGPR), mycorrhiza, N-fixing cyanobacteria, plant disease controlling beneficial bacteria, stress-tolerant endophytes, and biodegrading microbes. The term PGPR is currently functional to a wide spectrum of strains that have, in common, the ability to promote plant growth following inoculation onto seeds and subterranean plant parts (Kloepper et al. 1988; Bhowmik and Singh 2004).

Several other examples which prove that rhizospheric microbes enhance sustainable crop production are mentioned in Table 28.2 and depicted in Fig. 28.3.

Table 28.2 Sustainable crop production through rhizospheric microbes

Bacterial strain	Crop	Influence of inoculation on crops	References
Bacillus subtilis and *Bacillus licheniformis*	Soybean (*Glycine max* L.)	Improved photosynthetic parameters and radiation use efficiency during drought stress, thereby increasing crop growth and yield	Mondani et al. (2019)
Endophytes fungus *Paecilomyces formosus* LHL 10 *Bacteria Sphingomonas* sp. LK11	Soybean (*Glycine max* L.)	Improved physiological and photosynthetic parameters and macronutrient uptake as well as modulates the gene expression levels to increase gibberellins levels	Bilal et al. (2018)
Sphingomonas spp. LK11	Soybean (*Glycine max* L.)	Enhance the synthesis of phytohormones and trehalose and thereby improving crop growth and yield under drought stress	Asaf et al. (2017)
Pseudomonas fluorescens	Turmeric (*Curcuma longa*)	Improves the growth and curcumin content in turmeric	Kumar et al. (2016)
Pseudomonas fluorescens	Blackberries (*Rubus fruticosus*)	Improves fruit quality in blackberries	García-Seco et al. (2013)
Pseudomonas putida, *Pseudomonas fluorescens*	Black henbane (*Hyoscyamus niger*)	Improved alkaloid content and yield	Ghorbanpour et al. (2013)
Pseudomonas sp., *Erwinia* sp., *Pantoea* sp., and *Rhizobium* sp.	Lotus (*Lotus tenuis*)	Enhanced growth in lotus	Angus et al. (2013)
Pseudomonas fluorescens, *Azospirillum brasilense*	Marigold (*Tagetes*)	Increases shoot fresh weight, root dry weight, leaf number, and node number	del Rosario Cappellari et al. (2013)
Mesorhizobium sp. *Pseudomonas aeruginosa*	Chickpea (*Cicer arietinum*)	Enhanced grain and straw yield by increasing root shoot dry weight with the increased uptake of N and P	Verma et al. (2013)
Bacillus sp.	Cotton (*Gossypium hirsutum*)	Increase in plant height, number of bolls per plant, and boll weight and soil available phosphorus.	Qureshi et al. (2012)
Rhizobium and *Pseudomonas* sp.	Mothbean (*Vigna aconitifolia*)	Increase in root, shoot length	Sharma et al. (2013a, b)
P. putida, *P. fluorescens*	Spinach (*Spinacia oleracea*), pepper (*Piper nigrum*)	Increases plant heights	Hou and Oluranti (2013)

(continued)

Table 28.2 (continued)

Bacterial strain	Crop	Influence of inoculation on crops	References
Pseudomonas putida, *Pseudomonas fluorescens*	Rice (*Oryza sativa*)	Enhanced iron content in grains	Sharma et al. (2013a, b)
Pantoea agglomerans, *Burkholderia anthina*	Tomato (*Solanum lycopersicum*)	Increased plant height, root length, shoot and root dry weight, phosphorus uptake, and available phosphorus content	Walpola and Yoon (2013)
Pseudomonas sp. strain PAC and *Serratia* sp. strain CMR165	Rice (*Oryza sativa*)	Promoted plant growth and uptake of phosphate by increasing phosphate solubilization and could be used as biofertilizers to optimize phosphate fertilization	Nico et al. (2012)
Pseudomonas putida	Tomato (*Solanum lycopersicum*)	Increases plant growth and yield	Shen et al. (2012)
Pseudomonas sp.	Maize (*Zea mays*)	Increases plant height and dry weight	Jarak et al. (2012)
Pseudomonas aeruginosa	Tomato (*Solanum lycopersicum*)	Improves fruit yield	Dashti et al. (2012)
PGPR	Soybean (*Glycine max* L.)	Enhances soybean productivity	Salama et al. (2011)
Pseudomonas putida	Maize (*Zea mays*)	Increases grain yield	Dadnia and Moaveni (2011)
Pseudomonas fluorescens	Mustard (*Brassica* sp.)	Increases growth and yield attributes	Aeron et al. (2011)
Pseudomonas putida	Cherry trees (*Prunus sp.*)	Enhances fruit setting as well as vegetative growth of plants	Karakurt et al. (2011)
Bacillus sp.	Sunflower (*Helianthus annuus*)	Increase in growth, yield, and quality of plant, oil yield	Ekin (2010)
Gluconacetobacter sp. and *Burkholderia* sp.	Cowpea (*Vigna unguiculata*)	Improved nodulation, root and shoot biomass, straw and grain yield as well as nitrogen and phosphorus uptake	Linu et al. (2009)
Pseudomonas fluorescens	Wheat (*Triticum aestivum*)	Improves seed yield and shoot dry mass	Behn (2008)
PGPR	Cucumber (*Cucumis sativus*)	Enhances root growth	Bae et al. (2007)
Pseudomonas fluorescens, *Bacillus megaterium*	Chickpea (*Cicer arietinum*)	Increase in plumule and radicle length	Sharma et al. (2007)

(continued)

Table 28.2 (continued)

Bacterial strain	Crop	Influence of inoculation on crops	References
Pseudomonas sp., *Burkholderia caryophylli*	Wheat (*Triticum aestivum*)	Improved growth and yield of wheat	Shaharoona et al. (2007)
Pseudomonas corrugate	Maize (*Zea mays*)	Increased grain yield in maize	Kumar et al. (2007)

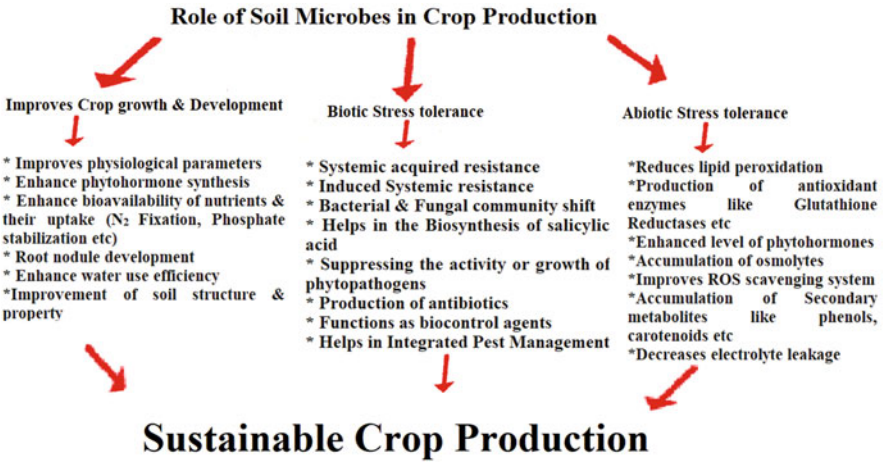

Fig. 28.3 Soil microbes in sustainable crop production

(c) *Microbe-mediated biotic stress tolerance:* Soil microflora assist uptake of nutrients from soil which results in enhanced yield as well as disease reduction or suppression. As given in Table 28.3 there are several examples of soil rhizobacteria which assist in disease inhibition. *Bacillus subtilis* has the potential for disease reduction, and more than 20 antibiotics are produced by them as depicted in Fig. 28.3. Efficacy of *Bacillus* spp. has been reported in different crop plants like tomato, chilli, brinjal, etc. to control different pathogens like *Colletotrichum acutatum, C. capsici, C. gloeosporioides, Pythium aphanidermatum,* and *R. solani* (Abdul et al. 2007). *Pseudomonas* spp. exhibit antifungal activity against *Pyricularia oryzae, R. solani, Xanthomonas oryzae* pv. *oryzae, and F. oxysporum* f. sp. *udum* under in vitro and in in vivo as well (Vidhyasekaran et al. 2001). Several soil-borne antagonists including *Trichoderma* spp. are reported to control fungal wilt of tomato caused by *F. oxysporum f.* sp. *lycopersici* (Singh et al. 2015a, b).

(d) *Microbe-mediated abiotic stress tolerance:* Abiotic stress is also one of the limiting factors that affects agricultural productivity. Crop plants function to mitigate the adverse effect of external pressure caused by edaphic or environmental condition by altering some physiological and biochemical changes

Table 28.3 Microbe-mediated biotic stress tolerance in crops

Disease causing agents	Crop	Biocontrol agents	References
Pseudomonas syringae *Xanthomonas fragariae* *Xanthomonas arboricola* *Xanthomonas campestris* *Xanthomonas axonopodi* spv. *vignicola*	Chickpea (*Cicer arietinum*) Cowpea (*Vigna unguiculata*)	*Pseudomonas aeruginosa* *Pseudomonas fluorescens* *Lactobacillus plantarum*	Kanthaiah and Velu (2019), Daranas et al. (2019), Corrêa et al. (2014)
Pseudomonas syringae pv. *Lachrymans* *Pseudocercospora griseola*	Common bean (*Phaseolus vulgaris* L.) Cucumber (*Cucumis sativus* L.)	*Ochrobactrum pseudintermedium* *Pantoea agglomerans*	Akbaba and Ozaktan (2018)
Alternaria alternata	Chickpea (*Cicer arietinum*) Lentil (*Lens culinaris*) Pea (*Pisum sativum*) Faba bean (*Vicia faba* L.)	*Trichoderma viride* *Trichoderma harzianum*	Surekha et al. (2013) and Kayim et al. (2018)
Fusarium oxysporum f. sp. *pisi* *Fusarium oxysporum* f. sp. *lentis* *Fusarium oxysporum* f. sp. *ciceris*	Pea (*Pisum sativum*) Lentil (*Lens culinaris*) Chickpea (*Cicer arietinum*)	*Bacillus cereus* *Streptomyces* spp.	Corrêa et al. (2014) and Anusha et al. (2019)
Sclerotinia sclerotiorum, *Sclerotinia trifoliorum*, *Sclerotinia minor*	Common bean (*Phaseolus vulgaris* L.)	*Bacillus subtilis* *Pseudomonas fluorescens*	Khater (2010), Sabaté et al. (2018), and Zhang and Xue (2010)
Sclerotium rolfsii	Lentil (*Lens culinaris*)	*Trichoderma viride*, *Trichoderma virens,* and *Trichoderma harzianum*	Kushwaha et al. (2018)
Erysiphe flexuosa	Cowpea (*Vigna unguiculata*)	*Glomus versiforme* and *Trichoderma harzianum*	Omomowo et al. (2018)
Fusarium oxysporum f. sp. *lentis*	Lentil (*Lens culinaris Medikus* subsp. *Culinaris* L.)	*Trichoderma viride*, *Trichoderma koningi,* and *Trichoderma harzianum*	Tiwari et al. (2018)

(continued)

Table 28.3 (continued)

Disease causing agents	Crop	Biocontrol agents	References
Fusarium oxysporum f. sp. *ciceri*	Chickpea (*Cicer arietinum* L.)	*Trichoderma harzianum*	Nirmalkar et al. (2017)
Myrothecium, Anthracnose, and *Rhizoctonia*	Soybean (*Glycine max* L.)	*Trichoderma viride*	Kuchlan et al. (2017)
Fusarium oxysporum f. sp. *ciceri*	Chickpea (*Cicer arietinum*)	*Trichoderma viride* and *Trichoderma harzianum*	Patole et al. (2017)
Fusarium solani	Faba bean (*Vicia faba* L.)	*Trichoderma harzianum*	Habtegebriel and Boydom (2016)
Ascochyta rabiei *Ascochyta lentis*	Chickpea (*Cicer arietinum* L.) Lentil (*Lens culinaris*)	*Pantoea agglomerans* *Bacillus* sp.	Liu et al. (2016)
Rhizoctonia solani	Bean (*Phaseolus vulgaris* L.)	*T. harzianum* T019	Mayo et al. (2015)
Stemphylium botryosum	Lentil (*Lens culinaris*)	*Trichoderma viride*, *Trichoderma harzianum*	Subedi et al. (2015)

in them failing which limits the crop growth and productivity. Certain microorganisms were known that have the capabilities to neutralize the harmful effect caused due to such abiotic stresses. They can modulate the various physiological and biochemical process in the plants and thereby allowing the plants to cope up with the harmful effect of these conditions. Some of the examples of microbe-mediated abiotic stress tolerance are given in Table 28.4 and depicted in Fig. 28.3.

(e) *Role of BSMs in sustainable agriculture:* Biofertilizers are those materials that contain living microorganisms that colonize the rhizosphere of the plants and raise the supply or convenience of principal nutrients and/or growth stimulus to the target crop (Bhattacharjee and Dey 2014). They are applied in the agricultural fields as replacement for conventional fertilizers. Biofertilizers are gaining impetus due to the maintenance of soil health, minimizing environmental pollution and cut down on the use of chemicals in the agriculture (Saeed et al. 2015). Various beneficial microbes have been used as biofertilizers for different crops to enhance their growth and productivity (Table 28.2). PGPRs, mainly N_2 fixing, phosphate and potassium solubilizers are observed as a sustainable way out to advance plant-nutrient uptake and crop production (Bhattacharjee and Dey 2014) (Fig. 28.3). According to an estimate, farmers usually need to apply at least 100 kg of N_2 per hectare (Deaker et al. 2004), whereas the use efficiency is generally below 40%, meaning that most applied fertilizer either washes out or is lost to the atmosphere. According to an estimate, cyanobacteria in symbiotic

Table 28.4 Microbe-mediated abiotic stress tolerance in plants

Abiotic stress	Crop	Microorganisms	Tolerance strategy	References
Salt	Soybean (*Glycine max* L. *Merrill*)	*Funneliformis mosseae, Rh. Intraradices*, and *C. etunicatum*	Improved nodule formation, leghemoglobin content, nitrogenase activity, and auxin synthesis, thereby protects soybean from salt-induced membrane damage, reduced the production of hydrogen peroxide, reduced the production of TBARS, and reduced lipid peroxidation and ultimately improved performance of soybean	Hashem et al. (2019)
Drought	Soybean (*Glycine max* L.)	*Bacillus subtilis* and *Bacillus licheniformis*	Improved photosynthetic parameters and radiation use efficiency	Mondani et al. (2019)
Drought	Soybean (*Glycine max* L.)	*Bradyrhizobium japonicum* and *AM Fungi*	Improved root system and increases photosynthetic efficiency	Takács et al. (2018)
Salinity	Soybean (*Glycine max* L.)	Endophytic bacterium *Pseudomonas putida* PIR3C and *Raoultella terrigena* PCM8	Physiological parameters were improved and bacterium enhanced ACC deaminase activity and lowers the ethylene levels	Simarmata et al. (2018)
Drought	Soybean (*Glycine max* L.)	*Bacillus subtilis* UFGS1, *Bacillus thuringiensis* UFGS2, and *Bacillus cereus* UFGRB2 and UFGRB3	Enhanced photosynthetic and physiological performance as well as differential expression of drought stress genes such as Gmp5cs, Gmgols, Gmdreb1a, and Gmereb	Martins et al. (2018)
Aluminum and zinc stresses	Soybean (*Glycine max* L.)	Endophytes fungus *Paecilomyces formosus* LHL 10 *Bacteria Sphingomonas* sp. LK11	Improved physiological and photosynthetic parameters, increased antioxidant enzymes and macronutrient uptake as well as modulates the gene expression levels to increase gibberellins levels	Bilal et al. (2018)

(continued)

Table 28.4 (continued)

Abiotic stress	Crop	Microorganisms	Tolerance strategy	References
Salinity	Soybean (*Glycine max* L.)	*Bacillus amyloliquefaciens* H-2-5	Enhanced solubilization of phosphates and accelerates the production of GAs, salicylic acid, jasmonic acid, and proline	Kim et al. (2017)
Drought	Soybean (*Glycine max* L.)	*Sphingomonas* spp. LK11	Enhances the synthesis of phytohormones and trehalose as well as modulates the gene expression of (GmDREBa and GmDREB2) and MYB (myeloblastosis) transcription factor (GmMYBJ1)	Asaf et al. (2017)
Oxidative stress	Soybean (*Glycine max* L.)	*Bacillus aryabhattai* SRB02	ABA-induced stomatal closure and improved levels of IAA, jasmonic acid, and antioxidant enzymes	Park et al. (2017)
Drought	*Medicago truncatula*	*Sinorhizobium medicae*	Increased root nodulation and nutrient acquisition of nutrient during drought stress	Staudinger et al. (2016)
Drought	*Cicer arietinum* L.	*Pseudomonas putida MTCC5279(RA)*	Accumulation of osmolytes, ROS scavenging ability, and expression of stress responsive genes	Tiwari et al. (2016)
Salt	*Glycine max*	*Pseudomonas simiae*	4-nitroguaiacol and quinoline promote soybean seed germination	Vaishnav et al. (2016)
Cd and Pb	Marigold (*Calendula officinalis* L.)	*Claroideoglomus claroideum Funneliformis mosseae*	Accumulation of secondary metabolites (phenols, flavonoids, carotenoids) and enhanced antioxidant capacity	Hristozkova et al. (2016)
Cd and Zn	*Helianthus annuus* L.	*Glomus fasciculatum* and *Pseudomonas putida*	Increased plant dry biomass of plants. Accumulation of Zn and Cd in root and shoot	Mani et al. (2015)
Cd and Pb	*Lettuce* (Lactuca sativa)	*Bradyrhizobium japonicum*	IAA production enhances the growth and increased the shoot root lengths and dry biomass	Seneviratne et al. (2016)

Cd and Pb	*Gladiolus grandiflorus* L.	*Thiobacillus thiooxidans* and *Pseudomonas putida*	Promotes root length, plant height, dry biomass of the plant, and enhanced accumulation of Cd and Pb	Mani et al. (2016)
Cd, Zn, and Cu	*Sedum*	*Bacillus pumilus E2S2*	Production of IAA, siderophores, ACC deaminase, and solubilization of phosphorus. Increased water extractable Cd and Zn contents in soil, improved plant growth and metal uptake	Ma et al. (2015)
Salt	*Glycine max* L. *Merrill*	*Pseudomonas koreensis* strain AK-1	Reduction in Na^+ level and increase in K^+ level	Kasotia et al. (2015)
Cd, AS, Cu, Pb, and Zn toxicity	*Miscanthus sinensis*	*Pseudomonas koreensis* AGB-1	ACC deaminase, IAA production	Babu et al. (2015)
Hg toxicity	*Phragmites australis*	*Photobacterium* spp.	IAA, mercury reductase activity	Mathew et al. (2015)
Salinity	Barley (*Hordeum vulgare* L.)	*Hartmannibacter diazotrophicus* E19	Increased root and shoot dry weight. ACC deaminase activity and lowers ethylene content	Suarez et al. (2015)
Salinity	*Lettuce* (*Lactuca sativa*)	*Azospirillum*	Promotes higher biomass, ascorbic acid content, antioxidant content, and a lower browning intensity	Fasciglione et al. (2015)
Zn	*Brassica juncea*	*Pseudomonas brassicacearum and rhizobium leguminosarum*	Induced metal chelation, toxicity attenuation, and microbial-assisted phytoremediation	Adediran et al. (2015)
Cd, Pb, and Zn	*Polygonum pubescens*	*Enterobacter* sp. JYX7 and *Klebsiella* sp. JYX10	Production of IAA, siderophores, ACC deaminase, solubilized inorganic phosphate improved phytoremediation efficiency	Jing et al. (2014)
Cd, Zn, and Cu	*Solanum nigrum* L.	*Pseudomonas* spp. Lk9	Improved soil Fe, P, and heavy metal availability, shoot dry biomass, and	Chen et al. (2014)

(continued)

Table 28.4 (continued)

Abiotic stress	Crop	Microorganisms	Tolerance strategy	References
			uptake of Cd, Zn and Cu biosurfactants. Production of siderophores and organic acids that induce growth and metal uptake by Cd hyperaccumulator	
Drought	*Lavandula dentate*	*Bacillus thuringiensis*	IAA induced higher proline and K-content improved nutritional, physiological, and metabolic activities and decreased glutathione reductase (GR) and ascorbate peroxidase (APX) activity	Armada et al. (2014)
Zn toxicity	*Triticum aestivum*	*Pseudomonas aeruginosa*	Improved biomass, N, P uptake, and total soluble protein	Sarker et al. (2014)
Heat	*Triticum aestivum*	*Bacillus amyloliquefaciens, Azospirillum brasilense*	Lowers the regeneration of ROS, preactivation of heat shock transcription factors, changes in metabolome	El-Daim et al. (2014)
Drought	*Zea mays*	*Burkholderia phytofirmans Enterobacter* sp. FD17	Increases photosynthesis, root and shoot biomass under drought conditions	Naveed et al. (2014)
Salt	*Oryza sativa*	Root-associated plant growth-promoting rhizobacteria (PGPR)	Expression of salt stress-related *RAB18* gene	Jha et al. (2014)
Salt	*Oryza sativa, Triticum aestivum, Zea mays, Gossypium hirsutum*	Cyanobacteria and cyanobacterial extracts	Phytohormones as elicitor molecule	Singh (2014)
Salinity	Barley and oats	*Acinetobacter* spp. *and Pseudomonas* sp.	Production of enzyme ACC deaminase lower ethylene and IAA promote plant growth	Chang et al. (2014)
Salinity and drought	Soybean	*Pseudomonas putida* H-2–3	Lowers the level of abscisic acid and salicylic acid and a higher level of jasmonic acid content. Improved antioxidant scavenging system	Kang et al. (2014)

Salinity	Rice GJ-17 (*Oryza sativa*)	*Pseudomonas pseudoalcaligenes* and *Bacillus pumilus*	Lowering the reactive oxygen species (ROS) and reduces lipid peroxidation	Jha and Subramanian (2014)
Cd, Ni, As, Cu, Pb, and Zn	*Alnus firma*	*Bacillus thuringiensis* GDB-1	Production of phytohormones, siderophore, (ACC) deaminase, and solubilization of phosphorus. Increased biomass, chlorophyll content, and nodulation	Babu et al. (2013)
Drought	*Capsicum annum*	*Bacillus licheniformis* strain K11	Synthesis of stress-related genes and proteins	Lim and Kim (2013)
Heat and drought	*Dichanthelium lanuginosum, Solanum lycopersicum*	*Curvularia protuberata* isolate Cp4666D	Colonization of roots	De Zelicourt et al. (2013)
Arsenic toxicity	*Brassica juncea*	*Staphylococcus arlettae*	Increased soil dehydrogenases and phosphatase	Srivastava et al. (2013)
Salinity	Rice (*Oryza sativa*)	*Bacillus amyloliquefaciens* NBRISN13 (SN13)	Modulating differential transcription in a set of at least 14 genes	Nautiyal et al. (2013)
Zn toxicity	*Sinapis alba*	*Enterobacter intermedius* MH8b	ACC deaminase, IAA, hydrocyanic acid, P solubilization	Płociniczak et al. (2013)
Drought	Maize (*Zea mays*)	*Azospirillum lipoferum*	Increased accumulation of soluble sugar, free amino acids, and proline	Bano et al. (2013)
Pb	*Calopogonium mucunoides*	*Glomus etunicatum*	Promoting plant nutrient (phosphorus, sulfur) acquisition, attenuating the negative effects of Pb on membranes, and contributing to the reduction of ROS generation	De Souza et al. (2012)
Drought	Rice (*Oryza sativa* L.)	*Trichoderma harzianum*	Promotes root growth independent of water status and delay drought response	Shukla et al. (2012a)

(continued)

Table 28.4 (continued)

Abiotic stress	Crop	Microorganisms	Tolerance strategy	References
Drought	*Cucumis sativa*	*Bacillus cereus* AR156, *B. subtilis* SM21, and Serratia sp. XY21	Production of monodehydroascorbate, proline, and antioxidant enzyme, expression of genes	Wang et al. (2012)
Salinity	*Capsicum annuum*	*Azospirillum brasilense* and *Pantoea dispersa* (co-inoculation)	High stomatal conductance and photosynthesis.	Del Amor and Cuadra-Crespo (2012)
Salinity	Groundnut (*Arachis hypogaea* L.)	*Brachybacterium saurashtrense* (JG-06), *Brevibacterium casei* (JG-08), and *Haererohalobacter* (JG-11)	Higher K^+/Na^+ ratio and higher Ca^{2+}, phosphorus, and nitrogen content. Higher root and shoot auxin concentration	Shukla et al. (2012a, b)
Salinity	*Brassica napus* (canola) and maize (*Zea mays*)	*Pseudomonas putida* UW4	Modulation of plant protein differential expression and ACC deaminase activity	Cheng et al. (2012)
Salinity	Mung bean (*Vigna radiata*)	*Rhizobium* and *Pseudomonas*	Increased ACC deaminase activity that improves growth, nodulation, and yield of mung bean	Ahmad et al. (2011)
Drought	Maize (*Zea mays*)	*Bacillus* spp.	Increased accumulation of proline, sugars, free amino acids, and decreased electrolyte leakage	Vardharajula et al. (2011)E

association contribute 7–80 kg N_2 /ha/year, free living 15 kg N_2 /ha/year, and associative (endophytic) bacteria 36 kg N_2/ha/year (Elkan 1992). It has been experiential that cereal crops may gain up to 30% of their N_2 from BNF when fertilized with high percentage of phosphorus and potassium as well as with microelements (Pedraza 2008; Mmbaga et al. 2014). In some studies, rhizobium inoculation also showed the biocontrol potential against soil-borne phytopathogenic fungi. Among non-symbiotic N_2-fixing bacteria, the most extensively studied genus is *Azospirillum*. In addition to escalating plant nitrogen content, it also improves plant growth by production of phytohormones such as auxins, cytokinins, and gibberellins (Steenhoudt and Vanderleyden 2000). Recently, legumes and their association with rhizobia and AMF have also been recognized for better nitrogen and phosphate uptake by plants and gaining importance in agroecosystems (Kaschuk et al. 2011). This tripartite association was not only effective in nodule formation, AM colonization, nitrogen fixation but also supported the faba bean plants growth under alkalinity stress (Abd-Alla et al. 2014). PSB and AMF are reported since long to solubilize insoluble phosphates and help in increasing yield of several crops (Fernández et al. 2007; Shahab et al. 2009). Studies designate that use of rock phosphate in combination with PSB could decrease 50% cost of accumulation of chemical fertilizers. There are several publications demonstrating that PSB-based inoculation increases P content of sugarcane, mung bean (Vikram and Hamzehzarghani 2008), maize (Oliveira et al. 2009), rice (Sarkar et al. 2012), and wheat (Sarker et al. 2014). PGPRs are known to have the capability of iron uptake in low-iron condition and enhance plant productivity (Saha et al. 2016). In plants, zinc is observed as a necessary micronutrient (Sauchelli 1969) but only very little amount remains available to plant, due to its transformation to different chemical forms (Mandal and Mandal 1987). Various studies have indicated that the release of insoluble and fixed forms of Zn by zinc solubilizing bacteria is an important aspect of increasing soil. Zn availability through the production and excretion of organic acids was studied by Singh et al. 2005. PGPFs have been extensively studied for solubilization of insoluble zinc compounds both in vitro and in vivo conditions. However, some genera of PGPRs such as *Acinetobacter, Bacillus, Gluconacetobacter,* and *Pseudomonas* have been reported to solubilize insoluble zinc. About 40% of the potential global crop yield is destroyed by pests (invertebrates, plant pathogens, and weeds) before it is harvested and another 20% is destroyed post-harvest (Chandler et al. 2010). However, for sustainable agriculture, use of chemical-based pesticides should be surrendered because of their unenthusiastic shock on the environment. Workers around the world are now using biopesticides and trying to minimize the use of conventional chemical pesticides. Biopesticides are pesticides originated from naturally present materials such as animals, plants, bacteria, and minerals. Worldwide, approximately 1400 biopesticide products are being sold (Marrone 2009) and their number is increasing day by day. For the universal position of biopesticide use and guideline as well as

ease of use, one can see review by Mishra et al. (2015) and Arora et al. (2016). BCAs can be grouped into three broad categories, namely bacterial, fungal, and viral. Among the most widely used bacterial pesticides are the strains of *Bacillus thuringiensis* (Bt), accounting for approximately 90% of the biopesticide market in the USA (Chattopadhyay et al. 2004), several species of *Pseudomonas* showed biocontrol potential against phytopathogens (Lugtenberg and Kamilova 2009). For example, biopesticides containing *Pseudomonas fluorescence* and *Pseudomonas syringae* have been used at large scale now. Fungal biopesticides include *Trichoderma harzianum*, used against plant pathogens, which is an antagonist of several soil-borne fungi such as *Rhizoctonia, Pythium, Fusarium,* and other phytopathogens (Hartmann et al. 2009). *Beauveria bassiana* and *Metarhizium anisopliae* are in nature occurring entomopathogenic fungi measured as good BCA and infect-sucking pests as well as *Nezara viridula L.* (green vegetable bug) and *Creontiades* sp. (green and brown mirids) (Sosa-Gómez and Moscardi 1998). Currently, most usually used microbial biopesticides are of biofungicides (*Bacillus, Trichoderma,* and *Pseudomonas*) bioherbicides (*Phytophthora*), and bioinsecticides (Bt) (Gupta and Dikshit 2010). Data point towards that among the biopesticide promoted for all crop types, bacterial biopesticides assert about 74%, fungal biopesticides about 10%, viral biopesticides about 5%, predator biopesticides about 8%, and "other" biopesticides about 3% (Thakore 2006).

28.5 Challenges and Future Prospects

The excessive use of chemical fertilizers and pesticides and various other anthropogenic activities is deliberately destroying agroecosystems and the balance of our planet. As a result, loss of soil fertility and crop productivity has generated awareness among agriculturists, and their consent of using BSMs in agroecosystems has gained thrust for enhancing plant productivity and soil eminence in its inhabitant form. Soil microbes are gifted with a variety of means to work as capable candidates in the field of sustainable agriculture and environment management. It has been analyzed that in properly managed-agriculture systems, BSMs can act as biofertilizers, biocontrol agents, and soil improvers. BSMs containing inoculum may replace synthetic chemicals, which result in environmental hazards and pose a serious toxicological threat to the ecosystem. Beneficial microbes used in biocontrol tend to have high-target specificity and are environment-friendly. The role of BSMs in environmental sustainability can be expanded if we get success in finding some unrevealed concepts related to their ecology, population dynamics, and functionality over a range of environments. As in near future, the global human population will increase further and, in this situation, agricultural sector would be dependent even more on BSMs to increase agricultural production in an eco-friendly manner.

28.6 Conclusion

Use of effective microbes can increase the soil quality for disease suppression by rendering the soil-borne diseases suppressive. To make sure the long-term outcome and adjustment of microbes in sustaining soil well-being by farmers and suggestion at viable level, more research is requisite. Improved knowledge of microbe-based symbiosis in plants can supply possible ways of developing sustainable agriculture in order to make certain human and animal food production with minimal risk to the environment.

Use of BSMs in sustainable agriculture and environment management proposes innumerable paybacks. Their exploitation in the form of biofertilizers and biopesticides is flattering and providing significant aid to the agroecosystems. Their potential to survive in callous environmental conditions makes them capable candidates in different types of stress management, whereas their catabolic miscellany can be used in the elimination of intractable pollutants. Our perception of the BSMs response in agroecosystems is increasing, and their potentially momentous effects on environment reinstallation are also strengthening and cooperatively helping to obtain the goal of sustainable development. Impact of climate change specifically in agriculture sector may also be done by the application of BSMs. However, with climate change outlook, efficient use of BSMs necessitates more exploration and it has been realized that clarification of mechanisms involved in their communication with plants in extreme conditions may endow with an enhanced chance of their enormous application in environmental sustainability.

References

Abd-Alla MH, Issa AA, Ohyama T (2014) Impact of harsh environmental conditions on nodule formation and dinitrogen fixation of legumes. Advances in biology and ecology of nitrogen fixation 9. https://doi.org/10.5772/56997

Abdul S, Mansoor A, Abdul K, Singh P, Suman K, Alok K, Abdul S, Kumar SA, Darokar MP, Shukla AK, Padmapriya T (2007) Novel strain of *Bacillus* as a bioinoculant. United States Patent Application 20070092491

Adediran GA, Ngwenya BT, Mosselmans JFW, Heal KV, Harvie BA (2015) Mechanisms behind bacteria induced plant growth promotion and Zn accumulation in *Brassica juncea*. J Hazard Mater 283:490–499

Aeron A, Dubey RC, Maheshwari DK, Pandey P, Bajpai VK, Kang SC (2011) Multifarious activity of bioformulated *Pseudomonas fluorescens* PS1 and biocontrol of *Sclerotinia sclerotiorum* in Indian rapeseed (*Brassica campestris* L.). Eur J Plant Pathol 131(1):81–93

Ahmad M, Zahir ZA, Asghar HN, Asghar M (2011) Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. Can J Microbiol 57(7):578–589

Akbaba M, Ozaktan H (2018) Biocontrol of angular leaf spot disease and colonization of cucumber (*Cucumis sativus* L.) by endophytic bacteria. Egypt J Biol Pest Control 28(1):14

Amaresan N, Murugesan S, Kumar K, Sankaranarayanan A (eds) (2020) Microbial mitigation of stress response of food legumes. CRC Press, Boca Raton, FL

Angus AA, Lee A, Lum MR, Shehayeb M, Hessabi R, Fujishige NA, Yerrapragada S, Kano S, Song N, Yang P, De Los Santos PE (2013) Nodulation and effective nitrogen fixation of

Macroptilium atropurpureum (siratro) by *Burkholderia tuberum*, a nodulating and plant growth promoting beta-proteobacterium, are influenced by environmental factors. Plant Soil 369 (1–2):543–562

Antoun H, Prévost D (2005) Ecology of plant growth promoting rhizobacteria. In: PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 1–38

Anusha BG, Gopalakrishnan S, Naik MK, Sharma M (2019) Evaluation of *Streptomyces spp.* and *Bacillus* spp. for biocontrol of *Fusarium* wilt in chickpea (*Cicer arietinum* L.). Arch Phytopathol Plant Protect 52(5-6):417–442

Armada E, Roldán A, Azcon R (2014) Differential activity of autochthonous bacteria in controlling drought stress in native Lavandula and Salvia plants species under drought conditions in natural arid soil. Microb Ecol 67(2):410–420

Arnon DI, Stout PR (1939) The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol 14(2):371

Arora NK, Verma M, Prakash J, Mishra J (2016) Regulation of biopesticides: global concerns and policies. In: Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 283–299

Asaf S, Khan AL, Khan MA, Imran QM, Yun BW, Lee IJ (2017) Osmoprotective functions conferred to soybean plants via inoculation with *Sphingomonas* sp. LK11 and exogenous trehalose. Microbiol Res 205:135–145

Babu AG, Kim JD, Oh BT (2013) Enhancement of heavy metal phytoremediation by *Alnus firma* with endophytic *Bacillus thuringiensis* GDB-1. J Hazard Mater 250:477–483

Babu AG, Shea PJ, Sudhakar D, Jung IB, Oh BT (2015) Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal (loid)-contaminated mining site soil. J Environ Manag 151:160–166

Bae YS, Park KS, Lee YG, Choi OH (2007) A simple and rapid method for functional analysis of plant growth-promoting rhizobacteria using the development of cucumber adventitious root system. Plant Pathol J 23(3):223–225

Bahme JB, Schroth MN (1987) Spatial-temporal colonization patterns of a rhizobacterium on underground organs of potato. Phytopathology 77(7):1093–1100

Baker KF, Cook RJ (1974) Biological control of plant pathogens. W. H. Freeman and Company, San Francisco, CA

Bano Q, Ilyas N, Bano A, Zafar N, Akram A, Hassan F (2013) Effect of *Azospirillum* inoculation on maize (*Zea mays* L.) under drought stress. Pak J Bot 45(S1):13–20

Barak R, Chet I (1990) Lectin of *Sclerotium rolfsii*: its purification and possible function in fungal-fungal interaction. J Appl Bacteriol 69(1):101–112

Bashan Y, De-Bashan LE (2005) Plant growth-promoting. In: Encyclopedia of soils in the environment, vol 1. Academic Press, Amsterdam, pp 103–115

Behn O (2008) Influence of *Pseudomonas fluorescens* and *arbuscular mycorrhiza* on the growth, yield, quality and resistance of wheat infected with *Gaeumannomyces graminis*. J Plant Dis Prot 115(1):4–8

Bélanger RR, Avis TJ (2002) Ecological processes and interactions occurring in leaf surface fungi. The American Phytopathological Society, Saint Paul, MN, pp 193–208

Berg G, Zachow C, Müller H, Philipps J, Tilcher R (2013) Next-generation bio-products sowing the seeds of success for sustainable agriculture. Agronomy 3(4):648–656

Bhattacharjee R, Dey U (2014) Biofertilizer, a way towards organic agriculture: a review. Afr J Microbiol Res 8(24):2332–2343

Bhowmik SN, Singh CS (2004) Mass multiplication of AM inoculum: effect of plant growth-promoting rhizobacteria and yeast in rapid culturing of *Glomus mosseae*. Curr Sci 86:705–709

Bilal S, Shahzad R, Khan AL, Kang SM, Imran QM, Al-Harrasi A, Yun BW, Lee IJ (2018) Endophytic microbial consortia of phytohormones-producing fungus *Paecilomyces formosus* LHL10 and bacteria *Sphingomonas* sp. LK11 to *Glycine max* L. regulates physio-hormonal changes to attenuate aluminum and zinc stresses. Front Plant Sci 9:1273

Brahmaprakash GP, Sahu PK (2012) Biofertilizers for sustainability. J Indian Inst Sci 92(1):37–62

Bulgarelli D, Schlaeppi K, Spaepen S, Van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838

Burris RH, Roberts GP (1993) Biological nitrogen fixation. Annu Rev Nutr 13(1):317–335

Chandler D, Grant WP, Greaves J (2010) Biopesticides: pest management and regulation. CABI, Wallingford

Chang P, Gerhardt KE, Huang XD, Yu XM, Glick BR, Gerwing PD, Greenberg BM (2014) Plant growth-promoting bacteria facilitate the growth of barley and oats in salt-impacted soil: implications for phytoremediation of saline soils. Int J Phytoremediation 16(11):1133–1147

Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK (2004) Turmeric and curcumin: biological actions and medicinal applications. Curr Sci 87:44–53

Chen L, Luo S, Li X, Wan Y, Chen J, Liu C (2014) Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas* sp. Lk9 on soil heavy metals uptake. Soil Biol Biochem 68:300–308

Cheng Z, Woody OZ, McConkey BJ, Glick BR (2012) Combined effects of the plant growth-promoting bacterium *Pseudomonas putida* UW4 and salinity stress on the *Brassica napus* proteome. Appl Soil Ecol 61:255–263

Corrêa BO, Schafer JT, Moura AB (2014) Spectrum of biocontrol bacteria to control leaf, root and vascular diseases of dry bean. Biol Control 72:71–75

Dadnia MR, Moaveni P (2011) Response of corn (*Zea mays* L.) production in response to PGPRs under different rates of phosphorus. Res Crops 12(2):352–355

Daranas N, Roselló G, Cabrefiga J, Donati I, Francés J, Badosa E, Spinelli F, Montesinos E, Bonaterra A (2019) Biological control of bacterial plant diseases with *Lactobacillus plantarum* strains selected for their broad-spectrum activity. Ann Appl Biol 174(1):92–105

Dashti NH, Ali NY, Cherian VM, Montasser MS (2012) Application of plant growth-promoting rhizobacteria (PGPR) in combination with a mild strain of cucumber mosaic virus (CMV) associated with viral satellite RNAs to enhance growth and protection against a virulent strain of CMV in tomato. Can J Plant Pathol 34(2):177–186

De Souza LA, de Andrade SAL, de Souza SCR, Schiavinato MA (2012) Arbuscular mycorrhiza confers Pb tolerance in *Calopogonium mucunoides*. Acta Physiol Plant 34(2):523–531

De Zelicourt A, Al-Yousif M, Hirt H (2013) Rhizosphere microbes as essential partners for plant stress tolerance. Mol Plant 6(2):242–245

Deaker R, Roughley RJ, Kennedy IR (2004) Legume seed inoculation technology—a review. Soil Biol Biochem 36(8):1275–1288

Del Amor FM, Cuadra-Crespo P (2012) Plant growth-promoting bacteria as a tool to improve salinity tolerance in sweet pepper. Funct Plant Biol 39(1):82–90

del Rosario Cappellari L, Santoro MV, Nievas F, Giordano W, Banchio E (2013) Increase of secondary metabolite content in marigold by inoculation with plant growth-promoting rhizobacteria. Appl Soil Ecol 70:16–22

Doran JW, Sarrantonio M, Janke R (1994) Strategies to promote soil quality and health. In: Soil biota: management in sustainable farming systems. CSIRO, Melbourne, pp 230–237

Dudeja SS, Singh NP, Sharma P, Gupta SC, Chandra R, Dhar B, Bansal RK, Brahmaprakash GP, Potdukhe SR, Gundappagol RC, Gaikawad BG (2011) Biofertilizer technology and pulse production. In: Bioaugmentation, biostimulation and biocontrol. Springer, Berlin, Heidelberg, pp 43–63

Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10(1):1–9

Ekin Z (2010) Performance of phosphate solubilizing bacteria for improving growth and yield of sunflower (*Helianthus annuus* L.) in the presence of phosphorus fertilizer. Afr J Biotechnol 9 (25):3794–3800

El-Daim IAA, Bejai S, Meijer J (2014) Improved heat stress tolerance of wheat seedlings by bacterial seed treatment. Plant Soil 379(1–2):337–350

Elkan C (1992, May) Reasoning about action in first-order logic. In: Proceedings of the binennial conference—Canadian Society for Computational Studies of Intelligence. Canadian Information Processing Society, Mississauga, pp 221–227

Fasciglione G, Casanovas EM, Quillehauquy V, Yommi AK, Goñi MG, Roura SI, Barassi CA (2015) *Azospirillum* inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. Sci Hortic 195:154–162

Fernández LA, Zalba P, Gómez MA, Sagardoy MA (2007) Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol Fertil Soils 43(6):805–809

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320(5878):889–892

García-Seco D, Bonilla A, Algar E, García-Villaraco A, Mañero JG, Ramos-Solano B (2013) Enhanced blackberry production using *Pseudomonas fluorescens* as elicitor. Agron Sustain Dev 33(2):385–392

Ghorbanpour M, Hatami M, Khavazi K (2013) Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid production of *Hyoscyamus niger* under water deficit stress. Turk J Biol 37(3):350–360

Gupta S, Dikshit AK (2010) Biopesticides: an ecofriendly approach for pest control. J Biopest 3:186

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent *pseudomonads*. Nat Rev Microbiol 3(4):307–319

Habtegebriel B, Boydom A (2016) Biocontrol of faba bean black root rot caused by *Fusarium solani* using seed dressing and soil application of *Trichoderma harzianum*. J Biol Control 30 (3):169–176

Hartmann A, Schmid M, Van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321(1-2):235–257

Hashem A, Abd-Allah EF, Alqarawi AA, Wirth S, Egamberdieva D (2019) Comparing symbiotic performance and physiological responses of two soybean cultivars to arbuscular mycorrhizal fungi under salt stress. Saudi J Biol Sci 26(1):38–48

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311(1-2):1–18

Hou MP, Oluranti BO (2013) Evaluation of plant growth promoting potential of four rhizobacterial species for indigenous system. J Cent South Univ 20(1):164–171

Hristozkova M, Geneva M, Stancheva I, Boychinova M, Djonova E (2016) Contribution of arbuscular mycorrhizal fungi in attenuation of heavy metal impact on *Calendula officinalis* development. Appl Soil Ecol 101:57–63

Jarak M, Mrkovački N, Bjelić D, Jošić D, Hajnal-Jafari T, Stamenov D (2012) Effects of plant growth promoting rhizobacteria on maize in greenhouse and field trial. Afr J Microbiol Res 6 (27):5683–5690

Jha Y, Subramanian RB (2014) PGPR regulate caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. Physiol Mol Biol Plants 20(2):201–207

Jha Y, Sablok G, Subbarao N, Sudhakar R, Fazil MT, Subramanian RB, Squartini A, Kumar S (2014) Bacterial-induced expression of RAB18 protein in *Oryza sativa* salinity stress and insights into molecular interaction with GTP ligand. J Mol Recognit 27(9):521–527

Jing YX, Yan JL, He HD, Yang DJ, Xiao L, Zhong T, Yuan M, Cai XD, Li SB (2014) Characterization of bacteria in the rhizosphere soils of *Polygonum pubescens* and their potential in promoting growth and Cd, Pb, Zn uptake by *Brassica napus*. Int J Phytoremediation 16 (4):321–333

Kang SM, Radhakrishnan R, Khan AL, Kim MJ, Park JM, Kim BR, Shin DH, Lee IJ (2014) Gibberellin secreting rhizobacterium, *Pseudomonas putida* H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. Plant Physiol Biochem 84:115–124

Kanthaiah K, Velu RK (2019) Characterization of the bioactive metabolite from a plant growth promoting rhizobacteria *Pseudomonas aeruginosa* VRKK1 and exploitation of antibacterial behaviour against *Xanthomonas campestris* a causative agent of bacterial blight disease in cowpea. Arch Phytopathol Plant Prot. https://doi.org/10.1080/03235408.2018.1557883

Karakurt H, Kotan R, Dadasoglu F, Aslantas R, Şahin F (2011) Effects of plant growth promoting rhizobacteria on fruit set, pomological and chemical characteristics, color values, and vegetative growth of sour cherry (*Prunus cerasus* cv. Kütahya). Turk J Biol 35(3):283–291

Kaschuk G, Alberton O, Hungria M (2011) Quantifying effects of different agricultural land uses on soil microbial biomass and activity in Brazilian biomes: inferences to improve soil quality. Plant Soil 338(1–2):467–481

Kasotia A, Varma A, Choudhary DK (2015) *Pseudomonas*-mediated mitigation of salt stress and growth promotion in *Glycine max*. Agric Res 4(1):31–41

Kayim M, Yones AM, Endes A (2018) Biocontrol of *Alternaria alternata* causing leaf spot disease on Faba bean (*Vicia faba* L.) using some *Trichoderma harzianum* isolates under in vitro condition. Harran Tarımve Gıda Bilimleri Dergisi 22(2):169–178

Khater MMMN (2010) Biological control of *Sclerotinia Sclerotiorum* - the causal agent of white basal rot disease of beans (*Phaseolus* vulgaris). Doctoral dissertation, Alexandria University

Kim MJ, Radhakrishnan R, Kang SM, You YH, Jeong EJ, Kim JG, Lee IJ (2017) Plant growth promoting effect of *Bacillus amyloliquefaciens* H-2-5 on crop plants and influence on physiological changes in soybean under soil salinity. Physiol Mol Biol Plants 23(3):571–580

Kloepper JW, Hume DJ, Scher FM, Singleton C, Tipping B, Laliberte M, Frauley K, Kutchaw T, Simonson C, Lifshitz R, Zaleska I (1988) Plant growth-promoting rhizobacteria on canola (rapeseed). Plant Dis 72(1):42–46

Kuchlan MK, Kuchlan P, Husain SM (2017) Effect of foliar application of growth activator, promoter and antioxidant on seed quality of soybean. Legume Res 40(2):313–318

Kumar B, Trivedi P, Pandey A (2007) Pseudomonas corrugata: a suitable bacterial inoculant for maize grown under rainfed conditions of Himalayan region. Soil Biol Biochem 39 (12):3093–3100

Kumar A, Meena R, Meena VS, Bisht JK, Pattanayak A (2016) Towards the stress management and environmental sustainability. J Clean Prod 100(137):821–822

Kushwaha SK, Kumar S, Chaudhary B (2018) Efficacy of Trichoderma against *Sclerotium rolfsii* causing collar rot disease of lentil under in vitro conditions. J Appl Nat Sci 10(1):307–312

Lim JH, Kim SD (2013) Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. Plant Pathol J 29(2):201

Linu MS, Stephen J, Jisha MS (2009) Phosphate solubilizing Gluconacetobacter sp., *Burkholderia* sp. and their potential interaction with cowpea (*Vigna unguiculata* (L.) Walp.). Int J Agric Res 4 (2):79–87

Liu N, Xu S, Yao X, Zhang G, Mao W, Hu Q, Feng Z, Gong Y (2016) Studies on the control of *ascochyta blight* in field peas (*Pisum sativum* L.) caused by *Ascochyta pinodes* in Zhejiang Province, China. Front Microbiol 7:481

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Ma Y, Oliveira RS, Nai F, Rajkumar M, Luo Y, Rocha I, Freitas H (2015) The hyperaccumulator *Sedum plumbizincicola* harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil. J Environ Manag 156:62–69

Mandal LN, Mandal B (1987) Transformation of zinc fractions in rice soils. Soil Sci 143 (3):205–212

Mani D, Kumar C, Patel NK (2015) Integrated micro-biochemical approach for phytoremediation of cadmium and zinc contaminated soils. Ecotoxicol Environ Saf 111:86–95

Mani D, Kumar C, Patel NK (2016) Integrated micro-biochemical approach for phytoremediation of cadmium and lead contaminated soils using *Gladiolus grandiflorus* L cut flower. Ecotoxicol Environ Saf 124:435–446

Marrone PG (2009) Barriers to adoption of biological control agents and biological pesticides. In: Integrated pest management. Cambridge University Press, Cambridge, UK, pp 163–178

Martins SJ, Rocha GA, de Melo HC, de Castro Georg R, Ulhôa CJ, de Campos Dianese É, Oshiquiri LH, da Cunha MG, da Rocha MR, de Araújo LG, Vaz KS (2018) Plant-associated bacteria mitigate drought stress in soybean. Environ Sci Pollut Res 25(14):13676–13686

Mathew DC, Ho YN, Gicana RG, Mathew GM, Chien MC, Huang CC (2015) A rhizosphere-associated symbiont, *Photobacterium* spp. strain MELD1, and its targeted synergistic activity for phytoprotection against mercury. PLoS One 10(3):e0121178

Mayo S, Gutierrez S, Malmierca MG, Lorenzana A, Campelo MP, Hermosa R, Casquero PA (2015) Influence of *Rhizoctonia solani* and *Trichoderma* spp. in growth of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. Front Plant Sci 6:685

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37 (5):634–663

Miller RM, Jastrow JD (2000) Mycorrhizal fungi influence soil structure. In: Arbuscular mycorrhizas: physiology and function. Springer, Dordrecht, pp 3–18

Mishra J, Tewari S, Singh S, Arora NK (2015) Biopesticides: where we stand? In: Plant microbe's symbiosis: applied facets. Springer, New Delhi, pp 37–75

Mitchell CW (1973) Soil classification with particular reference to the seventh approximation. J Soil Sci 24(4):411–420

Mmbaga GW, Mtei KM, Ndakidemi PA (2014) Extrapolations on the use of rhizobium inoculants supplemented with phosphorus (P) and potassium (K) on growth and nutrition of legumes. Agric Sci 5(12):1207

Mondani F, Khani K, Honarmand SJ, Saeidi M (2019) Evaluating effects of plant growth-promoting rhizobacteria on the radiation use efficiency and yield of soybean (*Glycine max*) under water deficit stress condition. Agric Water Manag 213:707–713

Motsara MR, Bhattacharyya P, Srivastava B (1995) Biofertiliser technology, marketing and usage: a sourcebook-cum-glossary. Fertiliser Development and Consultation Organization, New Delhi

Nautiyal CS, Srivastava S, Chauhan PS, Seem K, Mishra A, Sopory SK (2013) Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol Biochem 66:1–9

Naveed M, Hussain MB, Zahir ZA, Mitter B, Sessitsch A (2014) Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. Plant Growth Regul 73 (2):121–131

Nico M, Ribaudo CM, Gori JI, Cantore ML, Curá JA (2012) Uptake of phosphate and promotion of vegetative growth in glucose-exuding rice plants (*Oryza sativa*) inoculated with plant growth-promoting bacteria. Appl Soil Ecol 61:190–195

Nirmalkar VK, Said PP, Kaushik DK (2017) Efficacy of fungicides and bio-agents against *Pyricularia grisea* in paddy and yield gap analysis thought frontline demonstration. Int J Curr Microbiol App Sci 6(4):2338–2346

Oliveira CA, Sa NM, Gomes EA, Marriel IE, Scotti MR, Guimaraes CT, Schaffert RE, Alves VM (2009) Assessment of the mycorrhizal community in the rhizosphere of maize (*Zea mays* L.) genotypes contrasting for phosphorus efficiency in the acid savannas of Brazil using denaturing gradient gel electrophoresis (DGGE). Appl Soil Ecol 41(3):249–258

Omomowo IO, Fadiji AE, Omomowo OI (2018) Assessment of bio-efficacy of *Glomus versiforme* and *Trichoderma harzianum* in inhibiting powdery mildew disease and enhancing the growth of cowpea. Ann Agric Sci 63(1):9–17

Park YG, Mun BG, Kang SM, Hussain A, Shahzad R, Seo CW, Kim AY, Lee SU, Oh KY, Lee DY, Lee IJ (2017) *Bacillus aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. PLoS One 12(3):e0173203

Parr JF, Hornick SB, Kaufman DD (1994) Use of microbial inoculants and organic fertilizers in agricultural production. ASPAC Food & Fertilizer Technology Center, Taiwan

Patole SP, Shankara K, Pradhan RS, Dhore SB (2017) Isolation and characterization of toxin from *Alternaria helianthi* inciting blight in sunflower. Int J Curr Microbiol App Sci 6(10):2892–2896

Pedraza RO (2008) Recent advances in nitrogen-fixing acetic acid bacteria. Int J Food Microbiol 125(1):25–35

Płociniczak T, Sinkkonen A, Romantschuk M, Piotrowska-Seget Z (2013) Characterization of *Enterobacter intermedius* MH8b and its use for the enhancement of heavy metals uptake by Sinapis alba L. Appl Soil Ecol 63:1–7

Qureshi MA, Ahmad ZA, Akhtar N, Iqbal A, Mujeeb F, Shakir MA (2012) Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth. J Anim Plant Sci 22(1):204–210

Rathore US, Singh SK, Kumar S (2018a) Identification of resistant sources for *Alternaria Blight* of Pigeonpea (*Cajanus cajan* L. Millsp). Int J Curr Microbiol Appl Sci 7(4):3380–3393

Rathore US, Singh SK, Kumar S, Saloni R (2018b) Application of botanicals for effective management of *Alternaria blight* of pigeon pea. J Pharmacog Phytochem 2:328–338

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321 (1-2):305–339

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17(4–5):319–339

Sabaté DC, Brandan CP, Petroselli G, Erra-Balsells R, Audisio MC (2018) Biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary on common bean by native lipopeptide-producer Bacillus strains. Microbiol Res 211:21–30

Saeed KS, Ahmed SA, Hassan IA, Ahmed PH (2015) Effect of bio-fertilizer and chemical fertilizer on growth and yield in cucumber (*Cucumis sativus*) in green house condition. Pak J Biol Sci 18 (3):129–134

Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P (2016) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res 23(5):3984–3999

Salama AB, Hamed ER, Shehata HS (2011) Effect of plant growth promoting rhizobacteria (PGPR) on the healthy and productivity of soy bean plant. Planta Medica 77(12):1299

Sarkar A, Islam T, Biswas G, Alam S, Hossain M, Talukder N (2012) Screening for phosphate solubilizing bacteria inhabiting the rhizoplane of rice grown in acidic soil in Bangladesh. Acta microbiologica et immunologica Hungarica 59(2):199–213

Sarker A, Talukder NM, Islam MT (2014) Phosphate solubilizing bacteria promote growth and enhance nutrient uptake by wheat. Plant Sci Today 1(2):86–93

Sauchelli V (1969) Trace elements in agriculture. Van Nostrand, New York

Scher FM, Baker R (1980) Mechanism of biological control in a *Fusarium*-suppressive soil. Phytopathology 70(5):412–417

Scher FM, Dupler M, Baker R (1984) Effect of synthetic iron chelates on population densities of *Fusarium oxysporum* and the biological control agent *Pseudomonas putida* in soil. Can J Microbiol 30(10):1271–1275

Schippers B, Bakker AW, Bakker PA (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annu Rev Phytopathol 25(1):339–358

Schroth MN, Hancock JG (1982) Disease-suppressive soil and root-colonizing bacteria. Science 216(4553):1376–1381

Seneviratne M, Gunaratne S, Bandara T, Weerasundara L, Rajakaruna N, Seneviratne G, Vithanage M (2016) Plant growth promotion by *Bradyrhizobium japonicum* under heavy metal stress. S Afr J Bot 105:19–24

Shahab S, Ahmed N, Khan NS (2009) Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. Afr J Agric Res 4(11):1312–1316

Shaharoona B, Jamro GM, Zahir ZA, Arshad M, Memon KS (2007) Effectiveness of various *Pseudomonas* spp. and *Burkholderia caryophylli* containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* I.). J Microbiol Biotechnol 17(8):1300

Sharma K, Dak G, Agrawal A, Bhatnagar M, Sharma R (2007) Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. J Herbal Med Toxicol 1(1):61–63

Sharma A, Shankhdhar D, Shankhdhar SC (2013a) Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria. Plant Soil Environ 59(2):89–94

Sharma S, Gaur RK, Choudhary DK (2013b) Solubilization of inorganic phosphate (Pi) and plant growth-promotion (PGP) activities by root-nodule bacteria isolated from cultivated legume, moth bean (*Vigna aconitifolia* L.) of the great Indian Thar desert. Res J Biotechnol 8(3):4–10

Shen M, Kang YJ, Wang HL, Zhang XS, Zhao QX (2012) Effect of plant growth-promoting rhizobacteria (PGPRs) on plant growth, yield, and quality of tomato (*Lycopersicon esculentum* Mill.) under simulated seawater irrigation. J Gen Appl Microbiol 58(4):253–262

Shiferaw B (2004) Harnessing improved BNF for the poor: assessment of technological, policy and Institutional constraints and research needs. In: Symbiotic nitrogen fixation: challenges and future prospects for application in tropical agroecosystems. Science Publishers, New Hampshire, p 1

Shukla N, Awasthi RP, Rawat L, Kumar J (2012a) Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by Trichoderma harzianum under drought stress. Plant Physiol Biochem 54:78–88

Shukla PS, Agarwal PK, Jha B (2012b) Improved salinity tolerance of *Arachis hypogaea* (L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria. J Plant Growth Regul 31 (2):195–206

Simarmata R, Ngadiman N, Rohman S, Simanjuntak P (2018) Amelioration of salt tolerance in soybean (*Glycine Max.* L) by plant-growth promoting endophytic bacteria produce 1-aminocyclopropane-1-carboxylase deaminase. J Trop Gen Bot 22(2):81–93

Singh S (2014) A review on possible elicitor molecules of cyanobacteria: their role in improving plant growth and providing tolerance against biotic or abiotic stress. J Appl Microbiol 117 (5):1221–1244

Singh B, Natesan SKA, Singh BK, Usha K (2005) Improving zinc efficiency of cereals under zinc deficiency. Curr Sci 88:36–44

Singh HR, Deka M, Das S (2015a) Enhanced resistance to blister blight in transgenic tea (*Camellia sinensis* [L.] O. Kuntze) by overexpression of class I chitinase gene from potato (*Solanum tuberosum*). Funct Integr Genomics 15(4):461–480

Singh RK, Rai N, Singh M, Saha S, Singh SN (2015b) Detection of tomato leaf curl virus resistance and inheritance in tomato (*Solanum lycopersicum* L.). J Agric Sci 153(1):78–89

Sosa-Gómez DR, Moscardi F (1998) Laboratory and field studies on the infection of stink bugs, *Nezara viridula, Piezodorus guildinii,* and *Euschistus heros* (*Hemiptera: Pentatomidae*) with *Metarhizium anisopliae* and *Beauveria bassiana in* Brazil. J Invertebr Pathol 71(2):115–120

Srivastava S, Verma PC, Chaudhry V, Singh N, Abhilash PC, Kumar KV, Sharma N, Singh N (2013) Influence of inoculation of arsenic-resistant *Staphylococcus arlettae* on growth and arsenic uptake in *Brassica juncea* (L.) Czern. Var. R-46. J Hazard Mater 262:1039–1047

Staudinger C, Mehmeti-Tershani V, Gil-Quintana E, Gonzalez EM, Hofhansl F, Bachmann G, Wienkoop S (2016) Evidence for a rhizobia-induced drought stress response strategy in *Medicago truncatula*. J Proteome 136:202–213

Steenhoudt O, Vanderleyden J (2000) *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24 (4):487–506

Stutz EW, Défago G, Kern H (1986) Naturally occurring fluorescent *Pseudomonads* involved in suppression. Phytopathology 76:181–185

Suarez C, Cardinale M, Ratering S, Steffens D, Jung S, Montoya AMZ, Geissler-Plaum R, Schnell S (2015) Plant growth-promoting effects of *Hartmannibacter diazotrophicus* on summer barley (*Hordeum vulgare* L.) under salt stress. Appl Soil Ecol 95:23–30

Subedi S, Shrestha SM, Bahadur KG, Thapa RB, Ghimire SK, Neupane S, Nessa B (2015) Botanical, chemical and biological management of *Stemphylium botryosum* blight disease of lentil in Nepal. Indian Phytopathol 68(4):415–423

Surekha CH, Neelapu NRR, Kamala G, Prasad BS, Ganesh PS (2013) Efficacy of *Trichoderma viride* to induce disease resistance and antioxidant responses in legume *Vigna Mungo* infested by *Fusarium oxysporum* and *Alternaria alternata*. Int J Agric Sci Res 3(2):285–294

Takács T, Cseresnyés I, Kovács R, Parádi I, Kelemen B, Szili-Kovács T, Füzy A (2018) Symbiotic effectivity of dual and tripartite associations on soybean (*Glycine max* L. Merr.) cultivars inoculated with *Bradyrhizobium japonicum* and AM fungi. Front Plant Sci 9:1631

Thakore Y (2006) The biopesticide market for global agricultural use. Ind Biotechnol 2(3):194–208

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK, Johri BN (2005) Diversity of plant growth and soil health supporting bacteria. Curr Sci 89:136–150

Timmusk S (2003) Mechanism of action of the plant growth promoting bacterium *Paenibacillus polymyxa*. Doctoral dissertation, Acta Universitatis Upsaliensis

Tiwari S, Lata C, Chauhan PS, Nautiyal CS (2016) *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. Plant Physiol Biochem 99:108–117

Tiwari N, Ahmed S, Kumar S, Sarker A (2018) Fusarium wilt: a killer disease of lentil. In: Fusarium-plant diseases, pathogen diversity, genetic diversity, resistance and molecular markers. BoD – Books on Demand, Norderstedt

Vaishnav A, Kumari S, Jain S, Varma A, Tuteja N, Choudhary DK (2016) PGPR-mediated expression of salt tolerance gene in soybean through volatiles under sodium nitroprusside. J Basic Microbiol 56(11):1274–1288

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. Eur J Plant Pathol 103(9):753–765

Van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81(7):728–734

Van Wees SC, Van der Ent S, Pieterse CM (2008) Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol 11(4):443–448

Vardharajula S, Zulfikar Ali S, Grover M, Reddy G, Bandi V (2011) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6(1):1–14

Verma JP, Yadav J, Tiwari KN, Kumar A (2013) Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. Ecol Eng 51:282–286

Vidhyasekaran P, Kamala N, Ramanathan A, Rajappan K, Paranidharan V, Velazhahan R (2001) Induction of systemic resistance by *Pseudomonas fluorescens* Pf1 against *Xanthomonas oryzae* pv. *Oryzae* in rice leaves. Phytoparasitica 29(2):155

Vikram A, Hamzehzarghani H (2008) Effect of phosphate solubilizing bacteria on nodulation and growth parameters of green gram (*Vigna radiata* L. Wilczek). Res J Microbiol 3(2):62–72

Walpola BC, Yoon MH (2013) Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake. Afr J Microbiol Res 7(3):266–275

Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX, Wang YP, Guo JH (2012) Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS One 7(12):e52565

Weller DM, Raaijmakers JM, Gardener BBM, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40 (1):309–348

Zhang JX, Xue AG (2010) Biocontrol of sclerotinia stem rot (*Sclerotinia sclerotiorum*) of soybean using novel *Bacillus subtilis* strain SB24 under control conditions. Plant Pathol 59(2):382–391

Molecular Mechanisms Deciphering Cross-Talk Between Quorum Sensing Genes and Major Iron Regulons in Rhizospheric Communities

29

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Abstract

Growth and development of plants are mostly attributed with beneficial rhizospheric microbial communities colonizing in their roots which can efficiently alter the overall root morphology, modulate plant growth, and enhance the uptake of several valuable minerals. Plant growth-promoting rhizobacteria (PGPRs), an important habitant of rhizosphere, are recognized as one of the major contributor for promoting plant health. Colonization of these PGPRs at the root interface is facilitated by means of quorum sensing, a density dependent gene regulation system present in bacteria. This was initially witnessed in bioluminescence process displayed by *Vibrio harveyi* which was attributed as a density dependent process that first time revealed the association of quorum sensing networks in microbial systems. The components of these quorum sensing networks are wired in series or in parallel circuits as represented by *Pseudomonas aeruginosa* and *Vibrio harveyi,* respectively, to give a final architecture for bacterial dialogues. There are various quorum sensing regulatory systems such as LuxIR in *Vibrio harveyi*, PQS and Las/Rhl systems in *Pseudomonas* spp.

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These QS systems work in synchronicity with other systems due to the overlapping regulons and common regulators such as *fur* that regulates collaboration of iron acquisition with quorum sensing. Ferric uptake regulator (Fur) functions in a density dependent manner for iron homeostasis in bacteria by sensing their intracellular iron levels. Moreover, PQS, a signalling molecule for bacterial communication is also associated with the synthesis of siderophore pyoverdine establishing a link between quorum sensing and iron acquisition. Biofilm formation and synthesis of virulence factors seen in *Vibrio vulnificus* are some other examples of quorum sensing. However, further studies are required to explore about the detailed molecular chemistry employed for bacterial conversation and its involvement with other cellular processes.

Keywords

Quorum sensing · Rhizosphere · Biofilm · Siderophore synthesis · Iron sequestration

29.1 Introduction

Ecosystem constitutes "Bubble of Life", representing interactions between living and non-living components, allowing all possible communications among diverse organisms and their surroundings. This bubble of life signifies a distinct microbial community where the survival as an individual is somehow reliant upon a group of varied organisms, which implies the formation of microbial consortium. In order to maintain and regulate the growth of the consortium, a wired molecular network is required for communication among inter as well as the intra species. In bacteria this co-ordinated behaviour of population structuring is achieved by collectively synchronizing specific set of genes in a density dependent manner, termed as "Quorum sensing" (QS). Bacteria possess an attribute of quorum sensing that allows them to make a transit from unicellular to multicellular state by synchronizing and coordinating their activities in response to change in cell density and species composition of surrounding microbial consortia (Papenfort and Bassler 2016). Molecular mechanism underlying the process of quorum sensing involves production of small diffusible chemical signalling molecules called autoinducers. In response to increasing bacterial population density, these signalling molecules are secreted extracellularly and accumulated to reach a threshold where they are detected in order to initiate signal transduction cascade, resulting in synchronized gene expression and co-ordinated change in behaviour of the population (Rehman and Leiknes 2018; Schauder et al. 2001). There are several QS systems identified namely, LuxI-LuxR in *Vibrio fischeri*, LasI-LasR and RhlI-RhlR QS in *Pseudomonas aeruginosa*, TraI-TraR QS in *Agrobacterium tumefaciens*, which have been vastly deciphered till date (Hawver et al. 2016). Plant growth-promoting rhizobacteria (PGPR) are known to be an inevitable component of rhizosphere exerting positive effects on plant growth by directly fixing the problems related to

nutrient solubilization, nitrogen fixation, and synthesis of growth regulators. However, they are also reported to employ indirect mechanisms to promote plant growth by inducing growth of mycorrhizae; eliminating toxins and pathogens from the niche of their host plant (Bhattacharyya and Jha 2012).

Notably, quorum sensing also provides the command over colonization, for plant growth stimulation and biocontrol, an important aspect of these PGPRs. However, this co-operative behaviour also proves helpful in nutrient acquisition where sometimes iron becomes a limiting factor. This iron limitation can be conferred to the formation of insoluble oxides under aerobic conditions; chelation by host plants to avoid infection or its sequestration by competing microbes (Sexton and Schuster 2017). Quorum sensing is a key to regulate ecological behaviour of environmental bacteria as they play an important role in iron uptake and biofilm formation (Zhang et al. 2018).

In PGPRs, this is evident by the production of CDPs (cyclodipeptides) and siderophores, high affinity iron chelator molecules required for acquisition of insoluble iron forms, which corroborates an inter connection between quorum sensing and iron acquisition. Further bioinformatics analysis also confirms the presence of highly homologous protein sequences between non-ribosomal protein synthases coding for CDP and PvdD, a synthase coding for pyoverdine siderophore. PvdD synthesis is thought to be under the control of LasR AHL (*N*-acylhomoserine lactone) QS system. LasR is the component of LasI-LasR QS system where it acts as cognate response regulator for LasI that functions to regulate the synthesis of AHL autoinducers (Steindler et al. 2009). These findings suggest a strong link between cell–cell communication and iron acquisition (Rosier et al. 2018). Here in this chapter we will decipher the molecular networks connecting quorum sensing and iron acquisition in case of rhizosphere associated bacteria.

29.2 Bacterial Distribution in Rhizospheric Zone

Soil ecosystem comprises a diverse range of microorganisms that establishes a variety of interactions with plants. Rhizosphere, a major constituent of the soil ecosystem is an interface representative of interaction between plant roots, soil, and inhabiting microflora. Rhizosphere perfectly demonstrates the microbial diversity with a density of 10^{11} cells per gram of soil (Wu et al. 2018). The interactions found in rhizosphere are either positive as in the case of plant growth-promoting bacteria (PGPR), nitrogen-fixing bacteria, or mycorrhiza that displays a symbiotic relationship with plants or they are negative where pathogenic microorganisms are said to cause crop damage and loss of yield. These plant–microbe interactions are accomplished by a set of biochemical molecules secreted by both plants and microorganisms. Plants secrete certain organic compounds to attract and sustain microorganisms while microorganisms secrete signalling molecules to communicate with plants as well as other bacteria in surroundings (Sharma et al. 2003). Plant growth-promoting bacteria (PGPR) are mostly reported to have agronomic significance because of their beneficial interactions with plants. These bacteria are either

found in close association with roots, rhizosphere or they may interact as an endophyte. PGPR influence plant growth in two different ways. It is either by providing them with the compounds such as phytohormones and facilitating the uptake of nutrients or by waving off the deleterious effects of pathogens surrounding the plants. PGPRs play an essential role either as biocontrol agent or they may also exhibit role as microbial antagonists. This antagonistic behaviour includes synthesis of antibiotics, bacteriocins, and mostly siderophores (Beneduzi et al. 2012). Siderophore production plays a significant role in improving the plant health by increasing their iron uptake where plants are known to recognize the microbial siderophores. Also, these siderophores have tendency to fight back the pathogens. As described by Kloepper et al. (1980), *P. putida* B10 strain can suppress the growth of *Fusarium oxysporum* in iron deficit conditions via siderophore mediated signalling which suggests a versatile role of siderophore in enhancing plant growth (Kloepper et al. 1980). However, to specifically establish these beneficial effects, PGPRs need to colonize themselves around plants in a precise and co-ordinated manner, which is done by employing quorum sensing networks. Quorum sensing was initially described in *Vibrio fischeri* and *Vibrio harveyi* where it was thought to play an important role in regulation of bioluminescence.

29.3 Quorum Sensing: Cross-Talk Among Rhizobacteria

Rhizospheric bacteria possess density dependent quorum sensing systems in order to captivate their niche around the plants. These quorum sensing systems team up with other regulatory networks to establish a hold in rhizosphere adapting to its microenvironment. This can be exemplified with an example of *Pseudomonas aeruginosa*, an important member of rhizosphere community possesses three different types of QS systems, namely Rhl, Las, quinolone-based QS systems. Las and Rhl are AHL mediated QS systems where LasR, a transcriptional regulator works in collaboration with its corresponding AHL signal N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) synthesized by LasI. Rhl QS system comprises the components same as Las system, RhlR and RhlI with AHL signal N-Butyryl-L-homoserine lactone (C4-HSL). Apart from these AHL signals, there is a chemically distinct signal produced by *Pseudomonas spp*. which is quinolone based. The quinolone-based QS system acts via *Pseudomonas* quinolone signal (PQS) which is chemically described as 2-heptyl-3-hydroxy-4(1*H*)-quinolone. The QS systems mentioned above are interlinked to each other with PQS serving as a connecting link between Las and Rhl systems. Let us consider PQS as subject of study in order to understand these overlapping regulatory networks of quorum sensing (McGrath et al. 2004). PQS, an alkylquinolone is synthesized from its precursor HHQ (2-Heptyl-4-Quinolone). The unique characteristic of HHQ is that it can induce its own expression. Biosynthesis of PQS initiates under the control of cluster of genes such as *pqsABCDE, phnAB, and pqsH.* Here PqsA functions for the first step of PQS synthesis where it is responsible for the production of anthraniloyl-coenzyme A, using anthranilate as substrate. PqsD synthesizes 2-aminobenzoylacetate (2-ABA)

from anthraniloyl-coenzyme A and malonyl-coenzyme. PqsE is crucial for the synthesis of 2-ABA as it catalyses the hydrolysis of 2-hydroxylaminobenzoylacetate. Finally, PqsBC catalyses the reaction where octanoyl-coenzyme A and (2-ABA) undergo condensation to give HHQ, a precursor for PQS. Once the PQS is synthesized, it binds to pqsR, a protein that acts as a transcriptional regulator for *pqsABCDE* operon. PqsR is linked to Las and Rhl systems where Las positively regulates PqsR expression and Rhl regulates the same negatively (Baker et al. 2017; Lin et al. 2018). *Albeit,* PQS is an important signalling molecule for quorum sensing, it also plays a significant role in iron acquisition. PQS acts as an iron trap as it binds to the extracellular Fe^{+3} in a non-deliverable manner to the cells mimicking the condition of iron scarcity prompting the expression of siderophore synthesis genes such as that of pyoverdine and pyochelin in case of *Pseudomonas aeruginosa*. In an experiment, four *lux* gene reporter fusions were constructed to confirm the contribution of PQS in iron acquisition. Here the reporter was fused to the PQS regulated genes *pvdE* and *pvdS*, these genes are required for synthesis and regulation of pyoverdine. The fusions were also made using *pqsA* gene, first gene in the *pqsABCDE* operon and to the *lecA* gene that plays an important role in biofilm formation and is believed to be dependent on quorum sensing. These fusions were then introduced in the *pqsA* mutants to analyse control of PQS over all four fusions. When the iron-sufficient LB medium was provided, all four fusions were strongly prompted by PQS suggesting the stimulation of biofilm formation and virulence by the co-ordinated efforts of PQS and iron. Also, the quantitative real time PCR based study supports the findings that application of PQS upregulates the expression of *pvdA* and *pchE* genes required for siderophore synthesis. This suggests that PQS holds a significant role in the regulation and synthesis of pyoverdine as well as pyochelin (Diggle et al. 2007). Moreover, mutations in *pqsR* gene show downregulation of genes coding for pyocyanin, elastase, exoproteins, and lectins that are required for virulence and biofilm formation; it hinders *pqsABCDE, phnAB* expression, and also AQ synthesis (Lin et al. 2018). This explains how the density dependent population control has their inter-regulatory networks linked to the process of iron acquisition and virulence.

Another interesting example includes inter-generic behaviour of density dependent siderophore production. Enterochelin, a siderophore produced by *Escherichia coli* can be secreted as public goods for the use by Ent non-producers or can be stored for private use by *Escherichia coli*. These *ent* gene clusters operate under the control of *fur* regulon. Study suggests that at low cell densities, these Ent producers show optimum growth but they do not support the growth of Ent non-producers suggesting that at low cell densities of *E. coli*, only small fractions of public enterochelin is produced for Ent non-producers (e.g. *Pseudomonas aeruginosa*) in low iron conditions (Scholz and Greenberg 2015). This uniqueness of enterochelin production by *E. coli* is suffice to connect the dots between quorum sensing and iron acquisition (Table 29.1).

Table 29.1 Types of quorum sensing (QS) systems utilized by various microorganism for facilitating cellular communication

SI no.	Organism name	QS type	Key regulatory functions	References
1.	*Pseudomonas aeruginosa*	LasIR-RhlIR type, coupled with PQS system	Role in regulating lectin, catalase, exotoxin A, pyocyanin, and pyoverdine activity	Lin et al. (2018)
2.	*Yersinia pseudotuberculosis*	Two distinct LuxIR homologues, namely YpsRI and YtbRI. Mutations in *ypsI* and *ypsR*	Regulatory control of quorum sensing over motility and cellular aggregation in temperature-dependent manner	Atkinson et al. (1999)
3.	*Agrobacterium tumefaciens*	TraI-TraR and their respective regulators, TrlR and TraM which are operative under opine control	Facilitates the conjugal transfer of Ti plasmid by mustering the donor cells resulting in an increased transformation efficiency	Piper and Von Bodman (1999)
4.	*Rhizobium etli* CNPAF512	Two major quorum sensing systems, namely *raiRI* and *cinRI*	Role in growth inhibition, nitrogen fixation, and symbiosomal development	Sanchez-contreras et al. (2007) and Whitehead et al. (2001)
5.	*R. leguminosarum* bv. viciae	rhiR and rhiI, homologues of *luxR* and *luxI,* respectively	Augment cell-density dependent communication is known to regulate the process of nodulation	Sanchez-contreras et al. (2007) and Whitehead et al. (2001)
6.	*Bradyrhizobium japonicum*	Employs branched acyl-HSL and *BjaI* system	Repression of *nod* genes also required during the early intermediate stages of symbiosis.	Lindemann et al. (2011)
7.	*Sinorhizobium meliloti* Rm1021	Harbours *sinR/sinI* locus that encodes for various AHLs ranging from C_{12}-HSL to C_{18}-HSL	Facilitates symbiosis with *Medicago sativa*, important role in node invasion through regulation of exopolysaccharides	Marketon et al. (2003) and Capela et al. (2006)
8.	*Ralstonia solanacearum*	*solI* and *solR*type that co-ordinates with *phc* genes	Regulates the release of virulence factors such as exopolysaccharides and provides flagellar	Hikichi et al. (2017)

(continued)

Table 29.1 (continued)

SI no.	Organism name	QS type	Key regulatory functions	References
			motility via expression of *flic* gene	
9.	*Xanthomonas campestris*	RpfC-RpfG along with RavS-RavR system	Expression of *Xcc* virulence factors and exopolysaccharides via *clp* to establish a link between population density and environmental signals for an improved communication.	He et al. (2009)
10.	*Pseudomonas putida* PCL1445	*ppuI*, *rsaL,* and *ppuR*	Known to produce Putisolvin I and Putisolvin II, biosurfactants during stationary phase causing disruption of biofilm formed by another *Pseudomonas spp which* implies this to be a cell-density dependent process.	Lugtenberg and Bloemberg (2006)

29.3.1 Mode of Cellular Communication Employed During Quorum Sensing in Bacteria

Quorum sensing paves the way for communication among bacterial system which is density dependent and is known to be mediated through various signalling molecules and complex set of molecular circuits. Though gram-negative and gram-positive bacteria both utilize distinct autoinducers for cellular communications, yet they possess few common components for such interactions, which includes autoinducers (AI), cognate receptors for autoinducers, autoinducer synthases, and selected downstream components of signalling cascade (Mukherjee and Bassler 2019). These autoinducers can be categorized into: (i) N-acylhomoserine lactones (AHLs) synthesized by gram-negative bacteria; (ii) Autoinducer peptides (AIPs) synthesized by gram-positive bacteria; and (iii) Autoinducer-2 (AI-2), biochemically known as furanosyl borate diester, a global signalling molecule for interspecies communication (Hense and Schuster 2015).

Quorum sensing operates in gram-positive and gram-negative bacteria via two different component systems, viz. one-component QS systems and two-component QS systems, respectively. In one-component QS system, autoinducers synthesized by AI synthases are secreted extracellularly which are then diffused back to the cytoplasm via QS receptors. However, in case of two-component QS system, sensory molecules are coupled to the response regulators. Once the secreted

autoinducers are sensed by transmembrane receptors, they undergo auto-phosphorylation resulting in kinase mediated cascade signalling. These kinase-based receptors are unique to the phosphorelay systems associated with two-component QS systems (Hawver et al. 2016).

Several researchers have reported the role of ATP-binding cassette (ABC) transporters in post-translational modification of these autoinducers. ABC transporter aids in peptide modification with their concomitant export to the outside of the cell. Michiels et al. (2001) showed the involvement of comA exporter (ABC type) in proteolytic cleavage and translocation of pheromone peptides in gram-positive *Streptococcus pneumoniae.* They also identified the presence of ABC transporters in gram-negative bacteria such as *Rhizobium etli*, an organism found in close association with *Phaseolus vulgaris.* These organisms showed presence of bacteroid transporter A (BtrA) which had sequence similarity to the comA exporter of *S. pneumoniae* (Michiels et al. 2001). Both autoinducer synthesis and their uptake systems are the prerequisites for the successful accomplishment of quorum sensing.

29.3.2 Types of Network Architecture Employed in Bacteria During Quorum Sensing

Bacterial diversity is crucial to the plant growth and development. These bacteria at higher densities with co-operativity behave ecologically productive than at low cell densities. In order to achieve such high densities, bacteria employ quorum sensing systems to function in synchronicity. These quorum sensing systems are integrated with other networks to manage complexity at such high densities. Gram-negative bacteria are mostly known to employ Lux-I/R type system to establish their wide range of communication for regulation of bioluminescence. In this Lux-I/R type system, lux-I protein plays an important role in production of AHL (N-acylhomoserine lactone), a QS signalling molecule. This lux-I protein functions as AHL synthase that acts on S-adenosyl-methionine (SAM) to synthesize homoserine lactone ring of AHL while acyl chains come from lipid metabolism. Another protein Lux-R serves as a receptor for AHL whose binding turns Lux-R into a transcriptional regulator for control of gene expression (Reading and Sperandio 2006). This regulation is held through a network of architecture that leads to quorum sensing. These quorum sensing networks are either arranged in parallel or series architecture to build up a strong communication system between the cells which is elucidated next by referring individual examples in the next section.

29.3.2.1 Interconnected Network of QS Circuits Arranged in Parallel

Let us consider an example of *Vibrio harveyi*, a gram-negative bioluminescence displaying marine bacterium, and show parallel arrangement of QS circuits where three distinct autoinducers and their cognate receptors interact to generate a signal leading to a shared regulatory pathway. This type of arrangement ensures that the concomitant presence or absence of these signals is inevitable to regulate activation or repression of particular genes. In order to understand this, let us focus on distinct

autoinducers produced by *V. harveyi* which are categorized into: (i) HAI-1 (3OHC4-homoserine lactone); (ii) AI-2 (Furanosyl borate diester); (iii) CAI-1. Lux-N, Lux-Q and CqsS are their cognate receptors, respectively.

When the cell density is below threshold, autoinducers are found to be in negligible amounts thus causing Lux-N, Lux-Q, and CqsS to act as kinases resulting in auto-phosphorylation of these histidine kinase type receptors. The signals received then cause phosphorylation of Lux-U, a cytoplasmic protein. Lux-U in turn phosphorylates Lux-O, a response regulator that binds to DNA. Phosphorylated Lux-O along with transcription factor σ^{54} initiates transcription of genes coding for five small RNA (sRNA) called quorum regulatory RNA (Qrr). These sRNA in collaboration with Hfq which is an RNA chaperone involved in mRNA splicing destabilizes RNA coding for transcriptional activator Lux-R (Lux-R functions to activate transcription of luciferase operon *luxCD-ABE* required for bioluminescence). Therefore at low cell density, bioluminescence is not displayed by bacteria due to the missing signals required to activate Lux-R. Whereas at high cell density, when autoinducers cross threshold concentration, the three sensor proteins switch from kinases to phosphatases yielding unphosphorylated Lux-O. Lux-O, in its unphosphorylated form cannot function to express sRNA thereby inducing translation of *lux*R mRNA and synthesis of Lux-R protein allowing display of bioluminescence (Reading and Sperandio 2006; Waters and Bassler 2005). To conclude, the concurrent activation of all three receptors by their respective autoinducers is required to achieve the targeted gene expression.

29.3.2.2 Interconnected Network of QS Circuits Arranged in Series

Here we will consider an example of *Pseudomonas aeruginosa,* a gram-negative bacterium resident of soil and also an opportunistic organism responsible for causing lung infections. These organisms possess two interconnected LuxIR type circuits where these circuits function hierarchically rather than working in parallel arrangement. LasIR and RhlIR are the two circuit systems arranged in series for regulating gene expression in *P. aeruginosa*. In LasIR type system, Las-I acts as autoinducer synthase that helps in synthesizing *N*-3-oxo-dodecanoyl-homoserine lactone (3OC12-HSL). This autoinducer then binds to Las-R forming a complex involved in positive feedback loop for stimulating more production of AHL synthase. Las-R-AHL complex also induces expression of *rhlI* and *rhlR,* genes from another circuit. RhlI produces C4-homoserine lactone (C4-HSL) which in turn binds to RhlR leading to a complex formation that induces expression of a particular set of genes (Waters and Bassler 2005). Quorum sensing achieved by either of the two circuits leads to the collective behavioural adaptation by activation or repression of target genes. Moreover, study suggests that quorum sensing is not an independent process but is interconnected to a global network linking cell communication to a wide range of physiological processes (Schuster and Greenberg 2006) Multiple QS systems operating in *Pseudomonas* spp. are a representative of temporally controlled gene expression of target genes that are regulated by overlapping QS regulons. These target genes encode for factors such as elastases, *P. aeruginosa* aminopeptidase [PaAP], and other secondary metabolites like hydrogen cyanide, pyocyanin,

rhamnolipids required for establishing virulence and other team behaviour (Mellbye and Schuster 2014).

29.4 Microbial Mediated Iron Entrapment Strategy: Sequestering the Inevitable

Iron is considered to be one of the most inevitable elements on earth due to its dedicated role as a cofactor for enzymes involved in metabolism and respiration, the two fundamental processes required for survival of organisms. However, its accessibility becomes a limiting factor due to the aerobic and neutral pH conditions prevailing biologically. These conditions oxidize iron to ferric state which is insoluble in nature. Iron exists in two biologically relevant forms ferrous (Fe^{+2}) and ferric (Fe^{+3}), former being soluble while later being insoluble. Iron can also form a complex with other elements like ferric citrate, ferric phosphate. They also couple with proteins like transferrin in mammals or with plant associated pigments. Iron plays a chief role as cofactor for certain enzymes involved in free radical scavenging, it serves an important role in electron transport chain, metabolism of carbon via TCA cycle, DNA synthesis, RNA synthesis, and regulation of gene expression (Caza and Kronstad 2013; Khan et al. 2017).

There are various strategies employed by bacteria in order to meet their demand of iron under its limiting conditions. In certain strategies, bacteria modulate the pH of their extracellular environment in order to switch ferric form to ferrous which is a relatively soluble form, whereas some bacteria make use of RIA, i.e. Reductive Iron Assimilation. In RIA, the ferric state of iron is reduced to ferrous form and taken up by a complex or in option high affinity iron permeases are used, and heme capture, transferrin/lactoferrin transfer are other modes of iron capture beside siderophore iron acquisition. Siderophores are the low molecular weight (<10KDa), high affinity molecules secreted by microorganisms in order to sequester and uptake iron from the surroundings to combat iron scarcity. Siderophore mediated iron acquisition involves excretion of high affinity iron chelators that is produced under the strong regulation by Fur (Ferric uptake regulator) (Khan et al. 2017; Sheldon et al. 2016). Not all microorganisms have ability to produce siderophores, they utilize siderophores produced by other microorganisms, termed as xenosiderophores. *Saccharomyces cerevisiae*, *Cryptococcus neoformans,* and *Candida albicans* are examples of non-siderophore producers. They rely on other microorganisms for siderophore procurement. Whereas microorganisms like *Escherichia coli* synthesize their own siderophores and also utilize iron chelators produced by other fungi.

Siderophores are mainly classified into three types based upon their chemical nature: (i) Hydroxamate siderophore; (ii) Catecholate siderophore; (iii) Carboxylate siderophore. Hydroxamate siderophores are hydrophilic molecules made up of hydroxylated and acylated alkylamines in bacteria, whereas in fungi, they are made of alkylated and hydroxylated ornithine base. Fusarinine C produced by *Aspergillus nidulans* is an example of hydroxamate siderophore. Catecholate siderophores are lipophilic molecules made up of catecholates and hydroxyls.

These siderophores bind with Fe^{+2} by their hydroxyl or catechol ends. Enterobactin produced by *Escherichia coli* is a catecholate siderophore having highest affinity towards iron as compared to other siderophores available. Carboxylate siderophores are mostly produced by fungi and some exceptional bacteria. They possess carboxyl and hydroxyl groups for iron sequestration. These siderophores are made of citric acid and β-hydroxybutyrate that can bind and sequester iron. However, there are certain organisms synthesizing siderophores having both catecholate and hydroxylate. Such siderophores are termed as mixed siderophores. Here we can quote the example of heterobactin synthesized by *Rhodococcus erythropolis* (Khan et al. 2017).

29.4.1 Fur Regulated Siderophore-Dependent Iron Homeostasis

In order to maintain the correct level of iron, tight regulation is employed by bacteria to regulate its uptake from the environment. To establish this iron homeostasis, bacteria utilize Fe^{+2} itself to transcriptionally control expression of genes coding for iron acquisition proteins. However, bioavailability of Fe^{+2} becomes possible by secretion of siderophores in conditions of iron scarcity. As described by Gao et al. (2008), from his studies on *Yersinia pestis*, Fur (Ferric uptake regulator) protein coded by *fur* genes possesses dual role of being sensory as well as regulatory. When the intracellular iron levels are high, fur proteins interact with divalent form (Fe^{+2}) of iron resulting in changed configuration that can bind to DNA at its target sequences generally termed as Fur box. This binding inhibits the genes/operons that are required for iron synthesis and uptake. While in conditions of iron scarcity, the interaction between divalent iron and fur protein fails, causing no change in configuration. This prevents binding of fur protein to its target sequence, allowing genes required for iron uptake to be expressed (Gao et al. 2008).

Pyoverdine, a yellow-green coloured fluorescent siderophore secreted by pseudomonads in order to sequester Fe^{+3} from the environment is one of the well-suited examples for siderophore synthesis under fur control. Pseudomonads including *Pseudomonas aeruginosa*, *Pseudomonas putida,* and their varying strains found colonizing rhizosphere are the producers of pyoverdine type of siderophore (Lamont and Martin 2003).

PvdS gene is required for the synthesis of pyoverdine in *Pseudomonas aeruginosa* (Ringel and Brüser 2018). Cloning and characterization studies on PvdS gene explain it to be a member of RNA polymerase sigma factor family. Pyoverdine is synthesized only in the conditions of iron scarcity. Once the abundant level of iron in the bacteria is restored, fur proteins bind to PvdS promoter inhibiting the production of siderophores thereby maintaining iron homeostasis (Cunliffe et al. 1995). Studies analysed by Liu et al. (2016), on plant pathogen *Xanthomonas vesicatoria* show that introduction of mutations in the *fur* genes resulted in increased siderophore production while complementing the *fur* genes for the wild-type *fur* gene resulted in reduced siderophore production. This suggests negative regulation of fur over siderophore synthesis. However, there are also studies on other bacteria

indicating this reciprocal association between *fur* genes and siderophore production. This negative regulation can be subjected to the intracellular level of iron in the bacterial system as explained for the pyoverdine above (Liu et al. 2016).

29.4.2 FUR Mediated Iron Uptake and Transport

As siderophore synthesis being the first step towards fulfilling the iron requirement of the organism, the uptake of siderophore–iron complex is the next crucial step for utilization of iron by them. In case of gram-negative bacteria, TBDTs (TonB dependent transporters) are the first line proteins having higher affinity for iron chelators. These transporters require energy which they procure via proton motive force. TonB help these transporters in tapping the energy which they derive from electrochemical gradients generated across membranes. Iron–siderophore complex is then further carried from periplasm to cytoplasm by ABC (ATP-binding cassette) transporters (Aznar and Dellagi 2015; Barda et al. 2010; Lau et al. 2015). *Pseudomonas aeruginosa*, a gram-negative bacterium found colonizing rhizosphere possesses two $TonB_1$ outer membrane transporters, FiuA and FoxA. These two transporters aid in uptake of iron by interacting with ferrichrome, an iron chelator. These transporter proteins are coupled with accessory proteins for supply of energy required to transport iron–ferrichrome complex. FiuB and FoxB are the inner membrane proteins serving as permease that allows transport of iron–ferrichrome complex at inner membrane level. FiuC is a cytoplasmic protein that acts as *N*-acetyltransferase causing dissociation of iron from ferrichrome resulting in free iron and acetylated desferrichrome which is then recycled back to the extracellular environment.

FiuR and FoxR are said to be the regulators for sigma factors FiuI and FoxI. FiuR and FoxR interact with $TonB_1$ towards periplasmic end and towards the cytoplasmic end they interact with sigma factors to regulate expression of genes required for iron uptake under iron limiting conditions. These sigma factors interact with RNA polymerase to transcriptionally activate expression of genes coding for receptors of ferrichrome and other proteins essential for iron transport (Barda et al. 2010). Fur proteins play a significant role in regulating the expression of genes coding for ABC transporters and energy production. Fur proteins are also necessary for expression of TonB dependent transporters. Role of fur becomes evident by the fact that fur boxes are found to be present upstream to the *fiu* genes that are involved in iron uptake systems in case of *Pseudomonas aeruginosa* (Noinaj et al. 2011).

29.4.3 Quorum Sensing Enables FUR Dependent Iron Acquisition in Rhizospheric Bacteria

Rhizospheric bacteria work in complete synchronization to establish their structure below ground such that they can benefit plants in different ways. In order to settle in the complex environment of soil where nutrient levels keep on changing constantly,

these microbes possess overlapping regulatory systems at genetic level. These two processes not only show dependence on each other rather they co-ordinate to control other factors such as virulence. To exemplify the interconnection between two major global regulatory networks, i.e. quorum sensing and iron acquisition, we will focus on *Vibrio vulnificus* where Fur–iron complex collaborates with quorum sensing in guiding the siderophore synthesis and other factors such as virulence. In a study performed by Hwang Kim et al. (2013), *vvsAB,* a gene encoding for vulnibactin siderophore said to be under the control of *fur,* showed nil expression at low cell densities in iron limiting environment. Whereas in iron-suffice environment, *vvsAB* was expressed regardless of surrounding density. This relation between cell density and iron regulation can be elucidated with the help of SmcR, a molecule analogous to LuxR of *Vibrio harveyi* and is known to be the master regulator of cell-density dependent gene regulation. In conditions of iron sufficiency, Fur–iron complex binds to the sequence upstream to the *smcR* start site in order to induce its expression under high cell-density conditions. However, when the conditions are deprived of iron, fur does not interact with regulatory sequences of *smcR* thus allowing cell density alone to influence its expression. For this reason, *smcR* can be attributed as the mediator between quorum sensing and iron acquisition. Role of *fur* in *smcR* regulation was confirmed by western hybridization where polyclonal antibodies from rats were subjected against SmcR showing lower SmcR levels under iron rich conditions. Mutations in *fur* reversed the effects and showed increased production of SmcR. Once the function of *fur* was restored by complementation with wild-type plasmid, the SmcR production again decreased in presence of iron. This suggests the suppression of quorum sensing regulator SmcR by *fur* in conditions of iron sufficiency. This SmcR in turn controls the expression of *vvpE,* the encoder of elastase, a virulence factor (Hwang Kim et al. 2013). There are multiple examples where these interconnections can be further explained. In *pseudomonas syringae*, *fur* genes were reported to regulate the synthesis of *N*-acyl homoserine lactones and vice versa (Young et al. 2008). In *Pseudomonas aeruginosa*, *fur* is found to influence the production of PQS (*Pseudomonas* quinolone signal), gene responsible for the activation of virulence factors and *pqsABCDE* operon (Oglesby et al. 2008).

In this way microorganism facilitates in solubilizing the insoluble iron present in the soil through a complex interplay between quorum sensing and iron sequestering machinery. Further these available iron complexes can also be utilized by several transporters present in plants thus helping them to buffer iron for upcoming adverse situations. This is accomplished via indirectly making alterations across the root periphery and thus establishing a fine tune among various siderophores producing microbial community.

29.5 Conclusion

Rhizospheric bacteria such as those belonging to PGPRs play a significant role in healthy sustenance of plants. PGPRs show antagonistic behaviour towards pathogens while synchronizing themselves with healthy below ground flora to

function in synergism for benefit of the plants. The establishment of this complex community amongst rhizospheric organisms is achieved by quorum sensing, a cell-density dependent communication system. There are various QS systems found across the PGPR communities where Las, Rhl, PQS, Lux are few commonly employed systems. These QS systems show different architectures where components are wired together in either series or parallel arrangement. These QS systems do not function independently rather they collaborate with other systems, iron acquisition being one of them. Siderophore synthesis is one of the major iron-acquiring systems in microorganisms which works under the control of *fur* regulon. Fur regulates iron homeostasis by interacting with the DNA directly by sensing their intracellular levels of iron. Fur also plays an important role in iron uptake and transport which becomes evident by the fact that *fur* boxes are present upstream to the iron uptake genes. These processes co-ordinate to function for the establishment of virulence, biofilm formation and toxin elimination. Moreover, the involvement of metal ion (Fe^{+3}) in regulating the synthesis of siderophore also directs our attention to the riboswitch based gene expression regulation of iron homeostasis in bacteria. Study on root exudates by metabolomics approach is helping us to explore more about the chemistry behind bacterial conversation and its connection with other processes that are prerequisite for the survival of microorganisms needed for good health of the plants.

References

Atkinson S, Throup JP, Gordon SAB (1999) A hierarchical quorum-sensing system in Yersinia pseudotuberculosis is involved in the regulation of motility and clumping. Mol Microbiol 33:1267–1277

Aznar A, Dellagi A (2015) New insights into the role of siderophores as triggers of plant immunity: what can we learn from animals? J Exp Bot 66(11):3001–3010

Baker YR, Hodgkinson JT, Florea BI, Alza E, Galloway WRJD, Grimm L, Geddis SM, Overkleeft HS, Welch M, Spring DR (2017) Identification of new quorum sensing autoinducer binding partners in *Pseudomonas aeruginosa* using photoaffinity probes. Chem Sci 8(11):7403–7411

Barda Y, Mislin LA, Shanzer A, Schalk IJ (2010) The ferrichrome uptake pathway in Pseudomonas aeruginosa involves an iron release mechanism with acylation of the siderophore and recycling of the modified desferrichrome. J Bacteriol 192(5):1212–1220

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 4:1044–1051

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Capela D, Filipe C, Bobik C, Batut J, Bruand C, Plantes-microorganismes LI, Inra-cnrs UMR (2006) Sinorhizobium meliloti differentiation during symbiosis with alfalfa: a transcriptomic dissection. Mol Plant Microbe Interact 19(4):363–372

Caza M, Kronstad JW (2013) Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. Front Cell Infect Microbiol 3:1–23

Cunliffe HE, Merriman TR, Lamont IL (1995) Cloning and characterization of pvdS, a gene required for pyoverdine synthesis in Pseudomonas aeruginosa: PvdS is probably an alternative sigma factor. J Bacteriol 177(10):2744–2750

Diggle SP, Matthijs S, Wright VJ, Fletcher MP, Chhabra SR, Lamont IL, Kong X, Hider RC, Cornelis P, Cámara M, Williams P (2007) The Pseudomonas aeruginosa 4-quinolone signal

molecules HHQ and PQS play multifunctional roles in quorum sensing and iron entrapment. Chem Biol 14(1):87–96
Gao H, Zhou D, Li Y, Guo Z, Han Y, Song Y, Zhai J, Du Z, Wang X, Lu J, Yang R (2008) The iron-responsive fur regulon in Yersinia pestis. J Bacteriol 190(8):3063–3075
Hawver LA, Jung SA, Ng WL (2016) Specificity and complexity in bacterial quorum-sensing systems. FEMS Microbiol Rev 40(5):738–752
He Y, Boon C, Zhou L, Zhang L (2009) Co-regulation of Xanthomonas campestris virulence by quorum sensing and a novel two-component regulatory system RavS/RavR. Mol Microbiol 71:1464–1476
Hense BA, Schuster M (2015) Core principles of bacterial autoinducer systems. Microbiol Mol Biol Rev 79(1):153–169
Hikichi Y, Mori Y, Ishikawa S, Hayashi K, Ohnishi K (2017) Regulation involved in colonization of intercellular spaces of host plants in Ralstonia solanacearum. Front Plant Sci 8:1–6
Hwang Kim I, Wen Y, Son JS, Lee KH, Kim KS (2013) The fur-iron complex modulates expression of the quorum-sensing master regulator, SmcR, to control expression of virulence factors in Vibrio vulnificus. Infect Immun 81(8):2888–2898
Khan A, Singh P, Srivastava A (2017) Synthesis, nature and utility of universal iron chelator – Siderophore: a review. Microbiol Res 212–213:103–111
Kloepper JW, Leong J, Teintze M, Schrotht MN (1980) Pseudomonas siderophores: a mechanism explaining disease-suppressive soils. Curr Microbiol 4:317–320
Lamont IL, Martin LW (2003) Identification and characterization of novel pyoverdine synthesis genes in Pseudomonas aeruginosa. Microbiology 149:833–842
Lau CKY, Krewulak KD, Vogel HJ (2015) Bacterial ferrous iron transport: the Feo system. FEMS Microbiol Rev 40:273–298
Lin J, Cheng J, Wang Y, Shen X (2018) The Pseudomonas quinolone signal (PQS): not just for quorum sensing anymore. Front Cell Infect Microbiol 8:1–9
Lindemann A, Pessi G, Schaefer AL, Mattmann ME, Christensen QH, Kessler A (2011) Isovaleryl-homoserine lactone, an unusual branched-chain quorum-sensing signal from the soybean symbiont *Bradyrhizobium japonicum*. PNAS 108(40):16765–16770
Liu H, Dong C, Zhao T, Han J, Wang T (2016) Functional analysis of the ferric uptake regulator gene fur in Xanthomonas vesicatoria. PLoS One 11:1–13
Lugtenberg BJJ, Bloemberg GV (2006) The *ppuI-rsaL-ppuR* quorum-sensing system regulates biofilm formation of *Pseudomonas putida* PCL1445 by controlling biosynthesis of the cyclic lipopeptides Putisolvins I and II. J Bacteriol 188(8):2898–2906
Marketon MM, Glenn SA, Eberhard A, Gonza JE (2003) Quorum sensing controls exopolysaccharide production in *Sinorhizobium meliloti*. J Bacteriol 185(1):325–331
McGrath S, Wade DS, Pesci EC (2004) Dueling quorum sensing systems in *Pseudomonas aeruginosa* control the production of the *Pseudomonas quinolone* signal (PQS). FEMS Microbiol Lett 230(1):27–34
Mellbye B, Schuster M (2014) Physiological framework for the regulation of quorum sensing-dependent public goods in *Pseudomonas aeruginosa*. J Bacteriol 196(6):1155–1164
Michiels J, Dirix G, Vanderleyden J, Xi C (2001) Processing and export of peptide pheromones and bacteriocins in gram-negative bacteria. Trends Microbiol 9(4):164–168
Mukherjee S, Bassler BL (2019) Bacterial quorum sensing in complex and dynamically changing environments. Nat Rev Microbiol 17(6):371–382
Noinaj N, Barnard T, Susan Buchanan MG (2011) TonB-dependent transporters: regulation, structure, and function. Annu Rev Microbiol 64:43–60
Oglesby AG, Iii JMF, Lee J, Tomaras AP, Greenberg EP, Pesci EC, Vasil ML (2008) The influence of iron on *Pseudomonas aeruginosa* physiology a regulatory link between iron and quorum sensing. J Biol Chem 283(23):15558–15567
Papenfort K, Bassler BL (2016) Quorum sensing signal – response systems in gram-negative bacteria. Nat Publ Group 14(9):576–588

Piper KR, Von Bodman SB (1999) Hierarchical gene regulatory systems arising from fortuitous gene associations: controlling quorum sensing by the opine regulon in agrobacterium. Mol Microbiol 32:1077–1089

Reading NC, Sperandio V (2006) Quorum sensing: the many languages of bacteria. FEMS Microbiol Lett 254(1):1–11

Rehman ZU, Leiknes TO (2018) Quorum-quenching bacteria isolated from red sea sediments reduce biofilm formation by *Pseudomonas aeruginosa*. Front Microbiol 9:1–13

Ringel MT, Brüser T (2018) The biosynthesis of pyoverdines. Microb Cell 5(10):424–437

Rosier A, Medeiros FHV, Bais HP (2018) Defining plant growth promoting rhizobacteria molecular and biochemical networks in beneficial plant-microbe interactions. Plant Soil 428(1–2):35–55

Sanchez-contreras M, Bauer WD, Gao M, Robinson JB, Downie JA, Centre JI, Lane C, Nr N (2007) Quorum-sensing regulation in rhizobia and its role in symbiotic interactions with legumes. Philos Trans R Soc B Biol Sci 362:1149–1163

Schauder S, Shokat K, Surette MG, Bassler BL (2001) The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol Microbiol 41(2):463–476

Scholz RL, Greenberg EP (2015) Sociality in *Escherichia coli*: enterochelin is a private good at low cell density and can be shared at high cell density. J Bacteriol 197(13):2122–2128

Schuster M, Greenberg EP (2006) A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. Int J Med Microbiol 296(2–3):73–81

Sexton DJ, Schuster M (2017) Nutrient limitation determines the fitness of cheaters in bacterial siderophore cooperation. Nat Commun. https://doi.org/10.1038/s41467-017-00222-2

Sharma A, Sahgal M, Johri BN (2003) Microbial communication in the rhizosphere: operation of quorum sensing. Curr Sci 85(8):1164–1172

Sheldon JR, Laakso HA, Heinrichs DE (2016) Iron acquisition strategies of bacterial pathogens. Microbiol Spectr 32:1–32

Steindler L, Bertani I, De Sordi L, Schwager S, Eberl L, Venturi V (2009) LasI/R and RhlI/R quorum sensing in a strain of *Pseudomonas aeruginosa* beneficial to plants. Appl Environ Microbiol 75(15):5131–5140

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21(1):319–346

Whitehead NA, Barnard AML, Slater H, Simpson NJL, Salmond GPC (2001) Quorum-sensing in gram-negative bacteria. FEMS Microbiol Rev 25:365–404

Wu Z, Liu Q, Li Z, Cheng W, Sun J, Guo Z, Li Y, Zhou J (2018) Environmental factors shaping the diversity of bacterial communities that promote rice production. BMC Microbiol 18:51

Young J, Seung J, Oh J, Won J, Baik HS (2008) Functional analysis of the role of Fur in the virulence of *Pseudomonas syringae* pv. tabaci 11528: Fur controls expression of genes involved in quorum-sensing. Biochem Biophys Res Commun 366:281–287

Zhang Y, Gao J, Wang L, Liu S, Bai Z, Zhuang X, Zhuang G (2018) Environmental adaptability and quorum sensing: iron uptake regulation during biofilm formation by *Paracoccus denitrificans*. Appl Environ Microbiol 84(14):1–15

Exploring the Potential of Below Ground Microbiome: Mechanism of Action, Applications, and Commercial Challenges

30

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Abstract

Rhizospheric microorganisms affect the plant community and their composition in a particular niche in a larger way. The population density of below ground microbes acts as an indicator for above ground richness of flora and fauna. These PGPR affect the plants in a positive way through their direct and indirect plant growth promoting actions. Plant communicates with their associated microbial community through chemical signaling with the help of various signaling molecules. The knowledge of plant microbe interaction and communication has been greatly increased over the year. Due to the functional potential of these microorganisms and depleting crop production, it is utmost necessary to convert laboratory innovation into the potential commercial products. A huge amount of work has been done and still going on pertaining to microbial research using single microbes or the combination of two or more microbes, known as consortia. But the problem with these products lies with their efficacy in varying ecological conditions and the product shelf life in adverse environmental conditions. It is a high time when focus must be shifted towards the knowledge driven selection of suitable strains as well as development of suitable carrier and robust formulations. The current chapter signifies the importance of PGPR, their mechanism of action, advantage of microbial consortia, aspect of consortia engineering and their various applications. This chapter also reviews the fact that how these innovations face challenges after reaching to the farmer field in the form of formulations along with the other commercial challenges.

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PGPR · Microbial consortia · Plant microbe signaling · Sustainable agriculture · Commercial challenges · Microbial engineering

30.1 Introduction

Chemical fertilizers, a key towards global agriculture, mostly rely on three major nutrients, namely Nitrogen (N), Phosphorous (P), and Potassium (K), as an essential component responsible for sustaining global food production. According to a report the compound annual growth rate (CAGR) of ammonia consumption has grown two-fold since the last 10 years and similarly the demand of phosphorus has also increased five-fold since 2014 (FAO 2015). Increasing world population has demanded an extended use of chemical fertilizer for restoring food security, which has inadvertently disturbed the ecological balance, thereby polluting the natural environment. Another concern is the unavailability of nutrients in the soil, despite being present in an adequate amount, they still remain unavailable due to their insoluble forms and thus remain unable for plant uptake. Despite the fact, that these chemical fertilizers have inadvertently played a vital role in ensuring the global food security, still it is not advisable to recommend them as a sustainable solution for future agriculture. Moreover, incessantly declining nutrient assimilation efficiency and nutrient loss via soil and water runoff have further exacerbated the situation further leading to yield reduction, increased emergence of pests, pathogens causing disease that ultimately plays a significant impact in deterioration of flora and fauna (Savci 2012).

Therefore, it has become pertinent to use more sustainable and organic way of food production to reduce our dependency on chemical fertilizers. There are multiple ways by which one can reduce the usage of chemical fertilizers which includes crop rotation, conserving tillage practices, integrated pest and nutrient management along with application of advanced management skills can make agriculture much more sustainable (NRC 1989). One of the most potent way out using all these farming techniques is employing organic farming which relies on organic and bio-inputs. Organic farming could be a better option to enrich biodiversity which also assists in ensuring food safety thereby effectively preserving our ecosystem (Hassen et al. 2016).

Biofertilizer could be the most effective input for a successful organic farming which includes plant growth promoting rhizobacteria (PGPR), arbuscular mycorrhiza (AMF), blue green algae, and other kind of microbial fertilizers. Microbial fertilizer or biofertilizers, generally known as microbial inoculants, are beneficial soil microorganisms those are cultured and multiplied commercially using an artificial media as a culture or inoculum. Biofertilizer is reported to keep the soil environment balanced and healthy by multiple ways, namely, converting insoluble nutrients to a soluble form which is done through nitrogen fixation, phosphate and potassium solubilization and their mobilization, releasing plant growth promoting

hormones, increasing organic carbon content, releasing antibiotics, cyanides, lytic enzymes, siderophores. Further these biofertilizers are known to suppress the growth of pathogens and mitigate the effect of biotic and abiotic stress via activating the intrinsic systemic resistance and are also known to improve the nutrient use efficiency in plants (Fukami et al. 2017, 2018a, b). In a related study PGPR and a combination of PGPR & AMF and 70% fertilizer were known to significantly improve the N fixation and P uptake when compared to 100% fertilizer alone (Adesemoye et al. 2009). However, not all the bacteria can be designated as PGPR, reports recommend that bacteria displaying significant traits of growth promotion are to be used for commercial biofertilizer production.

Biofertilizer sector has shown impressive growth journey globally, Brazil is known to be the biggest consumer of microbial fertilizers followed by other countries like Argentina and India (Okon et al. 2015; Sruthilaxmi and Babu 2017). In the year 2006, Department of Agriculture and Cooperation, Government of India, New Delhi, vide their order Dated 24th March, 2006, later amended in 2009, included biofertilizers and organic fertilizers under section 3 of the Essential Commodities Act, 1955 (10 of 1955), in Fertilizer (Control) Order, 1985. Initially, only the carrier based solid biofertilizer was commercially developed however due to various technical problems in solid based biofertilizer the concept of liquid based biofertilizer came into picture. Since then, many new biofertilizers were appended in the existing list, latest being consortia biofertilizer, which is a mixture of NPK fixing/solubilizing bacteria and contains more than one bacteria in a single solution.

This chapter aims to highlight the plant growth promoting ability of microbes, their molecular mechanism of action. Major emphasis will be to elucidate the potential of microbial consortia and their applications in promoting sustainable agriculture and major challenges in commercialization of microbial based agri inputs which shall be discussed in the later part of the chapter.

30.2 Plant Growth Promotion: An Innate Ability of Microorganisms

Rhizosphere is a natural habitat for microorganisms, where they multiply and favor the plant growth, thereby increasing the productivity while also reducing the disease occurrence. The root rhizosphere is the most vibrant and nutrient enriched place, where soil microflora flourishes in response to root exudates released by the host plant. Plant growth promoting microorganisms (PGPMs) generally include bacteria and fungi such as *Azotobacter, Pantoea, Azospirillum, Pseudomonas, Bacillus, Rhizobium, Enterobacter, Paenibacillus, Flavobacter, Klebsiella, Gluconobacter, Penicillium, Streptomyces,* and *Trichoderma* (Nadeem et al. 2015). If we talk about bacteria, then these can be divided as symbiotic and free living bacteria. On the basis of habitat, they can be further divided as extracellular and intracellular. Extracellular bacteria are found in rhizospheric zone in soil, near root zone of plant, or even in extracellular spaces of root cell, while intracellular bacteria live inside the plant system. They enter inside the root cell through cell wall penetration and from organs

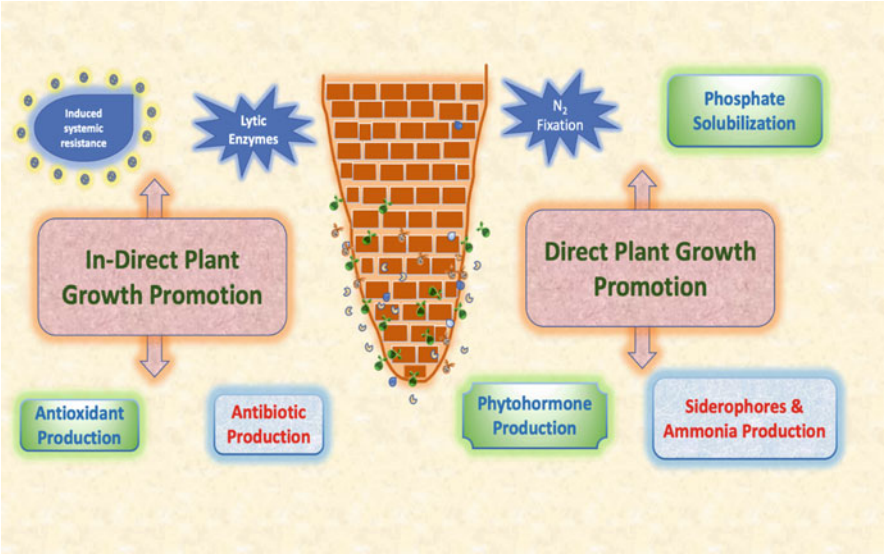

Fig. 30.1 Plant growth promotion activity exhibited by PGPR in Rhizospheric zone

like nodules in optimum conditions inside the plant system (Stamenković et al. 2018). These microorganisms are reported to change the whole microbiome of rhizosphere thus exhibiting plant growth promotion via solubilizing the insoluble nutrients while others act as bio-control agents and protect the plant from adverse environmental conditions by producing various hormones, enzymes, organic acids, antibiotics (Owen et al. 2015). In general, these growth promoting microbes exhibit direct and indirect mechanism of plant growth. Solubilization and acquisition of nutrients and essential minerals, via altering the plant hormone concentration, are considered as direct plant growth promoting mechanisms, whereas environmental stress mitigation, bio-control, and production of secondary molecules are assigned as indirect mechanism. The effect of these mechanisms in the form of yield enhancement and better plant health is reviewed by many researchers in various crops like cereals, pulses, horticultural crops, citrus fruits, plantation crops, ornamental plants, vegetables, and trees. It is interesting that plant can easily differentiate between these PGPMs and pathogenic microbes with the help of receptors through microbe associated molecular pattern (MAMP) which plays key role in microbe plant interaction and signaling (Finkel et al. 2017). It is reported that various molecules such as sugar, organic acids, flavonoids trigger the process of root nodulation and further release volatile chemicals via various signaling mechanism during a typical microbe plant communication. The type and composition of these signaling molecules are largely dependent on the microbial strain and the host plant (Qin et al. 2016). These signaling molecules act as a signal to initiate the process of nodulation or root colonization. After colonization, bacteria starts multiplying inside the plant cell and starts exhibiting the beneficial characteristics (Lugtenberg 2015) (Fig. 30.1).

The first commercialized bacterial culture was rhizobium especially formulated for leguminous plants. In the middle of 1990s, *Bacillus* spp. have gained so much attention as a seed dressing and were registered in more than seven crops and inoculated in the area of more than 2 million hectare (Backman et al. 1994). Later the chitosan based microbial formulation started to commercialize which had shown remarkable growth in tomato, pepper, and cucumber. Gradually many plant growth promoting bacterial and fungal cultures were commercialized and formulated as a single or mixed inoculum (Kavamura et al. 2013; Mumtaz et al. 2017). Among all bacterial cultures, *Pseudomonas* was reported to be most aggressive in colonizing various plants and exhibited broad spectrum antagonistic activities against a wide variety of pathogenic microbial hosts (Davison 1988). The damping off disease in cotton caused by *R. Solani* is controlled by antibiotic produced by *Pseudomonas fluorescens* (Howell and Stipanovic 1979). Furthermore, the combined formulation of *Bacillus polymyxa* and *Pseudomonas strica* was reported to increase grain yield and dry weight and nutrient uptake in sorghum (Alagawadi and Gaur 1992).

30.2.1 Potential Significance of Microbes in Sustainable Agriculture

Plant growth promoting rhizospheric microorganisms reside in the rhizospheric zone of the plant in the abundant numbers, i.e.10^{11} microbial cells per gram of root (Egamberdieva et al. 2008). Apart from them, approximately 30,000 prokaryotic species are also reported to be present in that zone. If we talk about the genome of whole microbial community residing around the plant, then it is larger than the genome of plant itself which is termed as microbiome (Mendes et al. 2013). These microbes interact synergistically with each other and their signaling governs the overall health of plant by accomplishing various mechanisms within the plant as well as outside the host plant, namely nutrient recycling, soil moisture maintenance, nutrient acquisition, pest control, regulation of abiotic stress, and organic matter decomposition (Berg et al. 2013). These microbial inoculants are a set of diverse microorganisms, which are inoculated in the soil to enhance the physicochemical property and microbial diversity of soil thus improving plant growth and enhancing overall crop productivity (Sahoo et al. 2014). Agriculturally important microbial communities having diverse group of microbes include nitrogen fixing bacteria and cyanobacteria, phosphate solubilizing/mobilizing bacteria and mycorrhiza, disease suppressive bacteria and fungus, stress tolerant microbes, and bio-degrading microorganisms (Singh et al. 2011).

Among PGPR, *Azotobacter*, *Azospirillum*, *Bacillus*, *Pantoea*, *Pseudomonas,* and *Rhizobium* are the most potent and efficient and are widely reported by many authors. Azotobacter, a free living bacteria, is reported to have nitrogen fixing ability and has a very important role in regulating global nitrogen cycle (Sahoo et al. 2014). Apart from the nitrogen fixation, *Azotobacter* is also reported to produce many vitamins like thiamine, riboflavin (Revillas et al. 2000), and phytohormones like

gibberellins, cytokinins, and indole acetic acid (Abd El-Fattah et al. 2013). Some of the species like *Azotobacter paspali*, *Azotobacter salinestris,* and *Azotobacter beijerinckii* also produce different types of siderophores (Collinson et al. 1987; Kannapiran and Sri Ramkumar 2011). *Azotobacter* spp. also exhibit bio-control traits against *Rhizoctonia solani* (Fatima et al. 2009), *Fusarium oxysporum* (Chauhan et al. 2012), *Macrophomina phaseolina* (Dubey et al. 2012). Azospirillum genus is microaerophilic nitrogen fixer which resides in the extracellular zone of roots. *Azospirillum brasilense* and *Azospirillum lipoferum* are the species which were discovered at first among all (Lin et al. 2015). Azospirillum is known to have several PGPR traits like siderophores production by *Azospirillum brasilense* (Bachhawat and Ghosh 1987) and *Azospirillum lipoferum* (Saxena et al. 1989), phytohormones production like abscisic acid (Perrig et al. 2007), IAA, ACCD, gibberellic acid (GA), and HCN production (Sahoo et al. 2014). It has also been reported for its antagonistic properties against pathogens like *Colletotrichum acutatum* (Tortora et al. 2011). Another important genera *Bacillus* also holds a significant position, as it is known to display PGP traits as well as bio-control properties against some virulent pathogens. *Bacillus subtilis,* being a model species of this genera, is extensively studied and described by (Graumann 2007). Many species like *B. megaterium* (Byers et al. 1967), *B. subtilis* (Ito and Neilands 1958), *B. amyloliquefaciens* (Niazi et al. 2014) are known for siderophores production. Other PGP properties like synthesis of ACCD, IAA (Kumar et al. 2014), ammonia (Ahmad et al. 2008), GA (Lenin and Jayanthi 2012), and production of various organic acids for solubilizing the insoluble phosphate (Pourbabaee et al. 2018) have been reported in this genera. Bio-control properties against several pathogenic microbes such as *Rhizoctonia solani*, *Fusarium oxysporum* (Kumar et al. 2014), *Macrophomina phaseolina* (Kesaulya et al. 2018), and *Ralstonia solanacearum* (Huang et al. 2016) have also been reported so far.

Pantoea genus is ideal for making commercial formulation related to medical, environmental, and agriculture. Various PGP properties like siderophores production by *Pantoea agglomerans, Pantoea eucalyptii* (Viruel et al. 2011), *Pantoea allii* (Pereira and Castro 2014), phosphate or nitrogen fixation (Kim et al. 2012), phytohormones synthesis like abscisic acid, IAA, and GA (Feng et al. 2006) are reported in various species of *Pantoea*.

Apart from these genera, other important bacteria responsible for growth promotion are *Pseudomonas* and *Rhizobium*. In *Pseudomonas* spp., properties, namely HCN and ammonia production (Subramanian and Satyan 2014), siderophores production (Kloepper et al. 1980), production of IAA, ACD, (Bona et al. 2017), phosphate solubilization (Subramanian and Satyan 2014), and nitrogen fixation (Pham et al. 2017) are being studied extensively. Pseudomonas is known for its antagonistic properties against various microorganisms like *Fusarium oxysporum* (Monali et al. 2018), *Pythium ultimum, Fusarium udum* (Sulochana et al. 2014), and *Erwinia amylovora* (Bahadou et al. 2018).

Genus Rhizobium, well-known nitrogen fixers, is commonly found in the nodules of leguminous plant roots. Apart from PGP traits like siderophore production (Eng-Wilmot and Van der Helm 1980), phosphate solubilization (Xing et al.

2016), nitrogen fixation (Datta et al. 2015), production of IAA and ACCD (Hernández et al. 2017), the antagonistic properties of *Rhizobium* spp. are also reported against some virulent pathogens like *Fusarium oxysporum* (Arfaoui et al. 2006), *Sclerotium rolfsii*, *Rhizoctonia solani*, *Macrophomina phaseolina,* and *Aspergillus niger* (Deboja and Manoja 2010) (Table 30.1).

30.3 Physiological Functions of Plant Growth Promoting Rhizobacteria

From the previous section, it is clear that microorganisms play a regulatory role in various biochemical and nutrient cycles of the plant system and proved to be beneficial for the plant health. It has been observed that the soil with high microbial diversity and increased organic matter has less requirement of fertilizer supplement when compared to conventionally managed soil (Bender et al. 2016). The molecular basis of plant microbe interaction is still not very well defined however due to modern approaches like Omics, it has been started to unfold gradually (Backer et al. 2018).

30.3.1 Microbes Mediated Nutrient Acquisition in Plants

The symbiotic relationship between Rhizobia and Leguminous plant is the most studied and explored plant microbe relationship. In this association, both partners are mutually benefitted as the plant provides shelter and reduced form of carbon to bacteria, whereas bacteria in turn fix the nitrogen for the plant. During this relationship both the partner undergo physiological changes. Due to colonization of Rhizobium, a new organ is formed inside the plant while bacteria from its rod shaped structure are converted into branched bacteroid (Oke and Long 1999). As a result of this association, globally 20–22 teragram nitrogen (Tg N) (Herridge et al. 2008) upto 40 Tg N (Galloway et al. 2008) nitrogen is being fixed annually. With the start of early twenty-first century, the commercialization of free living nitrogen fixing bacterial inoculants other than Rhizobia has gradually started. Among them, mainly are *Azospirillum* sp., *Azotobacter* sp., *Bacillus polymyxa, Burkholderia* sp., *Diazotrophicus* sp., *Gluconobacter* sp., and *Herbaspirillum sp*. (Vessey 2003). These bacterial inoculants are known to have broader host range than rhizobium.

The next most essential nutrient for plant growth after nitrogen is phosphorous (P) which is present in soil in a bulk amount however, being in insoluble form, it is not available readily to the plants. To overcome this difficulty the role of phosphorous solubilizing bacteria (PSB) comes in the picture as they efficiently solubilize the inorganic phosphorous, coupled with calcium, iron or aluminum, through the secretion of organic acid. PSB are also known to produce phytase which can extract reactive P from organic compounds (Backer et al. 2018). These bacteria are also reported to secrete HCN which is supposed to have adverse effect on pathogen. In contrary to this, Rijavec and Lapanje (2016) have debated the role of HCN for

Table 30.1 Types of PGPR and their contribution in improved crop health and yield.

Microbial Group	Name of microbes	Host plant	Key roles	References
Gram positive Bacteria	Bacillus sp.	Corn, soybean, wheat	Increased plant growth	Akinrinlola et al. (2018)
	Bacillus aryabhattai	Grape	Increased plant growth and yield significantly	Liu et al. (2016)
	Bacillus sp.	Tomato	Improved the plant growth, yield, and quality of fruit	Widnyana (2018)
	Bacillus drentensis	Mung bean	Improved the plant growth, yield, and abiotic stress resistance	Mahmood et al. 2016
	Bacillus subtilis, Bacillus amyloliquefaciens	Cucumber	Improved germination, seedling vigor, growth, and N content in root and shoot tissue	Islam et al. (2016)
Gram negative Bacteria	*Azotobacter chroococcum*	Maize	Reported increase in plant root and dry weight of plant	Sachin and Misra (2009)
	Pseudomonas aeruginosa	Fabba beans	Increased root and shoot dry weight	Haddoudi et al. (2017)
	Pseudomonas sp.	Tomato	Improved the plant growth, yield, and quality of fruit	Widnyana (2018)
	Enterobacter cloacae	Mung bean	Improved the plant growth, yield, and abiotic stress resistance	Mahmood et al. 2016
	Pseudomonas stutzeri, Stenotrophomonas maltophilia	Cucumber	Improved germination, seedling vigor, growth, and N content in root and shoot tissue	Islam et al. (2016)
	Pantoea and *Enterococcus*	Mung bean	Reduced Na concentration, enhanced antioxidants (ascorbic acid and glutathione)	Panwar et al. (2016)
	Rhizobium sp. BARIRGm901	Soybean	Increased the nodulation, growth, and yield of crop	Alam et al. (2015)
Fungi	*Mycorrhizae (Glomus* sp.)	Barley	Improved water use efficiency, increased yield content	Abdelhameid and Kenawey (2019)
	S. cerevisiae	In situ study	Increased Zn uptake	Martha-Paz et al. (2019)
Algae	Algal biochar	Maize	Improved physiology, dry and fresh weight of shoot and root under water deficit condition	Ullah et al. (2019)
	Cyanobacteria based compost	Cotton	Enhanced fresh weight, germination, and microbiological activities	Prasanna et al. (2015)

increasing the P availability via metal chelation and sequestration. The strains of phosphate solubilizer *Bacillus megaterium* have been formulated and commercialized by BioPower Lanka, Sri Lanka and strains like *Pseudomonas striata, B. megaterium,* and *B. polymyxa* had been commercialized by AgriLife, India (Mehnaz 2016).

In addition to nitrogen and phosphorous solubilizing bacteria, the potassium (K) solubilizing bacteria, namely *Bacillus mucilagenosus* are also being developed commercially which may prove imperative for K availability in plants. Unlike the macronutrients, sometimes micronutrients like Fe and Zn may also play an essential role in plant growth and can also prove to be growth limiting many times. Bacteria also play important role in iron sequestration by producing organic acids or siderophores and make it available for the plants. Therefore, Agri Life India has successfully developed the commercial strain of Fe mobilizing bacteria *Acidithiobacillus ferrooxidans* (Backer et al. 2018). Notably, solubilization of Fe in this strain has been reported through organic acid production rather than siderophores production (Bhatti and Yawar 2010). Similarly, Zn, Si solubilizing bacteria have been reported and commercialized till date which shows increase in uptake of micronutrient in the plants (Kumawat et al. 2017).

30.3.2 Signaling Between Plant Roots and PGPR

30.3.2.1 Signaling in Symbiotic Bacteria

(a) **Rhizobial NOD factors: Lipo-chitooligosaccharides (LCOs)**

In the previous part of this chapter, we have read about mutualistic relationship between Rhizobium and leguminous plants. Now, we will discuss about the molecular mechanism working behind this relationship. As *Rhizobia* encounters the host plant it so on initiates the colonizing process that results in the release of some flavonoids, which in turn, induces the Nod factors (Kondorosi et al. 1989). Nod factors are basically lipo-chitooligosaccharides (LCOs) which has β1,4 linked polymers of N-acetylglucosamine (GlcNAc) as core of chitin. N-acyl moiety which has a long fatty acid chain is a key feature of LCO which make them different from that of chitin. These fatty acid side chains vary in their length, substitution groups which make them unique between the *Rhizobial* strains and their selective host range (Oldroyd 2013). LCOs are known to be involved in plant growth promoting activities like establishing mutual relationships between plant and bacterial strains thus improving the level of photosynthesis as well as enhancing plant resistance (Rey et al. 2013). These LCOs receptor of the leguminous plants are identified as lysin motif containing receptor-like kinase family (Liang et al. 2014). These receptors are found in almost all bacterial genera except Archaea and elicit the signal by combining MAMPs including chitin (Antolín-Llovera et al. 2012). Being universal in nature, these LysM receptors, LCOs are significant signaling molecule in rhizospheric biology and affect the plant growth and trigger positive effects on plants (Rosier et al. 2018). In a transcriptomic study a significant alteration in the

level of gene expressions in response to LCO was reported where the growth and secondary metabolism related genes and their respective transcriptional factors were found to be upregulated, whereas stress inducing genes were downregulated in a maize treated with 10 nM LCO (Tanaka et al. 2015). Promoter fusion reporter constructs were subsequently generated for genes having strong positive responses to induction by LCO. Total four genes were identified and analyzed via promoter fusion reporter construct transformed to maize roots out of which the CaMB gene, that encodes calmodulin-binding protein, showed a three-fold enhancement in the expression level. It was primarily found to be localized in in the epidermal layer of roots, putatively role in ABA mediated hormone signaling, resulting in altering the root growth and development of maize roots.

(b) **Rhizobial Nop effectors type III secretion system (T_3SS)**

Pathogenic bacteria are known to exert their virulence effect through T_3SS system. However, this system is not confined up to pathogenic bacteria only but it is also present in various symbiotic and non-symbiotic bacteria. In rhizobia, it is a major signaling mechanism to suppress the host immunity, to identify the specific host and development of nodulation. Plant hormone signaling cascade is known to stimulate plant growth and provide immunity to the plant which is only possible through crosstalk between growth and defense mechanisms (Karasov et al. 2017). Lipopolysaccharides (LPS) and exopolysaccharides (EPS) are carbohydrate signaling molecule which are being used in suppressing the plant immunity for successful plant bacterial inoculation (Jones et al. 2008). Their interaction occurs through type III secretion system (T3SS) at plant cell membrane and in this way plant rhizobial interaction successfully takes place. Effector protein is directly delivered inside the cell under the mechanism called T3SS and is reported in pathogenic bacterial signaling system (Ji and Dong 2015). Many rhizobial genera like *Bradyrhizobium* and *Sinorhizobium* are reported to have this type of system. Effector protein in this case is termed as nodulating outer protein (NOP) and is transported through T3SS mechanism and bacteria secrete them in response to flavonoid (de Campos et al. 2011). It is reported that NOP acts as a key protein in suppressing the immune response of host plant. Moreover, a NOPL is reported to downregulate the transcription of PR proteins such as glucanases and chitinases which are further regulated by MAP-kinase pathway (Bartsev et al. 2004). The role of NOPL as a substrate of MAP-kinase responsible for host defense suppression is recently been reported by Ge et al. 2016.

30.3.2.2 Signaling in Non-Symbiotic Bacteria

Exometabolites produced by rhizospheric bacteria act as a signaling molecule which induces the positive effect on plant growth and immunity (Wiesel et al. 2014). Complete molecular pathways behind these physiological changes are not elucidated yet but it is clear that these secondary metabolites have a definite role in plant growth and immunity. Some of these metabolites are discussed in the following section:

(a) **Phytohormones**

As we have discussed earlier, PGPR has the innate ability of plant growth promotion and this is possible through various phytohormones or structurally similar molecules produced by bacteria (Spaepen 2015). These chemicals produced by the bacteria match the structures and functions of plant hormones such as gibberellic acid, auxin, and ethylene (Singh et al. 2011). Many researchers reported production of phytohormones like chemicals in various PGPR like *Bradyrhizobium japonicum, R. solanacearum, Erwinia chrysanthemi, B. amyloliquefaciens, and Paenibacillus polymyxa strain BFKC01*either for plant growth promotion or for suppression of pathogens (Zhou et al. 2016).

(b) **Bacterial Volatiles**

Plant and bacteria both are reported to produce these aromatic molecules called as volatile organic compounds (VOC). Recently, two authors Chung et al. (2016) and Audrain et al. (2015) have reported that these chemical compounds are having low molecular weight and they can be organic as well as inorganic in nature, therefore to be more specific, they termed them Bacterial Volatile Compounds (BVC). BVCs are known to have a role in plant growth promotion and induction of plant defense. In Blom et al. (2011)), conducted a large scale experiment on 42 rhizospheric bacterial strains and reported that *Burkholderia and Pseudomonas* were able to downregulated the ethylene production in response to the MAMP elicitor flg22. These molecules are also reported to have some role in expression of nutrient ion transporters. *Bacillus subtilis* GB03 is reported to augment the iron accumulation in *Arabidopsis* (Zhang et al. 2009).

(c) **Signaling through N-acyl homoserine lactones**

Quorum sensing is a density dependent technique used by the microorganisms in order to communicate among each other on inter/intra species level. It is a major communication mode among bacterial genera present in particular rhizo-microbiome (Lowery et al. 2008). Both gram positive and negative exhibit the quorum sensing technique for communication but the difference lies in the type of signaling molecule, their structure, and the response mechanism. Extracellular signal molecule also known as Auto Inducer (AI) is well studied in gram negative bacteria. Auto inducer is produced by AI synthase which further binds with transcription regulator protein and upregulates the expression of operon related to quorum sensing. Environmental condition acts as an inducer for the synthesis of AI and with the increase of population density, the signal becomes strong and a positive feedback traverses among the bacterial community (Rosier et al. 2018). Many rhizospheric bacteria use this signaling mechanism for their survival and other functions related to the environment (Venturi and Fuqua 2013). These activities are tightly regulated by AI concentration, its structure, and regulator protein. Many researchers also reported the involvement of QS in inter-species crosstalk (Rosier

et al. 2018). N-acyl homoserine lactones (AHL) act as a signal molecule for gram negative bacteria. AHL contains a lactone ring and an acyl side chain of different lengths, hydroxyl groups, and degree of saturation (Chernin 2011). These side chains can vary from C6 to C18 and it has been reported that long chains are mostly preferred among bacteria. For example, *rhizobia* contain C6 to C18 chains (Teplitski et al. 2003), while *Pseudomonas* exhibit C6 to C12 long chains of AHL molecule (Ortíz-Castro et al. 2008). These different AHL display different functions in the plant system (Schikora et al. 2016) right from the transpiration and stomatal control response in *Phaseolus vulgaris* L. (Joseph and Phillips 2003), increase in nodulation, flavonoid synthesis, hormone synthesis, and increasing plant defense through root inoculation with *S. meliloti*-specific 3-oxo-C14-HL.

Many more signaling molecules in plant PGPR interaction are yet to be discovered, which are able to stimulate plant growth, immune system, and nutrition related pathways. One such example of signal molecule is cyclodipeptides (CDPs) and their significant role in lateral root growth has been reported in *P. aeruginosa* (Ortiz-Castro et al. 2011).

30.4 Engineering Microbial Consortia: A New Avenue in PGPR Research

Microbial consortia are mix population of two or more genera of microbes which are proved to be efficient in environmental remediation, helping in food digestion, waste water treatment, and in sustainable agriculture. These microbes in mixed population can perform those tasks which are impossible for an individual stain. In natural ecosystem, these microbes reside in complex microbial communities called microbiome, where they take part in a complex global cycling of nitrogen, oxygen, and carbon. Use of these naturally occurring microbial flora is very common and ancient practice in food other industries (Bader et al. 2010). Each member of the group has to work hard to be remained in the community. Two bioleaching bacteria *Ferroplasma acidiphilum* and *Leptospirillum ferriphilum* which coexist in their natural habitat, i.e. acid mine drainage represent the excellent example of naturally occurring microbial consortia (Merino et al. 2015). They both oxidize iron and sulfur element symbiotically in their habitat (Merino et al. 2015, 2016). These natural consortia were further studied for understanding their metabolic process when both are growing symbiotically through metabolic model constructed using mixed culture of both of the bacteria (Merino et al. 2015). This study revealed that *Ferroplasma acidiphilum* uses organic matter for its growth produced by *Leptospirillum ferriphilum,* thereby helping *L. ferriphilum* by intoxicating the environment by consuming organic matter and lowering down their toxic concentration of organic matter for *L. ferriphilum*. (Merino et al. 2016). Second example of naturally occurring microbial consortia is the rumen bacteria which reside in the gut of herbivores. They work collaboratively to digest plant biomass directly to simple sugar by producing cellulolytic enzymes.

30.4.1 Recent Advances in Microbial Consortia Engineering

In this era, synthetic ecology is emerged as a new applied branch of synthetic biology (Fredrickson 2015). Many studies reveal that synthetic microbial consortia are also being constructed and functioning efficiently. These synthetic consortia mimic the synergies and interactions used by natural microbial communities for the efficient degradation.

These microbial consortia are designed specifically in a way that they may co-cultivate simultaneously for extensive metabolic engineering in various biotechnological applications and in greenhouse gas management (Hill et al. 2017).

New microbial inoculants are added for stimulating the native microbial network of the soil and in this way the beneficial and functional microbial groups are reactivated, whose availability usually diminishes over time, reasons may be excessive fertilization and exhaustive cropping system (Stringlis et al. 2018). This new microbiome protects the plant from various abiotic stresses (Van Oosten et al. 2017), heavy metal and pesticide pollution (Ventorino et al. 2014) via activating nitrogen fixation, augmenting macro and micronutrient solubilization/mobilization, initiating the production of beneficial microbial byproducts, namely exopolysaccharides and antibiotics for an enhanced plant protection.

It has been established from the research that these new microbial inoculants establish close association with native bacteria and fungi which are competent enough to survive with them. (Bonanomi et al. 2018). These bacteria and fungus consortia have the capability to build up the novel microbial communities (Ahmad et al. 2011; Lugtenberg 2016) and activate the PGP traits which are not possible through inoculation of single species.

To engineer the microbial consortia, it is necessary to screen and isolate the potential strains, compatibility analysis, screening for the potential characteristics, analysis and evaluation of the effects on native ecosystem, development of suitable production technology with effective formulation, and to reach it up to end user with all technical support (Kong et al. 2018). The basic mechanism regulating these complex inter or intrageneric interactions can only be elucidated through omics study. These studies can also provide some new mechanism that can also help in formulating new generation biostimulants (Fiorentino et al. 2018; Ventorino et al. 2018).

One example of synthetic microbial consortia is co-cultivation of a methanotrophic bacterium, *Methylomicrobium alcaliphilum* and a cyanobacterium, *Synechococcu*s PCC 7002 for transforming greenhouse gasses to microbial biomass through oxidative photosynthesis (Hill et al. 2017). Engineered microbial consortia give insights about metabolic coupling between oxidative photosynthesis and methane production. This type of co-cultivation model offered a platform for successfully transforming harmful greenhouse gases into microbial biomass and can be used to develop various such kind of products for a safe and sustainable future. In another example, three different strains, *Ketogulonicigenium vulgare, Gluconobacter oxydans, and Bacillus* spp. were employed to produce a vitamin C precursor-2-keto-l-gulonic acid (2-KLG) on industrial level via two step fermentation

technology (Guleria et al. 2017). In the above process, firstly fermenter process starts with *G. oxydans,* which converts D-Sorbitol to L-Sorbose by sorbitol dehydrogenase (SLDH). In the second step, *K. vulgare* and *B. megaterium* further convert this L-sorbose to L-sorbosone through L-sorbose dehydrogenase and it is further converted into 2-KLG by L-sorbone dehydrogenase. This is very high yielding process where the yield is said to be around 97% but is too complex to perform. To make it rather easy one step fermentation process, a consortium of *G. oxydans* and *K. vulgare* was redesigned and the product was achieved through one step fermentation with 87% yield in very less time which is at par with the previous process.

In this way, the potential of engineering the microbial communities strengthens its sustainable future ahead. It is expected that many synthetic consortia having multiple specialized microbial members or polycultures will be developed in near future to simplify the more complicated biosynthetic pathways. This new technology will provide an opportunity to study microbial ecosystem as a new area of research.

30.5 Challenges in Commercializing Microbial Based Agro-Inputs

The growing literature of agro-input indicates that there is emerging demand of microbial based products in agriculture. In many developing countries, they are being used effectively in large scale. In the country supporting organic agriculture, this microbial inoculum is growing rapidly and has a remarkable role in sustainable agriculture. But the limiting factor in commercialization of these microbial products is their specificity, selectivity, and time requirement for reaching the market. Furthermore they are known to be very selective to their niche and the environmental factors. When they are released in the open environment, where multiple factors work simultaneously, their quality and efficacy remain in question. On the contrary, chemical based agro-inputs have broad spectrum and are least affected with the environmental factors. In addition to these challenges, these products also face the registration and other legal regulation related challenges.

30.5.1 Challenges with Registration and Legal Framework

In India, policy reforms need to be simple and realistic so that it can meet the needs of consumer and producer that will ultimately affect the overall supply chain. There is urgent need to regularize the policy framework of these products in such a way so that infrastructure can be provided in rural area and farmer can achieve good returns and can lead to minimization of wastage (Sundar 2016). Now in response to the rapid change in the trend of global agribusiness, the priority of market has been more focused towards quality and diversification rather than increased quantity of the product. The legal regulation related to agribusiness has touched the highest standards. It is approaching all the area related to food health, environment

protection, safety standards, and child labor issues. It is more complicated in the segment of plant protection and bio-control products. These products sometimes reach the market in a span of 5–7 years including the registration process. In India, the registration process is a state affair in case of agro-inputs excluding fertilizers. Rules vary from one state to another and sometimes are very complex. It is very tough for an entrepreneur to wait for such a long time for releasing a single product. It is a high time when Government of India should take strong initiatives to regularize agro-inputs especially biostimulants.

30.5.2 Challenges Regarding Product Quality and Efficacy

It is evident that the efficacy of microbial products under field condition is in question. It is inconsistent after some time of its release in the market and requires some new innovations and further lab studies. The problem lies with the production of microbes abundantly in a synthetic environment as it is not certain that how one bacteria will behave in community or in isolation. It is necessary to conduct quantitative studies about the traits or characters which are going to be affected when these microbes are kept under varying degree of stress conditions. So the real challenge begins when the microbial products are applied for the crop improvement and they respond differently in different crops based on habitat and community structure of that particular plant roots. When we synthesize any microbial product in lab then our fundamental objective is to produce the specific trait which is going to induce specific biological activities in the target crop. This specific requirement needs innovative strategies targeting the product metabolism into the desired location. So the product to be effective, we have to understand the specific microbiome community residing in the root of target crop and the metabolic flux of the host plant. It is already accepted widely in medical science that biology is ultimately an information science (Nam et al. 2014; Rolfsson and Palsson 2015). Recent, technological advances have enabled us to understand and differentiate between genes and their response during microbe plant interaction. Using simple engineering technique, information technology, we can generate the data related to genes, signaling molecules involved, and their related metabolic pathways (Andrianantoandro et al. 2006; Pulendran et al. 2010). Through bioinformatics, these data are mined and are being used to create hypothesis behind certain biological changes. This type of systems biology approach is also being applied in vaccine science. Hence, in order to improve the inconsistency and efficacy of microbial products, the innovative molecular and genetic technologies along with bioinformatics should be utilized for reconstructing biochemical machinery. It can work as a base system using in-silico modeling followed by understanding and validating the plant microbe interaction.

This advanced system biology approach was used to study the vast microbial communities found in nature. As an example through such advanced studies, it could become possible to study microbial communities which form biofilm, which is basically a layer of protection and cellular balance system, wherein complex

microbial communities reside under natural conditions (Timmusk et al. 2015; Timmusk and Nevo 2011). This biofilm can be originated from a single bacterial species or from complex communities of several bacterial species. They coordinate for their metabolic activities and gene expression in a density dependent manner, a process called quorum sensing. Many researchers have reported that this biofilm represents a high level of coordination among cells. (Gestel et al. 2015). Another example is the signaling mechanism and molecules which are being reported in symbiotic as well as non-symbiotic plant system as described in the earlier part of this chapter.

30.6 Concluding Remarks

Through extensive research, it is now evident that the relationship between plant and microbiome is very historic and they are developed gradually through coevolution. This coevolution is only possible through interaction of microbes with plants. These interactions are the result of communication between the two through various types of signaling molecules. This process is constant and it is expected that some more new and novel relationships to be explored in coming time which will proved to be beneficial for crop yield and global food production. It is clearly known that these microbial strains exhibit some plant growth promoting characters which are beneficial for crop and sustainable agriculture but our understanding is in very primitive stage. The common and easiest way out to explore consortia is having very few members and analyzing the signal molecule they produce. Some products can be tested for plant growth promoting traits and the effect under adverse environment conditions like drought, heat, etc., while some can be analyzed for their ability to resist biotic stress and pest infestation. One should also keep in the mind that the regulatory framework of these products must be clear and easy because at this point of time the general perception of the people regarding bio-products is not very clear but it somewhat positive. The main reason behind this perception being positive is the adverse effect imposed by chemical inputs in the soil. Gradually these biological inputs are considered as an alternative to the chemical inputs. These naturally originated biostimulants are having great potential for agricultural benefit with regard to global food security and sustainable agriculture. It is definite that in this era of climate change these formulations will prove to be a boon if development and usage will be done consciously. There is urgent need of strict and serious intervention of government to deliver these formulations intact directly to the farmer's field. Mathematical modeling based on the engineered microbial consortia would help the rhizospheric microbes to deliver their best traits in crop production. This would confirm that the great potential of PGPB/PGPR science would discover its approach to expediting reproducible field application and sustainable food production under climate change conditions.

References

Abd El-Fattah DA, Eweda WE, Zayed MS, Hassanein MK (2013) Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of Azotobacter chroococcum inoculant. Ann Agric Sci 58(2):111–118

Abdelhameid NM, Kenawey KM (2019) Response of barley to bio fertilization with mycorrhiza and azotobacter under supplemental irrigation conditions at the North Western coast of Egypt. ASEJ 40:672–682

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58(4):921–929

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163(2):173–181

Ahmad I, Khan MSA, Aqil F, Singh M (2011) Microbial applications in agriculture and the environment: a broad perspective. In: Microbes and microbial technology. Springer, New York, pp 1–27

Akinrinlola RJ, Yuen GY, Drijber RA, Adesemoye AO (2018) Evaluation of Bacillus strains for plant growth promotion and predictability of efficacy by in vitro physiological traits. Int J Microbiol. https://doi.org/10.1155/2018/5686874

Alagawadi AR, Gaur AC (1992) Inoculation of Azospirillum brasilense and phosphate-solubilizing bacteria on yield of sorghum [*Sorghum bicolor* (L.) Moench] in dry land. Trop Agric 69:347–350

Alam F, Bhuiyan MAH, Alam SS, Waghmode TR, Kim PJ, Lee YB (2015) Effect of rhizobium sp. BARIRGm901 inoculation on nodulation, nitrogen fixation and yield of soybean (*Glycine max*) genotypes in gray terrace soil. Biosci Biotechnol Biochem 79(10):1660–1668

Andrianantoandro E, Basu S, Karig DK, Weiss R (2006) Synthetic biology: new engineering rules for an emerging discipline. Mol Syst Biol 2(1):2006–0028

Antolín-Llovera M, Ried MK, Binder A, Parniske M (2012) Receptor kinase signaling pathways in plant-microbe interactions. Annu Rev Phytopathol 50:451–473

Arfaoui A, Sifi B, Boudabous A, Hadrami IE, Cherif M (2006) Identification of rhizobium isolates possessing antagonistic activity against Fusarium oxysporum f. sp. ciceris, the causal agent of Fusarium wilt of chickpea. J Plant Pathol 88:67–75

Audrain B, Farag MA, Ryu CM, Ghigo JM (2015) Role of bacterial volatile compounds in bacterial biology. FEMS Microbiol Rev 39(2):222–233

Bachhawat AK, Ghosh S (1987) Iron transport in Azospirillum brasilense: role of the siderophore spirilobactin. Microbiology 133(7):1759–1765

Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473

Backman P, Brannen PM, Mahaffee WF (1994) Plant response and disease control following seed inoculation with *Bacillus subtilis*. In: Improving plant productivity with rhizosphere bacteria. Elsevier, New York, pp 3–8

Bader J, Mast-Gerlach E, Popović MK, Bajpai R, Stahl U (2010) Relevance of microbial coculture fermentations in biotechnology. J Appl Microbiol 109(2):371–387

Bahadou SA, Ouijja A, Karfach A, Tahiri A, Lahlali R (2018) New potential bacterial antagonists for the biocontrol of fire blight disease (Erwinia amylovora) in Morocco. Microb Pathog 117:7–15

Bartsev AV, Deakin WJ, Boukli NM, McAlvin CB, Stacey G, Malnoë P, Broughton WJ, Staehelin C (2004) NopL, an effector protein of rhizobium sp. NGR234, thwarts activation of plant defense reactions. Plant Physiol 134(2):871–879

Bender SF, Wagg C, van der Heijden MG (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol 31(6):440–452

Berg G, Zachow C, Müller H, Philipps J, Tilcher R (2013) Next-generation bio-products sowing the seeds of success for sustainable agriculture. Agronomy. https://doi.org/10.3390/agronomy3040648

Bhatti TM, Yawar W (2010) Bacterial solubilization of phosphorus from phosphate rock containing sulfur-mud. Hydrometallurgy 103(1–4):54–59

Blom D, Fabbri C, Connor EC, Schiestl FP, Klauser DR, Boller T, Eberl L, Weisskopf L (2011) Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. Environ Microbiol 13(11):3047–3058

Bona E, Cantamessa S, Massa N, Manassero P, Marsano F, Copetta A, Lingua G, D'Agostino G, Gamalero E, Berta G (2017) Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: a field study. Mycorrhiza 27(1):1–11

Bonanomi G, Lorito M, Vinale F, Woo SL (2018) Organic amendments, beneficial microbes, and soil microbiota: toward a unified framework for disease suppression. Annu Rev Phytopathol 56:1–20

Byers BR, Powell MV, Lankford CE (1967) Iron-chelating hydroxamic acid (schizokinen) active in initiation of cell division in *Bacillus megaterium*. J Bacteriol 93(1):286–294

Chauhan S, Wadhwa K, Vasudeva M, Narula N (2012) Potential of Azotobacter spp. as biocontrol agents against Rhizoctonia solani and Fusarium oxysporum in cotton (Gossypium hirsutum), guar (Cyamopsis tetragonoloba) and tomato (Lycopersicum esculentum). Arch Agron Soil Sci 58(12):1365–1385

Chernin LS (2011) Quorum-sensing signals as mediators of PGPRs' beneficial traits. In: Bacteria in agrobiology: plant nutrient management. Springer, Berlin, Heidelberg, pp 209–236

Chung JH, Song GC, Ryu CM (2016) Sweet scents from good bacteria: case studies on bacterial volatile compounds for plant growth and immunity. Plant Mol Biol 90(6):677–687

Collinson SK, Doran JL, Page WJ (1987) Production of 3,4-dihydroxybenzoic acid by Azomonas macrocytogenes and Azotobacter paspali. Can J Microbiol 33(2):169–175

Datta A, Singh RK, Tabassum S (2015) Isolation, characterization and growth of rhizobium strains under optimum conditions for effective biofertilizer production. Int J Pharm Sci Rev Res 32 (1):199–208

Davison J (1988) Plant beneficial bacteria. Biotechnology 6(3):282–286

de Campos SB, Deakin WJ, Broughton WJ, Passaglia LM (2011) Roles of flavonoids and the transcriptional regulator Tts I in the activation of the type III secretion system of Bradyrhizobium elkanii SEMIA587. Microbiology 157(3):627–635

Deboja S, Manoja D (2010) In vitro studies of antagonistic behaviour of rhizobium strain against phytopathogenic fungi. Natl Acad Sci Lett 33(1/2):17–20

Dubey RC, Maheshwari DK, Kumar V, Pandey RR (2012) Growth enhancement of Sesamum indicum L. by rhizosphere-competent Azotobacter chroococcum AZO2 and its antagonistic activity against Macrophomina phaseolina. Arch Phytopathol Plant Protect 45(4):437–454

Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10(1):1–9

Eng-Wilmot DL, Van der Helm D (1980) Molecular and crystal structure of the linear tricatechol siderophore, agrobactin. J Am Chem Soc 102(26):7719–7725

FAO, IFAD, WFP (2015) The state of food insecurity in the World. Meeting the 2015 international hunger targets. Taking stock of uneven progress. Rome, FAO

Fatima Z, Saleemi M, Zia M, Sultan T, Aslam M, Rehman R, Chaudhary MF (2009) Antifungal activity of plant growth-promoting rhizobacteria isolates against Rhizoctonia solani in wheat. Afr J Biotechnol 8(2):219

Feng Y, Shen D, Song W (2006) Rice endophyte Pantoea agglomerans YS19 promotes host plant growth and affects allocations of host photosynthates. J Appl Microbiol 100(5):938–945

Finkel OM, Castrillo G, Paredes SH, González IS, Dangl JL (2017) Understanding and exploiting plant beneficial microbes. Curr Opin Plant Biol 38:155–163

Fiorentino N, Ventorino V, Woo SL, Pepe O, De Rosa A, Gioia L, Romano I, Lombardi N, Napolitano M, Colla G, Rouphael Y (2018) Trichoderma-based biostimulants modulate rhizosphere microbial populations and improve N uptake efficiency, yield, and nutritional quality of leafy vegetables. Front Plant Sci 9:743

Fredrickson JK (2015) Ecological communities by design. Science 348(6242):1425–1427

Fukami J, Ollero FJ, Megías M, Hungria M (2017) Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth. AMB Express 7(1):153

Fukami J, Cerezini P, Hungria M (2018a) Azospirillum: benefits that go far beyond biological nitrogen fixation. AMB Express 8(1):73

Fukami J, de la Osa C, Ollero FJ, Megías M, Hungria M (2018b) Co-inoculation of maize with *Azospirillum brasilense* and *Rhizobium tropici* as a strategy to mitigate salinity stress. Funct Plant Biol 45(3):328–339

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320(5878):889–892

Ge YY, Xiang QW, Wagner C, Zhang D, Xie ZP, Staehelin C (2016) The type 3 effector NopL of Sinorhizobium sp. strain NGR234 is a mitogen-activated protein kinase substrate. J Exp Bot 67 (8):2483–2494

Gestel JV, Vlamakis H, Kolter R (2015) Division of labor in biofilms: the ecology of cell differentiation. Microbial Biofilms 110:67–97

Graumann P (2007) Bacillus: cellular and molecular biology. Caister Academic Press, Norfolk

Guleria S, Zhou J, Koffas MA (2017) Nutraceuticals (vitamin C, carotenoids, resveratrol). In: Industrial biotechnology: products and processes, vol 4. Wiley, Hoboken, NJ, pp 309–336

Haddoudi I, Sendi Y, Batnini M, Romdhane SB, Mhadhbi H, Mrabet M (2017) The bean rhizosphere Pseudomonas aeruginosa strain RZ9 strongly reduces Fusarium culmorum growth and infectiveness of plant roots. Span J Agric Res 15(2):21

Hassen AI, Bopape FL, Sanger LK (2016) Microbial inoculants as agents of growth promotion and abiotic stress tolerance in plants. In: Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 23–36

Hernández AG, de Moura GD, Binati RL, Nascimento FXI, Londoño DM, Mamede ACP, da Silva EP, de Armas RD, Giachini AJ, Rossi MJ, Soares CRFS (2017) Selection and characterization of coal mine autochthonous rhizobia for the inoculation of herbaceous legumes. Arch Microbiol 199(7):991–1001

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311(1–2):1–18

Hill EA, Chrisler WB, Beliaev AS, Bernstein HC (2017) A flexible microbial co-culture platform for simultaneous utilization of methane and carbon dioxide from gas feedstocks. Bioresour Technol 228:250–256

Howell CR, Stipanovic RD (1979) Control of Rhizoctonia solani on cotton seedlings with Pseudomonas fluorescens and with an antibiotic produced by the bacterium. Phytopathology 69 (5):480–482

Huang CN, Lin CP, Hsieh FC, Lee SK, Cheng KC, Liu CT (2016) Characterization and evaluation of Bacillus amyloliquefaciens strain WF02 regarding its biocontrol activities and genetic responses against bacterial wilt in two different resistant tomato cultivars. World J Microbiol Biotechnol 32(11):183

Islam S, Akanda AM, Prova A, Islam MT, Hossain MM (2016) Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Front Microbiol 6:1360

Ito T, Neilands JB (1958) Products of "low-iron fermentation" with *Bacillus subtilis*: isolation, characterization and synthesis of 2,3-dihydroxybenzoylglycine[1,2]. J Am Chem Soc 80 (17):4645–4647

Ji H, Dong H (2015) Key steps in type III secretion system (T3SS) towards translocon assembly with potential sensor at plant plasma membrane. Mol Plant Pathol 16(7):762–773

Jones KM, Sharopova N, Lohar DP, Zhang JQ, Vanden Bosch KA, Walker GC (2008) Differential response of the plant Medicago truncatula to its symbiont Sinorhizobium meliloti or an exopolysaccharide-deficient mutant. Proc Natl Acad Sci 105(2):704–709

Joseph CM, Phillips DA (2003) Metabolites from soil bacteria affect plant water relations. Plant Physiol Biochem 41(2):189–192

Kannapiran E, Sri Ramkumar V (2011) Inoculation effect of nitrogen-fixing and phosphate-solubilizing bacteria to promote growth of black gram (*Phaseolus mungo* Roxb; Eng). Ann Biol Res 2(5):615–621

Karasov TL, Chae E, Herman JJ, Bergelson J (2017) Mechanisms to mitigate the trade-off between growth and defense. Plant Cell 29(4):666–680

Kavamura VN, Santos SN, da Silva JL, Parma MM, Ávila LA, Visconti A, Zucchi TD, Taketani RG, Andreote FD, de Melo IS (2013) Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. Microbiol Res 168(4):183–191

Kesaulya H, Hasinu JV, Tuhumury GNC (2018, January) Potential of Bacillus spp produces siderophores in suppressing the wilt disease of banana plants. In IOP Conference Series: Earth and Environmental Science, vol 102, pp 012016, Semarang, Indonesia

Kim SN, Cho WK, Kim WI, Jee HJ, Park CS (2012) Growth promotion of pepper plants by Pantoea ananatis B1-9 and its efficient endophytic colonization capacity in plant tissues. Plant Pathol J 28(3):270–281

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Pseudomonas siderophores: a mechanism explaining disease-suppressive soils. Curr Microbiol 4(5):317–320

Kondorosi E, Gyuris J, Schmidt J, John M, Duda E, Hoffmann B, Schell J, Kondorosi A (1989) Positive and negative control of nod gene expression in Rhizobium meliloti is required for optimal nodulation. EMBO J 8(5):1331–1340

Kong W, Meldgin DR, Collins JJ, Lu T (2018) Designing microbial consortia with defined social interactions. Nat Chem Biol 14(8):821–829

Kumar A, Kumar A, Pratush A (2014) Molecular diversity and functional variability of environmental isolates of Bacillus species. SpringerPlus 3(1):312

Kumawat N, Kumar R, Kumar S, Meena VS (2017) Nutrient solubilizing microbes (NSMs): its role in sustainable crop production. In: Agriculturally important microbes for sustainable agriculture. Springer, Singapore, pp 25–61

Lenin G, Jayanthi M (2012) Indole acetic acid, gibberellic acid and siderophore production by PGPR isolates from rhizospheric soils of Catharanthus roseus. Int J Pharmacy Biol Arch 3:933–938

Liang Y, Tóth K, Cao Y, Tanaka K, Espinoza C, Stacey G (2014) Lipochitooligosaccharide recognition: an ancient story. New Phytol 204(2):289–296

Lin SY, Hameed A, Liu YC, Hsu YH, Lai WA, Shen FT, Young CC (2015) Azospirillum soli sp. nov., a nitrogen-fixing species isolated from agricultural soil. Int J Syst Evol Microbiol 65 (12):4601–4607

Liu M, Liu X, Cheng BS, Ma XL, Lyu XT, Zhao XF, Ju YL, Min Z, Zhang ZW, Fang YL (2016) Selection and evaluation of phosphate-solubilizing bacteria from grapevine rhizospheres for use as biofertilizers. Span J Agric Res 14(4):26

Lowery CA, Dickerson TJ, Janda KD (2008) Interspecies and interkingdom communication mediated by bacterial quorum sensing. Chem Soc Rev 37(7):1337–1346

Lugtenberg B (2015) Life of microbes in the rhizosphere. In: Principles of plant-microbe interactions. Springer, Cham, pp 7–15

Lugtenberg B (2016) Principles of plant-microbe interactions. Springer, New York

Mahmood S, Daur I, Al-Solaimani SG, Ahmad S, Madkour MH, Yasir M, Hirt H, Ali S, Ali Z (2016) Plant growth promoting rhizobacteria and silicon synergistically enhance salinity tolerance of mung bean. Front Plant Sci 7:876

Martha-Paz AM, Eide D, Mendoza-Cózatl D, Castro-Guerrero NA, Aréchiga-Carvajal ET (2019) Zinc uptake in the Basidiomycota: characterization of zinc transporters in Ustilago maydis. Mol Membr Biol 35(1):39–50

Mehnaz S (2016) An overview of globally available bioformulations. In: Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 267–281

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37 (5):634–663

Merino MP, Andrews BA, Asenjo JA (2015) Stoichiometric model and flux balance analysis for a mixed culture of Leptospirillum ferriphilum and Ferroplasma acidiphilum. Biotechnol Prog 31 (2):307–315

Merino MP, Andrews BA, Parada P, Asenjo JA (2016) Characterization of Ferroplasma acidiphilum growing in pure and mixed culture with Leptospirillum ferriphilum. Biotechnol Prog 32(6):1390–1396

Monali D, Subramanyan JN, Satyan KB (2018) Production, characterization and iron binding affinity of hydroxamate siderophores from rhizosphere associated fluorescent Pseudomonas. J Plant Prot Res. https://doi.org/10.24425/119116

Mumtaz MZ, Ahmad M, Jamil M, Hussain T (2017) Zinc solubilizing Bacillus spp. potential candidates for biofortification in maize. Microbiol Res 202:51–60

Nadeem SM, Naveed M, Ahmad M, Zahir ZA (2015) Rhizosphere bacteria for crop production and improvement of stress tolerance: mechanisms of action, applications, and future prospects. In: Plant microbes symbiosis: applied facets. Springer, New Delhi, pp 1–36

Nam H, Campodonico M, Bordbar A, Hyduke DR, Kim S, Zielinski DC, Palsson BO (2014) A systems approach to predict oncometabolites via context-specific genome-scale metabolic networks. PLoS Comput Biol 10(9):e1003837

National Research Council (1989) Alternative agriculture. National Academies Press, Washington, DC, 464 pp

Niazi A, Manzoor S, Asari S, Bejai S, Meijer J, Bongcam-Rudloff E (2014) Genome analysis of Bacillus amyloliquefaciens subsp. plantarum UCMB 5113: a rhizobacterium that improves plant growth and stress management. PLoS One 9(8):e104651

Oke V, Long SR (1999) Bacteroid formation in the rhizobium–legume symbiosis. Curr Opin Microbiol 2(6):641–646

Okon Y, Labandera-Gonzales C, Lage M, Lage P (2015) Agronomic applications of Azospirillum and other PGPR. In: Biological nitrogen fixation. Wiley, Hoboken, NJ, pp 921–932

Oldroyd GE (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11(4):252–263

Ortíz-Castro R, Martínez-Trujillo M, López-Bucio J (2008) N-acyl-L-homoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in *Arabidopsis thaliana*. Plant Cell Environ 31(10):1497–1509

Ortiz-Castro R, Díaz-Pérez C, Martínez-Trujillo M, Rosa E, Campos-García J, López-Bucio J (2011) Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. Proc Natl Acad Sci 108(17):7253–7258

Owen D, Williams AP, Griffith GW, Withers PJ (2015) Use of commercial bio-inoculants to increase agricultural production through improved phosphorous acquisition. Appl Soil Ecol 86:41–54

Panwar M, Tewari R, Nayyar H (2016) Native halo-tolerant plant growth promoting rhizobacteria Enterococcus and Pantoea sp. improve seed yield of Mungbean (Vigna radiata L.) under soil salinity by reducing sodium uptake and stress injury. Physiol Mol Biol Plants 22(4):445–459

Pereira SIA, Castro PML (2014) Diversity and characterization of culturable bacterial endophytes from Zea mays and their potential as plant growth-promoting agents in metal-degraded soils. Environ Sci Pollut Res 21(24):14110–14123

Perrig D, Boiero ML, Masciarelli OA, Penna C, Ruiz OA, Cassán FD, Luna MV (2007) Plant-growth-promoting compounds produced by two agronomically important strains of

Azospirillum brasilense, and implications for inoculant formulation. Appl Microbiol Biotechnol 75(5):1143–1150
Pham VT, Rediers H, Ghequire MG, Nguyen HH, De Mot R, Vanderleyden J, Spaepen S (2017) The plant growth-promoting effect of the nitrogen-fixing endophyte Pseudomonas stutzeri A15. Arch Microbiol 199(3):513–517
Pourbabaee AA, Shoaibi F, Emami S, Alikhani HA (2018) The potential contribution of siderophore producing bacteria on growth and Fe ion concentration of sunflower (*Helianthus* annuus L.) under water stress. J Plant Nutr 41(5):619–626
Prasanna R, Babu S, Bidyarani N, Kumar A, Triveni S, Monga D, Mukherjee AK, Kranthi S, Gokte-Narkhedkar N, Adak A, Yadav K (2015) Prospecting cyanobacteria-fortified composts as plant growth promoting and biocontrol agents in cotton. Exp Agric 51(1):42–65
Pulendran B, Li S, Nakaya HI (2010) Systems vaccinology. Immunity 33(4):516–529
Qin S, Zhou W, Li Z, Lyu D (2016) Effects of rhizobacteria on the respiration and growth of Cerasus sachalinensis Kom. seedlings. Span J Agric Res 14(2):12
Revillas JJ, Rodelas B, Pozo C, Martínez-Toledo MV, González-López J (2000) Production of B-group vitamins by two Azotobacter strains with phenolic compounds as sole carbon source under diazotrophic and adiazotrophic conditions. J Appl Microbiol 89(3):486–493
Rey T, Nars A, Bonhomme M, Bottin A, Huguet S, Balzergue S, Jardinaud MF, Bono JJ, Cullimore J, Dumas B, Gough C (2013) NFP, a LysM protein controlling Nod factor perception, also intervenes in Medicago truncatula resistance to pathogens. New Phytol 198(3):875–886
Rijavec T, Lapanje A (2016) Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front Microbiol 7:1785
Rolfsson Ó, Palsson BO (2015) Decoding the jargon of bottom-up metabolic systems biology. BioEssays 37(6):588–591
Rosier A, Medeiros FH, Bais HP (2018) Defining plant growth promoting rhizobacteria molecular and biochemical networks in beneficial plant-microbe interactions. Plant Soil 428(1–2):35–55
Sachin DN, Misra P (2009) Effect of Azotobacter chroococcum (PGPR) on the growth of bamboo (Bambusa bamboo) and maize (*Zea mays*) plants. Biofrontiers 1(1):24–31
Sahoo RK, Ansari MW, Dangar TK, Mohanty S, Tuteja N (2014) Phenotypic and molecular characterisation of efficient nitrogen-fixing Azotobacter strains from rice fields for crop improvement. Protoplasma 251(3):511–523
Savci S (2012) Investigation of effect of chemical fertilizers on environment. APCBEE Procedia 1:287–292
Saxena B, Vithlani L, Modi VV (1989) Siderophore-mediated transport of molybdenum in Azospirillum lipoferum strain D-2. Curr Microbiol 19(5):291–295
Schikora A, Schenk ST, Hartmann A (2016) Beneficial effects of bacteria-plant communication based on quorum sensing molecules of the N-acyl homoserine lactone group. Plant Mol Biol 90 (6):605–612
Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140 (3–4):339–353
Spaepen S (2015) Plant hormones produced by microbes. In: Principles of plant-microbe interactions. Springer, Cham, pp 247–256
Sruthilaxmi CB, Babu S (2017) Microbial bio-inoculants in Indian agriculture: ecological perspectives for a more optimized use. Agric Ecosyst Environ 242:23–25
Stamenković S, Beškoski V, Karabegović I, Lazić M, Nikolić N (2018) Microbial fertilizers: a comprehensive review of current findings and future perspectives. Span J Agric Res 16(1): e09R01
Stringlis IA, Zhang H, Pieterse CM, Bolton MD, de Jonge R (2018) Microbial small molecules–weapons of plant subversion. Nat Prod Rep 35(5):410–433
Subramanian J, Satyan K (2014) Isolation and selection of fluorescent pseudomonads based on multiple plant growth promotion traits and siderotyping. Chilean J Agric Res 74(3):319–325

Sulochana MB, Jayachandra SY, Kumar SA, Parameshwar AB, Reddy KM, Dayanand A (2014) Siderophore as a potential plant growth-promoting agent produced by Pseudomonas aeruginosa JAS-25. Appl Biochem Biotechnol 174(1):297–308
Sundar I (2016) Agribusiness scope, opportunities and challenges in India. EPRA Int J Econ Bus Rev 4(7):171
Tanaka K, Cho SH, Lee H, Pham AQ, Batek JM, Cui S, Qiu J, Khan SM, Joshi T, Zhang ZJ, Xu D (2015) Effect of lipo-chitooligosaccharide on early growth of C4 grass seedlings. J Exp Bot 66 (19):5727–5738
Teplitski M, Eberhard A, Gronquist MR, Gao M, Robinson JB, Bauer WD (2003) Chemical identification of N-acyl homoserine lactone quorum-sensing signals produced by Sinorhizobium meliloti strains in defined medium. Arch Microbiol 180(6):494–497
Timmusk S, Nevo E (2011) Plant root associated biofilms: perspectives for natural product mining. In: Bacteria in agrobiology: plant nutrient management. Springer, Berlin, Heidelberg, pp 285–300
Timmusk S, Kim SB, Nevo E, Abd El Daim I, Ek B, Bergquist J, Behers L (2015) Sfp-type PPTase inactivation promotes bacterial biofilm formation and ability to enhance wheat drought tolerance. Front Microbiol 6:387
Tortora ML, Díaz-Ricci JC, Pedraza RO (2011) Azospirillum brasilense siderophores with antifungal activity against Colletotrichum acutatum. Arch Microbiol 193(4):275–286
Ullah N, Ditta A, Khalid A, Mehmood S, Rizwan MS, Ashraf M, Mubeen F, Imtiaz M, Iqbal MM (2019) Integrated effect of algal biochar and plant growth promoting rhizobacteria on physiology and growth of maize under deficit irrigations. J Soil Sci Plant Nutr 9:1–11
Van Oosten MJ, Pepe O, De Pascale S, Silletti S, Maggio A (2017) The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chem Biol Technol Agric 4(1):5
Ventorino V, Sannino F, Piccolo A, Cafaro V, Carotenuto R, Pepe O (2014) Methylobacterium populi VP2: plant growth-promoting bacterium isolated from a highly polluted environment for polycyclic aromatic hydrocarbon (PAH) biodegradation. Sci World J. https://doi.org/10.1155/2014/931793
Ventorino V, Pascale A, Adamo P, Rocco C, Fiorentino N, Mori M, Faraco V, Pepe O, Fagnano M (2018) Comparative assessment of autochthonous bacterial and fungal communities and microbial biomarkers of polluted agricultural soils of the Terra dei Fuochi. Sci Rep 8(1):1–13
Venturi V, Fuqua C (2013) Chemical signaling between plants and plant-pathogenic bacteria. Annu Rev Phytopathol 51:17–37
Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255(2):571–586
Viruel E, Lucca ME, Siñeriz F (2011) Plant growth promotion traits of phosphobacteria isolated from Puna, Argentina. Arch Microbiol 193(7):489–496
Widnyana IK (2018) PGPR (plant growth promoting rhizobacteria) benefits in spurring germination, growth and increase the yield of tomato plants. In: Recent advances in tomato breeding and production. IntechOpen
Wiesel L, Newton AC, Elliott I, Booty D, Gilroy EM, Birch PR, Hein I (2014) Molecular effects of resistance elicitors from biological origin and their potential for crop protection. Front Plant Sci 5:655
Xing YX, Wei CY, Mo Y, Yang LT, Huang SL, Li YR (2016) Nitrogen-fixing and plant growth-promoting ability of two endophytic bacterial strains isolated from sugarcane stalks. Sugar Tech 18(4):373–379
Zhang H, Sun Y, Xie X, Kim MS, Dowd SE, Paré PW (2009) A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms. Plant J 58(4):568–577
Zhou C, Guo J, Zhu L, Xiao X, Xie Y, Zhu J, Ma Z, Wang J (2016) Paenibacillus polymyxa BFKC01 enhances plant iron absorption via improved root systems and activated iron acquisition mechanisms. Plant Physiol Biochem 105:162–173

Plant Growth-Promoting Rhizobacteria (PGPR): A New Perspective in Abiotic Stress Management of Crop Plants

31

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Abstract

Rapid intensification in agricultural production systems over the past few decades has affected the environment, leading to several consequences including poor soil health, pollution, and increased abiotic and biotic stress. The change in global climate with extreme weather conditions and erratic rains during the recent past has further aggravated the situation and imposed additional stress conditions that the plants have to encounter during their life cycle. Abiotic stresses, viz., drought, heat, and soil deterioration due to increasing soil acidity and salinity, and increasing metal toxicity in soil, affect plant growth and severely limit crop production. Several studies point to the beneficial microbes, especially plant growth-promoting rhizobacteria (PGPR), which play a pivotal role in aiding the plants to overcome abiotic stresses and retain their productivity. Beneficial soil bacteria either live symbiotically with plants in the rhizosphere or as endophytes inside of the host plants. They aid in plant growth directly by secretion of phytohormone, enzymes, and biological nitrogen fixation, solubilizing minerals or mineralizing organic phosphate and producing organic matter such as amino acids. The PGPR may also confer plants with immunity against invading pathogens through induction of disease resistance mechanisms, promote favorable symbiosis, and remove/degrade xenobiotics from soil and minimize abiotic stresses. The basic mechanisms by which PGPR help plants to cope against abiotic stress include lowering ethylene levels, production and accumulation of

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compatible solutes such as proline and glycine betaine, and decreasing the production of ROS. Thus, the use of PGPR is suitable for ameliorating the environmental stress encountered by the crop plants and can be considered as an important component of sustainable agricultural practices.

Keywords

PGPR · Abiotic stress · Phytohormone · Sustainable agriculture

31.1 Introduction

Plants encounter several stress conditions including biotic and abiotic stress during their life cycle. These stress conditions restrict plant growth, development, and productivity. The global climate change and its fallout experienced during the recent years have highlighted the importance of abiotic stress on crop plants. Abiotic stress, encompassing conditions such as drought, nutrient deficiency, temperature fluctuations, and adverse (acidic/alkaline) soil conditions, affect crop growth and productivity. Despite several technological advances such as development of improved crop varieties, genetic modification of plants, and intricate irrigation systems, abiotic stress continues to remain challenging for farmers and people connected to agricultural activity. They affect not only the productivity of agricultural crops but also limit choice of crops for cultivation as well as the microbial community structure and their activity in soil. As such, agricultural management practices and the development of crops/cultivars tolerant to adverse environmental conditions have garnered major scientific attention in recent years (Barrow et al. 2008; Eisenstein 2013). Although classical plant breeding techniques have led to the development of high-yielding, stress-tolerant crop varieties, the technique continues to be limited by the long time period required for development of a variety and involvement of labor. Chances of events leading to loss of important nontarget traits from gene pool and non-transferability to other crop systems continue to dodge the technique (Eisenstein 2013). Genetic modification of crops offers a faster technique for crop improvement. However, this technique has its own set of drawbacks including issues related to biosafety, bioethics, environmental impact, customer perception, etc. (Fedoroff et al. 2010). Both these approaches consider plants as independent entities with the ability of self-growth regulation through genetic code and cellular physiology (Coleman-Derr and Tringe 2014). Interestingly, soil bacteria, especially the ones closely associated with the rhizosphere, are reported to be crucial in the plants' physiological responses to environmental conditions (Budak et al. 2013; Cooper et al. 2014). During the recent years, microbe-based approaches have gained considerable momentum in mitigating abiotic stress encountered by the plants. Multiple evidence-based studies have reported that plant-associated microbes collectively termed as "plant growth-promoting rhizobacteria" not only enhance crop's ability to surmount the adverse abiotic stress but also enhance their productivity (Marulanda et al. 2009; Yang et al. 2009). Plant growth-promoting

rhizobacteria produce enzymes and metabolites while colonizing the root system that aids the plants in tackling various stress conditions (Pineda et al. 2013; Chauhan et al. 2015). These naturally occurring soil microbes can be successfully harnessed to improve crop production under rapidly changing climate (Yang et al. 2009; Nadeem et al. 2014). The advancements in different omics approaches encompassing metagenomics, transcriptomics, and proteomics have shed a better light in understanding the plant-microbes interaction at a molecular level leading to enhanced crop survival under various stress conditions (Yang et al. 2009; Grover et al. 2011).

31.2 Abiotic Stress

Abiotic stress is the negative impact of naturally or anthropogenically occurring activities (non-living factors) on living organisms. The effect of abiotic stresses on plants may be due to individual factors or compounding factors. Before delving into the details of microbe-mediated stress amelioration in plants, a brief summary of the various agronomically important abiotic stresses is discussed.

31.2.1 Drought

Drought stress is considered as one of the most influential factors on crop growth. The effect of drought on crop plants is highly devastating and has thus drawn the attention of researchers (Vinocur and Altman 2005; Naveed et al. 2014). Drought stress significantly reduces seed germination rate, seedling development, photosynthesis, stomatal conductance, and biomass, thereby leading to poor vegetative and reproductive growth. Several key factors such as hydrometeorological status, socioeconomic conditions, and stochastic tendency of water are taken into consideration while defining the term "drought" (American_Meteorological_Society_Council 2004). Drought is mainly categorized into four different types, viz., meteorological drought caused due to prolonged delay in precipitation, hydrological drought due to inadequacy in surface and underground water resources, socioeconomic drought resulting from failure of water management system to meet household and community water demand, and agricultural drought characterized by decline in soil moisture that results in crop stress and crop yield (Wilhite and Glantz 1985). It is projected that by 2050, more than 50% of the earth's arable lands will face drought-associated reduction in crop productivity (Vinocur and Altman 2005). The world population is expected to reach 9 billion by that time which urgently calls for efficient crop production strategies for food and nutritional security (Gatehouse et al. 2011; Foley et al. 2011). The development of drought-resistant crop varieties resistant to limited water resources is essential for sustaining crop productivity (Mancosu et al. 2015). The severity, frequency, and duration of drought under a changing climatic environment will impact the productivity of drought-sensitive crops such as cotton, soybean, corn, etc., to a greater extent in many crop-producing areas around the globe (Solomon et al. 2007; EEA 2011).

31.2.2 Temperature

Reduction in plant growth or reduced metabolic activity and cellular or tissue injury due to exposure to temperatures above or below the thermal thresholds is defined as suboptimal temperature stress (Greaves 1996). Temperature is the most important climatic factor that influences growth, development, and yield of crops. Plant growth and development involve numerous biochemical reactions that are sensitive to temperature. Effect of temperature stress is evident in seedling establishment, plant growth and total biomass (Hasanuzzaman et al. 2013b).

31.2.2.1 High Temperature

High temperature (HT) stress is a major abiotic stress that affects plant growth, metabolism, and productivity. Exposures to moderately high temperature for prolonged periods and extreme high temperature for a short duration are both detrimental to plant growth and development. High temperature causes morphological alterations and changes in the cellular level leading to decreased photosynthesis, pollen development, photosynthesis, grain and fruit development, quality, and genetically determined yield potential of the crop. However, the response of the crop plant to HT stress is dependent to a large extent on the plant type and duration of the heat. High temperature, even for short period, affects crop growth especially in temperate crops like wheat. High temperature injury arises from excessive respiration and starvation due to loss of food reserves (Levitt 1972; Hasanuzzaman et al. 2013a, b).

31.2.2.2 Low Temperature

Low temperature stress is a major challenge faced by the plants in many parts of the world (Wang et al. 2016). Low temperature stress can be of two types, viz., chilling and freezing. Plants exposed to a low temperature between 0 °C and 14 °C are said to be under chilling stress, while exposure to a low temperature below 0 °C is termed as freezing stress (Lyons 1973; Burke et al. 1976). The most common site implicated for chilling injury is the plasma membrane which may lead to cell leakage or disruption. The chilling stress leads to changes in membrane structure and composition and decreased protoplasmic streaming (Lyons 1973). Unseasonal frost occurring during the active growth period of the crop plant causes freezing damage. Freezing damage occurs primarily due to the formation of ice crystals, which damage cell structure when the temperature falls below 0 °C (Pearce 2001). Symptoms include desiccation or burning of foliage, water-soaked areas that progress to necrotic spots on leaves, dead or weakened shoot and root system, or split bark on stems or branches (Levitt 1972; Witt and Barfield 1982). Plants respond to cold temperatures by activating and modifying metabolic pathways that protect the cells from cold and freezing conditions. The protection strategies include accumulation of sugars that prevent ice formation and activation of a signal transduction pathway that leads to the activation of transcription factors and cold-responsive genes which in turn synthesize and recruit proteins that stabilize membranes and resist rupture (Yadav 2010b).

31.2.3 Salinity

Soil salinization is one of the major factors leading to soil degradation. Although most of the continents have problematic saline soils, it is predominantly a problem of arid and semiarid regions of the world. Saline soils are those that have saturated soil paste extracts with an ECe of more than 4 dSm^{-1}, ESP <15% and pH below 8.5 (Waisel 1972; Abrol 1986; Szabolcs 1994). The soil becomes saline when the rate of evaporation and transpiration exceeds rainfall and there is insufficient rain to leach away soluble salts from the root zone (Miller and Donahue 1995). In India alone, seven million hectares of land are affected by salt stress (reviewed in Shrivastava and Kumar 2015). Saline soils have a mixture of salts of chloride, sulfate, sodium, magnesium, and calcium ions with sodium chloride often dominant (Ashraf and Philip 2005; Zaman et al. 2018). Salinity of soil may also result from weathering of minerals and the soils originating from saline parent rocks, due to improper irrigation, deforestation, overgrazing, and intensive cropping (Ashraf 1994). The presence of excessive amount of soluble salts hinders or affects the normal functions of plant growth. Salt stress imposed an osmotic or water-deficit effect on plants that reduces the ability of plants to take up water which in turn leads to slower growth. Excessive salts in the soil are taken up by the roots along with water and solutes which enter the transpiration stream of plant and injure cells in the transpiring leaves, which lead to reduction in plant growth. Accumulation of Na^+ and Cl^- is toxic to plant cells as these ions affect enzyme activity (Shrivastava and Kumar 2015).

31.2.4 Soil Acidity

Soil is regarded as acidic when the presence of concentration of H^+ is higher in soil solution and at exchange sites. They are characterized by a soil pH value <7.0 (Dahlgren et al. 2008). Around 30% of the world's ice-free lands are acidic in nature with major portions in the tropical and subtropical regions (von Uexküll and Mutert 1995). Several factors, including acidic parent material and alumina silicate minerals, leaching caused heavy rainfall, application of acid-forming fertilizers, and presence of humus and other organic acids, lead to the formation of acidic soils. Acid soils are problematic due to the increased solubility and toxicity of Al, Mn, and Fe. In acidic soil with pH value <4.5, aluminum is solubilized into ionic forms and rapidly mobilizes across various microsites. Soluble Al ions inhibit root elongation by damaging cell lining of the root apex which leads to root pruning and a defective rooting system. The inability to reach the nutrients in the subsoils seriously impedes plant growth and development, a phenomenon which is widely known by the term "aluminum phytotoxicity" (Kochian 1995; Goodwin and Sutter 2009). Deficiency of Ca and Mg with reduced availability of P and Mo is another challenge that the plants encounter in acid soils. Low pH of the soil also reduced microbial activity and their functional abilities, which disrupt the nutrient cycle and affect plant growth (reviewed in Bian et al. 2013).

31.2.5 Heavy Metal Toxicity

Soil polluted with heavy metals is a matter of serious concern because of their detrimental effects on the plants. Heavy metals are the ill-defined subset of elements having a higher molecular weight that includes transition metals, some metalloids, lanthanides, and actinides with specific density greater than water, i.e., 5 g/cm^3 (Babula et al. 2008). They are classified as essential and nonessential. Based on their function in living organisms, heavy metals like zinc (Zn), nickel (Ni), manganese (Mn), and iron (Fe) are required by the plants for performing various physiological and biochemical functions and are classified as essential heavy metals. Heavy metals like lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr), and mercury (Hg) with no physiological role are regarded as nonessential heavy metals. The presence of heavy metals beyond critical limits is hazardous as they hinder the normal functioning of the living systems including plants (Yadav 2010a; Singh et al. 2011b). Soils contaminated with heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) and a few related metalloids such as arsenic (As) and nickel (Ni) are a major problem in agriculture and environment (Abbas et al. 2014). Sources of heavy metal contamination in agricultural fields include (1) phosphate fertilizers, (2) sewage sludge, (3) industrial discharges and smelters, and (4) contaminated water supply through irrigation (Passariello et al. 2002). Excessive amounts of reactive oxygen species (ROS) are generated upon exposure to heavy metals which directly causes oxidative stresses in plants (Mithöfer et al. 2004) and, indirectly, derails the electron transport chain (Qadir et al. 2004). In parallel, ROS also hinders the metabolism of essential elements and lipid peroxidation (Dong et al. 2006).

31.3 Plant Growth-Promoting Rhizobacteria: Ameliorating Agents of Abiotic Stress

Plants possess several adaptive traits that enable them to endure abiotic stresses. Such traits include physiological modifications of the plant system architecture consisting of the root and shoot and leaf, and modulations in relative water content (De Zelicourt et al. 2013; Nadeem et al. 2014; de Souza et al. 2015). In the past few decades, the role of microorganisms to alleviate abiotic stresses in plants has received attention, and several reports suggest that microbes have intrinsic metabolic and genetic capabilities to alleviate abiotic stresses in plants (Gopalakrishnan et al. 2015). Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that reside in the rhizosphere, at root surfaces on the phylloplane, as well as endophytes that directly or indirectly aid in improving the plant growth. Direct plant growth-promoting activities mediated by bacteria are mostly based on production and regulation of phytohormones (IAA, cytokinin, and gibberellins) and provision of essential mineral nutrition (secretion of organic acids for solubilization of minerals such as phosphates) to plants. Other mechanisms include nitrogen fixation and production of siderophore (iron sequestration) and exopolysaccharides (Saharan

and Nehra 2011; Gopalakrishnan et al. 2015). Plant growth-promoting bacteria also help plants in maintaining osmotic balance and improve antioxidant metabolism during stress conditions (Ilangumaran and Smith 2017; Backer et al. 2018). The indirect activities are related to prevention of diseases and other stresses. Induction of such microbe-mediated responses toward abiotic stress in plants is better known as induced systemic tolerance (IST) (Pieterse et al. 2014). Earlier, the beneficial effect of PGPR as bioinoculants as an alternative to chemical fertilizers, pesticides, and supplements for improving the growth and yield of agricultural crops has been reported (Chakraborty et al. 2006; Ashrafuzzaman et al. 2009; Beneduzi et al. 2012; Noumavo et al. 2013; Abd-Alla et al. 2014; Vejan et al. 2016). Evidences from agricultural practices clearly demonstrate that the PGPR not only improve yield of diverse crop plants including rice, maize, barley, and soybean but also help the plants to grow under stresses (Sen and Chandrasekhar 2014; Suarez et al. 2015). Microbial interactions with the host plants have multifarious benefits. At one end, microbes induce local or systemic stress alleviation response in plants under abiotic stress conditions, while at the other end, they help plants to maintain their growth and development through fixation, mobilization, and/or production of nutrients, hormones, and organic biostimulant compounds. Such multifaceted actions of microbial community render them suitable for management of abiotic stress mitigation in crop plants. A number of rhizospheric bacteria belonging to the genera, viz., *Aeromonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Enterobacter*, *Methylobacterium*, *Pantoea*, *Pseudomonas, Rhizobium*, etc., have been reported to promote plant growth under multiple abiotic stresses (Egamberdiyeva and Höflich 2003; Nadeem et al. 2007; Omar et al. 2009; Singh et al. 2011a; Meena et al. 2012, 2017; Tittabutr et al. 2013; Sahoo et al. 2013; Sorty et al. 2016). Such PGPR significantly modulate plant growth by increasing nutrient uptake (P, K, Zn) through solubilization of complex minerals, secretion of biologically active phytohormones, and suppression of pathogens through siderophores and fungal cell wall-lysing enzymes (Frey-Klett and Garbaye 2005; Hameeda et al. 2006).

31.3.1 PGPR: Modifiers of Plant System Architecture

Spatial distribution, magnitude, and overall topology of the root system are the principal components of root architecture (Vacheron et al. 2013). Root architecture exhibits a unique characteristic of plasticity in response to different physicochemical condition of the soil. This allows the plants to better adapt to different chemical and physical edaphic properties, particularly under drought conditions (Bacon et al. 2002; Yu et al. 2007). Root traits such as lateral and longer root length and deeper root system are more prominent in plants facing drought conditions (Comas et al. 2013). Drought resistance in plants positively correlates a deep and prolific root system as evidenced from many studies involving soybeans, chickpea, maize, and wheat (Tuberosa et al. 2003, 2007; Landi et al. 2010; Varshney et al. 2011; Hund et al. 2011; Wasson et al. 2012). PGPR treatment of plants alters the root architecture

by increasing total root surface area for improved water and mineral uptake (Timmusk et al. 2014). Thus, plants with a prolific and deeper root system are more tolerant to drought stress than plants with fewer roots. Pretreated maize seeds with *Alcaligenes faecalis* AF3 showed an increase in root length by 10% compared to the untreated control plants when exposed to drought conditions (Naseem and Bano 2014). *Burkholderia phytofirmans* strain PsJN significantly (58–70%) increased root biomass of maize cultivars Mazurka and Kale under drought stress (Naveed et al. 2014). Similarly, inoculation of plants with *Enterobacter* sp. strain FD resulted in 47% and 40% increase in root mass of Mazurka and Kaleo cultivars, respectively, compared to control plants under drought stress conditions (Naveed et al. 2014). Another bacterial isolate, *Bacillus thuringiensis* AZP2 promoted longer (at least by 2–3 times) and denser lateral root development in wheat plants as compared to the uninoculated plants (Timmusk et al. 2014).

Under limited water availability, plants reduce shoot growth to limit leaf surface area for transpiration which also allows the plants to allocate more essential solutes toward housekeeping functions such as maintaining the osmotic balance (Achard et al. 2006; Neumann 2008; Skirycz and Inzé 2010). Such adaptive response takes a toll on the overall plant size and yield, for which stunt shoot growth is considered as a counterproductive response (Neumann 2008; Claeys and Inzé 2013). Therefore, near-normal shoot growth under limited water availability is an important desirable quality while developing drought-resistant crop varieties (Neumann 2008). Efficient PGPR strains can help plants in maintaining near-normal shoot growths and proper crop productivity even under drought stress. Plant growth-promoting bacteria *Bacillus* spp.-treated maize plants had improved shoot growth under drought stress conditions (Vardharajula et al. 2011). Bio-inoculated plants had significantly better shoot length and biomass compared to non-inoculated control plants indicating the potential of PGPR to enhance plant growth performance under limited water availability (Timmusk et al. 2014). Pepper plants treated with *Bacillus licheniformis* K11 promoted 50% higher biomass and an increase of plant shoot length compared to non-treated plants (Lim and Kim 2013). Similar changes in shoot morphology in PGPR treated have also been reported in other crops such as sorghum, sunflower, wheat, green gram, mung bean, and maize (Arzanesh et al. 2011; Saravanakumar et al. 2011; Kasim et al. 2013; Castillo et al. 2013; Naseem and Bano 2014; Grover et al. 2014; Sarma and Saikia 2014).

31.3.2 PGPR: Enhances Relative Water Content in Crops

Relative water content (RWC) in plant leaves is an important yardstick for the evaluation of water status in plants as water is directly linked to metabolic activity. Limited cell expansion under stress conditions results in the loss of turgor which is clearly reflected through a decline in RWC (Castillo et al. 2013). Higher RWC helps in maintaining proper turgor pressure conducive for physiological responses to counteract drought-associated oxidative and osmotic stresses. Drought-tolerant

plant species and cultivars have an intrinsic ability to maintain high RWC even under adverse conditions (Jarvis and Jarvis 1963). It was reported that drought-sensitive sorghum plants when inoculated with PGPR, *Bacillus* spp. strain KB 129, exhibited 24% more RWC as compared to the non-inoculated control plant (Grover et al. 2014). Similar activities have also been observed in maize plants (Naveed et al. 2014). Although there is an observed correlation between PGPR activity and RWC status in crop plants, the underlying mechanism for this phenomenon is yet to be fully understood. Observations from an interaction study between maize plant and PGPR, *Azospirillum brasilense* BR11005, suggested that abscisic acid (ABA) secreted from the bacterial partner might have stimulated stomatal closure under water-limited condition, which in turn reduced transpiration rate on leaf surface area (Casanovas et al. 2002). However, according to Dodd and coworkers (2010), modulations in physiological processes were responsible for stomatal closure rather than increase secretion of ABA (Dodd et al. 2010). It is expected that further research will shed a better understanding of the molecular mechanism leading to bacterial-mediated drought tolerance in crop plants.

31.3.3 PGPR: Promoting Osmotic Adjustment in Crops Under Drought Condition

Osmotic adjustment is a key cellular level process that minimizes the negative effects of drought stress (Blum 2005). Compatible solutes consisting of both organic and inorganic solutes help in maintaining cell turgor with a steady RWC over time (Serraj and Sinclair 2002). Osmotic adjustment essentially protects biomolecules such as cellular proteins, enzymes, cell organelles, transporters and membranes under drought stress (Kiani et al. 2007; Huang et al. 2014). Solutes such as glycine betaine, sucrose, mannitol, malate, calcium, proline, etc., are integral to the process of osmotic adjustment. Proline, an important osmolyte that accumulates in plants after exposure to drought, is regarded as a key player in stabilizing subcellular structures, scavenging free radicals and maintaining redox potential (Hayat et al. 2012; Huang et al. 2014). That proline accumulation positively correlates to drought tolerance in plants is evident from drought stress studies in pea, chickpea, rice, and soybean (Mafakheri et al. 2010; Silvente et al. 2012; Lum et al. 2014). Many PGPR strains with proven records of proline production have been reported to induce drought tolerance in crops like maize, sorghum, potato, and mung bean (Gururani et al. 2013; Naseem and Bano 2014; Sarma and Saikia 2014). A consortium consisting of PGPR strains, viz., *Bacillus cereus* AR156, *Bacillus subtilis* SM21 and *Serratia* sp. XY21, when applied to cucumber plants led to three- to fourfold increase in proline content which protected the plants from over-dehydration as compared to untreated controls (Wang et al. 2012).

31.3.4 PGPR: Reducers of Antioxidant Stress in Plants

Environmental stresses, both biotic and abiotic, generate a spectrum of reactive oxygen species (superoxide radical, hydrogen peroxide, singlet oxygen, and hydroxyl radical) in plant tissues (Carvalho Cruz de 2008). These ROS cause oxidative damages to biomolecules and cellular structures leading to cell death (Hasanuzzaman et al. 2014). To counteract the cellular oxidative damage, plants have evolved enzymatic and nonenzymatic antioxidants that scavenge the ROS in a coordinated manner (Carvalho Cruz de 2008). Enzymatic antioxidants include enzymes such as superoxide dismutase, catalase, peroxidase, glutathione reductase, and ascorbate peroxidase, while nonenzymatic antioxidants include biomolecules such as vitamin C and E, polyphenols, carotenoids, and glutathione. A direct correlation between the expression level of antioxidative enzymes and the extent of drought stress offers an interesting approach for the assessment of drought stress in plants (Contour-Ansel et al. 2006; Guo et al. 2006). Quantification of antioxidative enzymes such as superoxide dismutase, catalase, and peroxidase provides vital clues to evaluate PGPR-mediated drought tolerance in plants. Studies have revealed that *Bacillus pumilus* str. DH-11 and *Bacillus firmus* str. 40 increased ROS-scavenging enzymes in potato plants under drought stress (Gururani et al. 2013). The treated plants were able to overcome drought stress with an observed catalase enzyme activity up to 1.8 times higher as compared to non-treated plants. Similar observations were also reported in green gram plants inoculated with *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* EPB (Saravanakumar et al. 2011). However, we are yet to understand whether the expression level of catalase depends on the physiological status of the studied plants or a prospective bacteria produces multiple ROS-scavenging enzymes under different field circumstances. The effect of PGPR to induce other non-reported ROS-scavenging mechanisms in different crops remains to be addressed.

31.3.5 PGPR: Regulating Plant Growth During Abiotic Stress

Plant growth regulators, especially phytohormones, work synergistically to control the plant growth and development during the life cycle of the plant. Important phytohormones such as auxins, gibberellins, cytokinins, ethylene, and abscisic acid are involved in the plant's stress response and defense mechanisms (Farooq et al. 2009). Under drought stress and a limited water budget, expression level of growth regulatory phytohormones and signal molecules increases to accommodate basic housekeeping cellular functions (Farooq et al. 2009). Therefore, prospective PGPR strains have to efficiently modulate the phytohormones to function under drought stress including downregulating ethylene production, homeostasis of cytokinins and abscisic acid, and IAA signaling (Belimov et al. 2009; Contesto et al. 2010; Dodd et al. 2010; Bresson et al. 2014). Studies have shown that such modulatory properties are mandatory for a prospective PGPR to establish itself in the field condition and to promote plant growth under actual drought stress.

Auxin, indole-3-acetic acid (IAA), is an important phytohormone associated with diverse cellular functions responsible for plant growth and development. This plant growth regulator is responsible for (1) vascular tissue differentiation, (2) lateral and adventitious root development, (3) cell division, (4) stem and root elongation, and (5) phototropism (Glick 1995). Interestingly, many of the PGPR are known for their ability to produce high levels of IAA which stimulates root development and influences root architecture. It is now understood that interaction with such bacteria leads to increased root hairs and root surface area, the sites known for active nutrient uptake. Treatment of clover (*Trifolium repens* L.) plants with PGPR (*P. putida* and *B. megaterium*) increased shoot and root biomass and water content under drought stress that correlated with increased IAA production (Marulanda et al. 2009). Treatment of *Arabidopsis* plants with PGPR *Phyllobacterium brassicacearum* strain STM196 resulted in increased lateral root length and modifications of the root architecture that led to the observed drought tolerance (Bresson et al. 2014). The increases in root length and modifications of the root architecture correlated with increased IAA concentrations in rhizobacteria-treated plants suggesting that bacterial-mediated drought tolerance may be partly mediated by IAA (Contesto et al. 2010).

Ethylene (ET), an important plant growth regulator, is highly expressed under various biotic and abiotic stress signals such as wounding, exposure to chemicals and metals, water stress, and phytopathogen infection (Johnson and Ecker 1998). Regulation of 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ET, has been an alternative for reducing the expression of ethylene and for maintaining near-normal plant growth under drought stress (Saleem et al. 2007). Bacteria with ACC deaminase enzyme activity can hydrolyze ACC, thereby regulating ethylene production. Such PGPR strains have been reported to cleave ACC into ammonia and alpha-ketobutyrate (Glick et al. 1998). The ACC deaminase producing *Pseudomonas* spp. reduced the symptoms of drought stress in pea plants (Arshad et al. 2008). Another ACC deaminase-producing bacteria, *Achromobacter piechaudii* ARV8, repressed the expression level of ethylene and recovered growth in treating tomato and pepper (Mayak et al. 2004). The PGPR strains with the ability to produce IAA and ACC deaminase have been reported to promote barley and oats growth, respectively, in hypersaline soils (Chang et al. 2014).

Abscisic acid (ABA) plays a key role in influencing physiological processes in plants under various environmental stress conditions (Cohen et al. 2008). It has been observed that elevated levels of ABA modulate physiological processes in plants for better survival and growth, particularly under drought stress (Farooq et al. 2009). The ABA enhances plant growth under drought through multiple mechanisms. One such widely accepted theory states that ABA modulates leaf transpiration and root hydraulic conductivity under limited water resources (Aroca et al. 2006; Parent et al. 2009), while other theories suggest that ABA regulates aquaporins which are known membrane proteins responsible for transfer of water across membranes (Kaldenhoff et al. 1996, 2008). In this regard, PGPR that elevate the concentration of ABA in plants have significant scope in agricultural practices for crop production under low water availability conditions. Early reports from Arkhipova and co-workers (2007)

(Arkhipova et al. 2007) showed that lettuce (*Lactuca sativa* L.) plants treated with *Bacillus* sp. had increased amounts of ABA when compared to non-treated control plants. A similar phenomenon of increased ABA content in *Arabidopsis* plants treated with PGPR strain *Azospirillum brasilense* Sp245 was also reported that could be correlated with the observed drought tolerance (Cohen et al. 2008).

Studies on the crosstalks among different candidate bioinoculants have yielded some interesting findings related to field survivability of both the microbes and the target plants. For example, the rhizobium, *Rhizobium tropici*, is sensitive to drought stress, when applied alone to bean (*Phaseolus vulgaris* L.), and significantly fails to fix N_2 under water-limited conditions. However, the same *Rhizobium* when co-inoculated with two strains of *P. polymyxa* resumes its N_2 fixation ability leading to better plant biomass and nodule formation (Figueiredo et al. 2008).

31.3.6 PGPR: Influencer of Nutrient Uptake in Plants

Application of rampant fertilizers is a key factor in the low nutrient uptake efficiency by crop plants. A major portion of the available phosphorus (up to 90%) readily reacts with iron (Fe^{3+}), aluminum (Al^{3+}), and calcium (Ca^{2+}) ions to form a relatively insoluble complex and precipitates, making it unavailable to plants (Bhattacharya 2019). In fact, inadequacy in soil nutrients is another major abiotic factor that threatens plant growth. From an agronomic point of view, PGPR with the ability to solubilize or uptake the supplied fertilizers can decrease agricultural inputs without any effect on plant nutrition. Such bacteria can efficiently solubilize and increase the available phosphate for plant uptake (Duarah et al. 2011; Sharma et al. 2013; Dinesh et al. 2013). This is further supported by the results of PGPR cutting down the rates of fertilizer application (Shaharoona et al. 2008; Adesemoye et al. 2009; Hemissi et al. 2019).

31.3.7 PGPR Improve Soil and Plant Health Through Exopolysaccharides

Bacterial exopolysaccharides (EPS) are high-molecular-weight complex polymers principally composed of sugar moieties arranged as repeating units within the polymer molecules, but it may also contain proteins and humic substances (Morgan et al. 1990; Nielsen and Jahn 1999; Deka et al. 2019). Bacterial EPS associated with soil forms soil aggregates which stabilize and provide a continuous water and nutrient balance for agricultural crops, and as such bacterial EPS improves crop productivity and also helps increasing physiochemical properties of soil (Ashraf et al. 2005; Batool and Hasnain 2005; Qurashi and Sabri 2012). It was reported that exopolysaccharides (EPS) production ability of *Pseudomonas* helps in root colonization, biofilm formation, soil health improvement and plant growth (Sen and Chandrasekhar 2014). Application of EPS-producing *Bacillus amyloliquefaciens* improved soil health (soil aggregation) as well as plant health (Deka et al. 2019).

Several other studies also reported that EPS-producing bacteria improve soil and plant health (Lynch and Bragg 1985; Gouzou et al. 1993; Amellal et al. 1998; Alami et al. 2000). Heat and salt stresses are the key factors for EPS production in many PGPR. The EPS produced from such bacteria are crucial in terms of biofilm production, plant protection during desiccation, and other abiotic stress conditions (Qurashi and Sabri 2012). The bacterial biofilm formed as a function of EPS contains sugars, oligo- and polysaccharides, and some other beneficial macromolecules, thereby facilitating an active hydrated micro-environment for efficient plant-microbe interaction (Chang et al. 2007).

31.3.8 PGPR: Ameliorating High-Risk Agricultural Inputs

Application of agricultural inputs such as pesticides, herbicides, fungicides, and many heavy metals above their critical concentration can not only affect soil health but also the microbial diversity at their microsite level. The PGPR are not exceptional to the adverse effects of these recalcitrant compounds for which it is desirable that prospective PGPR sequester the critical dose of the pesticide or enzymatically degrade it to nontoxic or lesser toxic compounds. A strain belonging to *Mesorhizobium* genus was found to exhibit multi-spectrum tolerance to insecticides such as imidacloprid and thiamethoxam; herbicides such as metribuzin and glyphosate; and fungicides, viz., hexaconazole, metalaxyl, and kitazin (Ahemad and Khan 2012). *Pseudomonas* has been reported to tolerate organophosphorus pesticides, viz., guthion, methyl parathion and dimethoate, and sulfonylurea, and, herbicides, viz., metsulfuron methyl, chlorsulfuron, and thifensulfuron methyl (Boldt and Jacobsen 1998; Nazarian and Mousawi 2005). A *Rhizobium* strain specific to chickpea and green gram is reported to tolerate aldrin (Juneja and Dogra 1978). Several species of PGPR *Azotobacter* were found to resist 1% to 5% of pesticides, viz., pendimethalin, chlorpyrifos, glyphosate, and phorate (Chennappa et al. 2014).

Few PGPR have developed a different strategy to tackle the adverse effects of these recalcitrant compounds which involve active degradation through enzymatic hydrolysis, a property that can be utilized for large-scale in situ microbial degradation approaches (Herman et al. 2005). *Pseudomonas diminuta* and *Flavobacterium* sp. hydrolyze different organophosphorus insecticides through the action of organophosphorus hydrolase (Dumas et al. 1989). Similarly, *Pseudomonas maltophilia* inactivates the herbicide dicamba through the secretion of dicamba monooxygenase (Herman et al. 2005). Sometimes, microbes employ a multi-step degradation strategy involving a cascade of enzymatic reaction. For example, *Pseudomonas* sp. strain ADP which can utilize atrazine as the sole carbon source breaks down the substrate through the action of three enzymes, viz., AtzA, AtzB, and AtzC. The first enzyme, i.e., AtzA, catalyzes the conversion of atrazine to nontoxic hydroxyl atrazine; which is used as a substrate by the second enzyme, AtzB, to convert into N-isopropyl cyanuric amide. In the third step, the enzyme AtzC catalyzes the conversion of N-isopropyl cyanuric amide into cyanuric acid and isopropylamine. This cyanuric

acid is finally utilized as a nitrogen source by many soil bacteria (De Souza et al. 1996; Wackett et al. 2002).

31.3.9 PGPR: Alleviating Acidic Soil-Associated Al Phytotoxicity in Plants

Many plants exhibit intrinsic physiological responses to overcome Al phytotoxicity that can be broadly categorized into external and internal responses. External responses include secretion of organic acids into the rhizosphere to celate and neutralize aluminum ions (Delhaize et al. 2012), while internal responses involve actively transporting the aluminum ions into the root system and sequestering into plant vacuoles (Ramgareeb et al. 2004). Interestingly, siderophore-producing PGPR can help plants in reducing aluminum bioavailability, thereby protecting the sensitive cell systems and tissues from their inhibitory effects. Such PGPR have already been demonstrated to support plant growth in heavy metal-contaminated soils and reported to induce metal stress-related genes in plants (Idris et al. 2004; Belimov et al. 2005; Dell'Amico et al. 2005; Barzanti et al. 2007; Jiang et al. 2008; Kuffner et al. 2010; Aizawa et al. 2010). However, till this date, very little is known about Al-resistant PGPR and their physiological support to plant growth. Strains of *Pseudomonas simiae*, *Chryseobacterium polytrichastri*, and *Burkholderia ginsengiterrae* isolated from the rhizosphere of diseased Korean ginseng roots induced high expression of Al stress-related genes, *AtAIP*, *AtALS3*, and *AtALMT1*, in *Arabidopsis thaliana* stressed by aluminum. These strains with auxin and siderophore production ability together with phosphate solubilization were able to support growth both in *A. thaliana* and Korean ginseng seedlings, with particular influence on the foliar expansion and chlorophyll contents (Farh et al. 2017). In addition, acid-tolerant bacteria having in vitro PGP activities have been reported earlier and found that the in vitro PGP activities highly decreased under acid stress condition (Goswami et al. 2017; Deka et al. 2019).

31.3.10 PGPR: Tackling Heavy Metal Toxicity in Soils

Conventional remediation processes involving physicochemical techniques have inherent issues of environmental impacts and large incurred costs (Quartacci et al. 2006). In this scenario, application of PGPR offers an eco-friendly and cost-effective alternative to remediate such contaminated soils and to promote plant growth by ameliorating metal-induced stress (Pandey et al. 2013; Pramanik et al. 2016). A candidate PGPR targeted for heavy metal-contaminated soils must have either of these two abilities: (1) bioaccumulation, that is, sequestering the metals in cell compartments, and (2) biotransformation, which is conversion of a metal from a toxic state to nontoxic or less toxic forms through alterations in the valence states (Chen et al. 2016; Pramanik et al. 2016). Examples of such PGPR are *Ochrobactrum*, *Bacillus*, *Raoultella*, *Klebsiella*, *Leifsonia*, and *Enterobacter*

(Garrett et al. 2010; Pandey et al. 2013; Chen et al. 2016; Pramanik et al. 2016, 2017; Ahmad et al. 2016; Mitra et al. 2018). Major constraints in the field application of these strains are as follows: (1) survival of the strain becomes uncertain in a heavy metal-contaminated soil typically containing multiple metals and chemicals, (2) these PGPR are exposed not only to heavy metals but also variability in soil edaphic factors such as pH and temperature, and (3) heavy metal tolerance does not warrant plant growth-promoting abilities (Pramanik et al. 2018a, b). Therefore, further screening approaches are needed to prospect promising multi-metal-resistant PGPR in future.

31.4 Conclusion

During the recent times, the impact of global climate change has raised serious concern on environmental stress conditions affecting agri-ecosystems, crop productivity, and soil health. Effects of the environmental stress factors discussed above can impact the crop plants at physiological, biological, and molecular levels leading to loss amounting to 30–50% of agricultural productivity. The current state of intensive use of high-energy agricultural input coupled with erratic pattern of stress conditions requires a futuristic and feasible alternative to conventional agricultural and remediation practices. Candidate rhizobacteria with numerous plant growth-promoting activities can be prospected as ecological engineers to counter climate change-induced stresses. Such bioinoculum can support enhanced production of quality food grains and cut 20–25% spending in agricultural inputs. Although several PGPR have been reported to aid plant growth under adverse conditions, results of such studies vastly differ from actual field based data where both the target plant and the bioinoculum face multiple and recurring stresses. Multiple stresses may evoke a completely unique set of responsive mechanisms different from stresses applied individually. Therefore, sustained research efforts are required to understand the physicochemical and molecular mechanisms underlying the plant-microbe interactions in real field conditions. Knowledge on such interactions will provide valuable insights to microbe-mediated stress amelioration strategies and supportive data to the current findings. In parallel, native microbial diversity should also be tested for the discovery of novel stress-tolerant PGPR and to formulate microbial consortia targeting multi-spectrum stresses.

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References

Abbas SS, Schaalan MF, Bahgat AK, El-Denshary ES (2014) Possible potentiation by certain antioxidants of the anti-inflammatory effects of diclofenac in rats. Sci World J 2014:731462. https://doi.org/10.1155/2014/731462

Abd-Alla MH, El-Enany A-WE, Nafady NA et al (2014) Synergistic interaction of rhizobium leguminosarum bv. viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (Vicia faba L.) in alkaline soil. Microbiol Res 169:49–58. https://doi.org/10.1016/J.MICRES.2013.07.007

Abrol IP (1986) Salt-affected soils: an overview. In: Chopra VL, Paroda SL (eds) Approches for incorporating drought and salinity resistance in crop plants. Oxford and IBH Publishing Company, New Delhi, pp 1–23

Achard P, Cheng H, De Grauwe L et al (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science 311:91–94. https://doi.org/10.1126/science.1118642

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929. https://doi.org/10.1007/s00248-009-9531-y

Ahemad M, Khan MS (2012) Effects of pesticides on plant growth promoting traits of Mesorhizobium strain MRC4. J Saudi Soc Agric Sci 11:63–71. https://doi.org/10.1016/j.jssas.2011.10.001

Ahmad I, Akhtar MJ, Asghar HN et al (2016) Differential effects of plant growth-promoting rhizobacteria on maize growth and cadmium uptake. J Plant Growth Regul 35:303–315. https://doi.org/10.1007/s00344-015-9534-5

Aizawa T, Ve NB, Vijarnsorn P et al (2010) Burkholderia acidipaludis sp. nov., aluminium-tolerant bacteria isolated from Chinese water chestnut (Eleocharis dulcis) growing in highly acidic swamps in South-East Asia. Int J Syst Evol Microbiol 60:2036–2041

Alami Y, Achouak W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing Rhizobium sp. strain isolated from sunflower roots. Appl Environ Microbiol 66:3393–3398. https://doi.org/10.1128/AEM.66.8.3393-3398.2000

Amellal N, Burtin G, Bartoli F, Heulin T (1998) Colonization of wheat roots by an exopolysaccharide-producing Pantoea agglomerans strain and its effect on rhizosphere soil aggregation. Appl Environ Microbiol 64:3740–3747. https://doi.org/10.1128/aem.64.10.3740-3747.1998

American_Meteorological_Society_Council (2004) AMS statement on meteorological drought. Bull Am Meteorol Soc 85:771–773

Arkhipova TN, Prinsen E, Veselov SU et al (2007) Cytokinin producing bacteria enhance plant growth in drying soil. Plant Soil 292:305–315. https://doi.org/10.1007/s11104-007-9233-5

Aroca R, Ferrante A, Vernieri P, Chrispeels MJ (2006) Drought, abscisic acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in Phaseolus vulgaris plants. Ann Bot 98:1301–1310. https://doi.org/10.1093/aob/mcl219

Arshad M, Shaharoona B, Mahmood T (2008) Inoculation with Pseudomonas spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (Pisum sativum L.) project supported by the Higher Education Commission, Islamabad, Pakistan (no. PIN 041 211534 A-031). Pedosphere 18:611–620. https://doi.org/10.1016/S1002-0160(08)60055-7

Arzanesh MH, Alikhani HA, Khavazi K et al (2011) Wheat (Triticum aestivum L.) growth enhancement by Azospirillum sp. under drought stress. World J Microbiol Biotechnol 27:197–205. https://doi.org/10.1007/s11274-010-0444-1

Ashraf M (1994) Breeding for salinity tolerance in plants. CRC Crit Rev Plant Sci 13:17–42. https://doi.org/10.1080/07352689409701906

Ashraf M, Philip H (2005) Abiotic stresses: plant resistance through breeding and molecular approaches. CRC Press, Boca Raton, FL

Ashraf M, Hasnain S, Hussain F (2005) Exo-polysaccharides (exopolysaccharide) producing biofilm bacteria in improving physico-chemical characteristics of the salt affected soils. In: Int. conf. environmentally sustainable development (ESDev-2005)

Ashrafuzzaman M, Hossen FA, Razi Ismail M et al (2009) Efficiency of plant growth promoting rhizobacteria (PGPR) for the enhancement of rice growth. Afr J Biotechnol 8:1247–1252. https://doi.org/10.1016/j.tiv.2009.05.015

Babula P, Adam V, Opatrilova R et al (2008) Uncommon heavy metals, metalloids and their plant toxicity: a review. Environ Chem Lett 6:189–213

Backer R, Rokem JS, Ilangumaran G et al (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 871:1473. https://doi.org/10.3389/fpls.2018.01473

Bacon MA, Davies WJ, Mingo D, Wilkinson S (2002) Root Signals. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half, 3rd edn. Monticello, New York, pp 461–470

Barrow JR, Lucero ME, Reyes-Vera I, Havstad KM (2008) Do symbiotic microbes have a role in regulating plant performance and response to stress? Commun Integr Biol 1:69–73. https://doi.org/10.4161/cib.1.1.6238

Barzanti R, Ozino F, Bazzicalupo M et al (2007) Isolation and characterization of endophytic bacteria from the nickel hyperaccumulator plant Alyssum bertolonii. Microb Ecol 53:306–316. https://doi.org/10.1007/s00248-006-9164-3

Batool R, Hasnain S (2005) Growth stimulatory effects of enterobacter and serratia isolated from biofilms on plant growth and soil aggregation. Biotechnology (Faisalabad) 4:347–353. https://doi.org/10.3923/biotech.2005.347.353

Belimov AA, Hontzeas N, Safronova VI et al (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.). Soil Biol Biochem 37:241–250. https://doi.org/10.1016/j.soilbio.2004.07.033

Belimov AA, Dodd IC, Hontzeas N et al (2009) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. New Phytol 181:413–423. https://doi.org/10.1111/j.1469-8137.2008.02657.x

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35:1044–1051

Bhattacharya A (2019) Changing environmental condition and phosphorus-use efficiency in plants. In: Changing climate and resource use efficiency in plants. Elsevier, Amsterdam, pp 241–305

Bian M, Zhou M, Sun D, Li C (2013) Molecular approaches unravel the mechanism of acid soil tolerance in plants. Crop J 1:91–104

Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Aust J Agric Res 56:1159. https://doi.org/10.1071/AR05069

Boldt TS, Jacobsen CS (1998) Different toxic effects of the sulfonylurea herbicides metsulfuron methyl, chlorsulfuron and thifensulfuron methyl on fluorescent pseudomonads isolated from an agricultural soil. FEMS Microbiol Lett 161:29–35. https://doi.org/10.1016/S0378-1097(98)00045-7

Bresson J, Vasseur F, Dauzat M et al (2014) Interact to survive: Phyllobacterium brassicacearum improves arabidopsis tolerance to severe water deficit and growth recovery. PLoS One 9:e107607. https://doi.org/10.1371/journal.pone.0107607

Budak H, Kantar M, Kurtoglu KY et al (2013) Drought tolerance in modern and wild wheat. Sci World J 2013:16. https://doi.org/10.1155/2013/548246

Burke MJ, Gusta LV, Quamme HA et al (1976) Freezing and injury in plants. Annu Rev Plant Physiol 27:507–528. https://doi.org/10.1146/annurev.pp.27.060176.002451

Carvalho Cruz de HM (2008) Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal Behav 3:156–165

Casanovas EM, Barassi CA, Sueldo RJ (2002) Azospirillum inoculation mitigates water stress effects in maize seedlings. Cereal Res Commun 30:343–350. https://doi.org/10.1007/BF03543428

Castillo P, Escalante M, Gallardo M et al (2013) Effects of bacterial single inoculation and co-inoculation on growth and phytohormone production of sunflower seedlings under water stress. Acta Physiol Plant 35:2299–2309. https://doi.org/10.1007/s11738-013-1267-0

Chakraborty U, Chakraborty B, Basnet M (2006) Plant growth promotion and induction of resistance in Camellia sinensis by Bacillus megaterium. J Basic Microbiol 46:186–195. https://doi.org/10.1002/jobm.200510050

Chang WS, Van De Mortel M, Nielsen L et al (2007) Alginate production by Pseudomonas putida creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. J Bacteriol 189(22):8290–8299

Chang P, Gerhardt KE, Huang XD et al (2014) Plant growth-promoting bacteria facilitate the growth of barley and oats in salt-impacted soil: implications for phytoremediation of saline soils. Int J Phytoremediation 16:1133–1147. https://doi.org/10.1080/15226514.2013.821447

Chauhan H, Bagyaraj DJ, Selvakumar G, Sundaram SP (2015) Novel plant growth promoting rhizobacteria-prospects and potential. Appl Soil Ecol 95:38–53

Chen Y, Chao Y, Li Y et al (2016) Survival strategies of the plant-associated bacterium Enterobacter sp. strain EG16 under cadmium stress. Appl Environ Microbiol 82:1734–1744. https://doi.org/10.1128/AEM.03689-15

Chennappa G, Adkar-Purushothama CR, Suraj U et al (2014) Pesticide tolerant Azotobacter isolates from paddy growing areas of northern Karnataka, India. World J Microbiol Biotechnol 30:1–7. https://doi.org/10.1007/s11274-013-1412-3

Claeys H, Inzé D (2013) The agony of choice: how plants balance growth and survival under water-limiting conditions. Plant Physiol 162:1768–1779

Cohen AC, Bottini R, Piccoli PN (2008) Azospirillum brasilense Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. Plant Growth Regul 54:97–103. https://doi.org/10.1007/s10725-007-9232-9

Coleman-Derr D, Tringe SG (2014) Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. Front Microbiol 5:283. https://doi.org/10.3389/fmicb.2014.00283

Comas LH, Becker SR, Cruz VMV et al (2013) Root traits contributing to plant productivity under drought. Front Plant Sci 4:442

Contesto C, Milesi S, Mantelin S et al (2010) The auxin-signaling pathway is required for the lateral root response of arabidopsis to the rhizobacterium phyllobacterium brassicacearum. Planta 232:1455–1470. https://doi.org/10.1007/s00425-010-1264-0

Contour-Ansel D, Torres-Franklin ML, Cruz De Carvalho MH et al (2006) Glutathione reductase in leaves of cowpea: cloning of two cDNAs, expression and enzymatic activity under progressive drought stress, desiccation and abscisic acid treatment. Ann Bot 98:1279–1287. https://doi.org/10.1093/aob/mcl217

Cooper M, Gho C, Leafgren R et al (2014) Breeding drought-tolerant maize hybrids for the US corn-belt: discovery to product. J Exp Bot 65:6191–6194. https://doi.org/10.1093/jxb/eru064

Dahlgren RA, Macías F, Arbestain MC et al (2008) Acidity. In: Chesworth W (ed) Encyclopedia of soil science. Springer, Dordrecht, pp 10–21

De Souza ML, Sadowsky MJ, Wackett LP (1996) Atrazine chlorohydrolase from Pseudomonas sp. strain ADP: gene sequence, enzyme purification, and protein characterization. J Bacteriol 178:4894–4900. https://doi.org/10.1128/jb.178.16.4894-4900.1996

de Souza R, Ambrosini A, Passaglia LMP (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38:401–419. https://doi.org/10.1590/S1415-475738420150053

De Zelicourt A, Al-Yousif M, Hirt H (2013) Rhizosphere microbes as essential partners for plant stress tolerance. Mol Plant 6:242–245. https://doi.org/10.1093/mp/sst028

Deka P, Goswami G, Das P et al (2019) Bacterial exopolysaccharide promotes acid tolerance in Bacillus amyloliquefaciens and improves soil aggregation. Mol Biol Rep 46:1079–1091. https://doi.org/10.1007/s11033-018-4566-0

Delhaize E, Ma JF, Ryan PR (2012) Transcriptional regulation of aluminium tolerance genes. Trends Plant Sci 17:341–348

Dell'Amico E, Cavalca L, Andreoni V (2005) Analysis of rhizobacterial communities in perennial Graminaceae from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. FEMS Microbiol Ecol 52:153–162. https://doi.org/10.1016/j.femsec.2004.11.005

Dinesh R, Anandaraj M, Kumar A et al (2013) Effects of plant growth-promoting rhizobacteria and NPK fertilizers on biochemical and microbial properties of soils under ginger (Zingiber officinale) cultivation. Agric Res 2:346–353. https://doi.org/10.1007/s40003-013-0080-8

Dodd IC, Zinovkina NY, Safronova VI, Belimov AA (2010) Rhizobacterial mediation of plant hormone status. Ann Appl Biol 157:361–379

Dong J, Wu F, Zhang G (2006) Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (Lycopersicon esculentum). Chemosphere 64:1659–1666. https://doi.org/10.1016/j.chemosphere.2006.01.030

Duarah I, Deka M, Saikia N, Deka Boruah HP (2011) Phosphate solubilizers enhance NPK fertilizer use efficiency in rice and legume cultivation. 3 Biotech 1:227–238. https://doi.org/10.1007/s13205-011-0028-2

Dumas DP, Caldwell SR, Wild JR, Raushel FM (1989) Purification and properties of the phosphotriesterase from Pseudomonas diminuta. J Biol Chem 264:19659–19665

EEA (2011) Europe's environment—an assessment of assesments. EEA, Copenhagen

Egamberdiyeva D, Höflich G (2003) Influence of growth-promoting on the growth of wheat in different soils and temperatures. Soil Biol Biochem 35:973–978. https://doi.org/10.1016/S0038-0717(03)00158-5

Eisenstein M (2013) Plant breeding: discovery in a dry spell. Nature 501:S7–S9. https://doi.org/10.1038/501S7a

Farh MEA, Kim YJ, Sukweenadhi J et al (2017) Aluminium resistant, plant growth promoting bacteria induce overexpression of Aluminium stress related genes in Arabidopsis thaliana and increase the ginseng tolerance against Aluminium stress. Microbiol Res 200:45–52. https://doi.org/10.1016/j.micres.2017.04.004

Farooq M, Wahid A, Kobayashi N et al (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212

Fedoroff NV, Battisti DS, Beachy RN et al (2010) Radically rethinking agriculture for the 21st century. Science 327:833–834

Figueiredo MVB, Burity HA, Martínez CR, Chanway CP (2008) Alleviation of drought stress in the common bean (Phaseolus vulgaris L.) by co-inoculation with paenibacillus polymyxa and rhizobium tropici. Appl Soil Ecol 40:182–188. https://doi.org/10.1016/j.apsoil.2008.04.005

Foley JA, Ramankutty N, Brauman KA et al (2011) Solutions for a cultivated planet. Nature 478:337–342. https://doi.org/10.1038/nature10452

Frey-Klett P, Garbaye J (2005) Mycorrhiza helper bacteria: a promising model for the genomic analysis of fungal-bacterial interactions. New Phytol 168:4–8. https://doi.org/10.1111/j.1469-8137.2005.01553.x

Garrett WS, Gallini CA, Yatsunenko T et al (2010) Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe 8:292–300. https://doi.org/10.1016/j.chom.2010.08.004

Gatehouse AMR, Ferry N, Edwards MG, Bell HA (2011) Insect-resistant biotech crops and their impacts on beneficial arthropods. Philos Trans R Soc Lond Ser B Biol Sci 366:1438–1452. https://doi.org/10.1098/rstb.2010.0330

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117. https://doi.org/10.1139/m95-015

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68. https://doi.org/10.1006/jtbi.1997.0532

Goodwin SB, Sutter TR (2009) Microarray analysis of Arabidopsis genome response to aluminum stress. Biol Plant 53:85–99. https://doi.org/10.1007/s10535-009-0012-4

Gopalakrishnan S, Sathya A, Vijayabharathi R et al (2015) Plant growth promoting rhizobia: challenges and opportunities. 3 Biotech 5:355–377

Goswami G, Deka P, Das P et al (2017) Diversity and functional properties of acid-tolerant bacteria isolated from tea plantation soil of Assam. 3 Biotech 7:229. https://doi.org/10.1007/s13205-017-0864-9

Gouzou L, Burtin G, Philippy R et al (1993) Effect of inoculation with Bacillus polymyxa on soil aggregation in the wheat rhizosphere: preliminary examination. In: Soil structure/soil biota interrelationships. Elsevier, Amsterdam, pp 479–491

Greaves JA (1996) Improving suboptimal temperature tolerance in maize- the search for variation. J Exp Bot 47:307–323. https://doi.org/10.1093/jxb/47.3.307

Grover M, Ali SZ, Sandhya V et al (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27:1231–1240. https://doi.org/10.1007/s11274-010-0572-7

Grover M, Madhubala R, Ali SZ et al (2014) Influence of *Bacillus* spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions. J Basic Microbiol 54:951–961. https://doi.org/10.1002/jobm.201300250

Guo Z, Ou W, Lu S, Zhong Q (2006) Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. Plant Physiol Biochem 44:828–836. https://doi.org/10.1016/j.plaphy.2006.10.024

Gururani MA, Upadhyaya CP, Baskar V et al (2013) Plant growth-promoting Rhizobacteria enhance abiotic stress tolerance in Solanum tuberosum through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. J Plant Growth Regul 32:245–258. https://doi.org/10.1007/s00344-012-9292-6

Hameeda B, Rupela O, Reddy G, Satyavani K (2006) Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of pearl millet (Pennisetum glaucum L). Biol Fertil Soils 43:221–227

Hasanuzzaman M, Kamrun N, Fujita M (2013a) Extreme temperature responses, oxidative stress and antioxidant defense in plants. In: Kourosh V, Leslie C (eds) Abiotic stress - plant responses and applications in agriculture. InTech, London, pp 169–205

Hasanuzzaman M, Nahar K, Alam M et al (2013b) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int J Mol Sci 14:9643–9684. https://doi.org/10.3390/ijms14059643

Hasanuzzaman M, Nahar K, Gill SS et al (2014) Drought stress responses in plants, oxidative stress, and antioxidant defense. In: Tuteja N, Gill SS (eds) Climate change and plant abiotic stress tolerance. Wiley-VCH Verlag GmbH, KGaA, Weinheim, pp 209–249

Hayat S, Hayat Q, Alyemeni MN et al (2012) Role of proline under changing environments: a review. Plant Signal Behav 7:1456–1466

Hemissi I, Hammami R, Hachana A et al (2019) Fertilizer-dependent efficiency of Mesorhizobium strain for improving growth, nutrient uptake and grain yield of durum wheat (Triticum turgidium L.) variety. J New Sci 61:3885–3891

Herman PL, Behrens M, Chakraborty S et al (2005) A three-component Dicamba O-demethylase from Pseudomonas maltophilia, strain DI-6: gene isolation, characterization, and heterologous expression. J Biol Chem 280:24759–24767. https://doi.org/10.1074/jbc.M500597200

Huang B, DaCosta M, Jiang Y (2014) Research advances in mechanisms of turfgrass tolerance to abiotic stresses: from physiology to molecular biology. CRC Crit Rev Plant Sci 33:141–189. https://doi.org/10.1080/07352689.2014.870411

Hund A, Reimer R, Messmer R (2011) A consensus map of QTLs controlling the root length of maize. Plant Soil 344:143–158. https://doi.org/10.1007/s11104-011-0735-9

Idris R, Trifonova R, Puschenreiter M et al (2004) Bacterial communities associated with flowering plants of the Ni hyperaccumulator Thlaspi goesingense. Appl Environ Microbiol 70:2667–2677. https://doi.org/10.1128/AEM.70.5.2667-2677.2004

Ilangumaran G, Smith DL (2017) Plant growth promoting rhizobacteria in amelioration of salinity stress: a systems biology perspective. Front Plant Sci 8:1–14. https://doi.org/10.3389/fpls.2017.01768

Jarvis PG, Jarvis MS (1963) The water relations of tree seedlings: IV. Some aspects of the tissue water relations and drought resistance. Physiol Plant 16:501–516. https://doi.org/10.1111/j.1399-3054.1963.tb08327.x

Jiang CY, Sheng XF, Qian M, Wang QY (2008) Isolation and characterization of a heavy metal-resistant Burkholderia sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere 72:157–164. https://doi.org/10.1016/j.chemosphere.2008.02.006

Johnson PR, Ecker JR (1998) The ethylene gas signal transduction pathway: a molecular perspective. Annu Rev Genet 32:227–254. https://doi.org/10.1146/annurev.genet.32.1.227

Juneja S, Dogra RC (1978) Effect of Aldrin on growth and oxidative metabolism of rhizobia. J Appl Bacteriol 44:107–115. https://doi.org/10.1111/j.1365-2672.1978.tb00781.x

Kaldenhoff R, Kölling A, Richter G (1996) Regulation of the Arabidopsis thaliana aquaporin gene AthH2 (PIPlb). J Photochem Photobiol B Biol 36:351–354. https://doi.org/10.1016/S1011-1344(96)07392-7

Kaldenhoff R, Ribas-Carbo M, Sans JF et al (2008) Aquaporins and plant water balance. Plant Cell Environ 31:658–666

Kasim WA, Osman ME, Omar MN et al (2013) Control of drought stress in wheat using plant-growth-promoting bacteria. J Plant Growth Regul 32:122–130. https://doi.org/10.1007/s00344-012-9283-7

Kiani SP, Talia P, Maury P et al (2007) Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. Plant Sci 172:773–787. https://doi.org/10.1016/j.plantsci.2006.12.007

Kochian L (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Annu Rev Plant Physiol 46:237–260

Kuffner M, De Maria S, Puschenreiter M et al (2010) Culturable bacteria from Zn- and Cd-accumulating Salix caprea with differential effects on plant growth and heavy metal availability. J Appl Microbiol 108:1471–1484. https://doi.org/10.1111/j.1365-2672.2010.04670.x

Landi P, Giuliani S, Salvi S et al (2010) Characterization of root-yield-1.06, a major constitutive QTL for root and agronomic traits in maize across water regimes. J Exp Bot 61:3553–3562. https://doi.org/10.1093/jxb/erq192

Levitt J (1972) Responses of plants to environmental stresses. Academic Press, New York

Lim JH, Kim SD (2013) Induction of drought stress resistance by multi-functional PGPR Bacillus licheniformis K11 in pepper. Plant Pathol J 29:201–208. https://doi.org/10.5423/PPJ.SI.02.2013.0021

Lum MS, Hanafi MM, Akmar ASN (2014) Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. J Anim Plant Sci 24:1487–1493

Lynch J, Bragg E (1985) Microorganisms and soil aggregate stability. Springer, New York

Lyons JM (1973) Chilling injury in plants. Annu Rev Plant Physiol 24:445–466. https://doi.org/10.1146/annurev.pp.24.060173.002305

Mafakheri A, Siosemardeh A, Bahramnejad B et al (2010) Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust J Crop Sci 4(8):580

Mancosu N, Snyder R, Kyriakakis G, Spano D (2015) Water scarcity and future challenges for food production. Water 7:975–992. https://doi.org/10.3390/w7030975

Marulanda A, Barea JM, Azcón R (2009) Stimulation of plant growth and drought tolerance by native microorganisms (AM Fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. J Plant Growth Regul 28:115–124. https://doi.org/10.1007/s00344-009-9079-6

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530. https://doi.org/10.1016/j.plantsci.2003.10.025

Meena KK, Kumar M, Kalyuzhnaya MG et al (2012) Epiphytic pink-pigmented methylotrophic bacteria enhance germination and seedling growth of wheat (Triticum aestivum) by producing phytohormone. Antonie Van Leeuwenhoek 101:777–786. https://doi.org/10.1007/s10482-011-9692-9

Meena KK, Sorty AM, Bitla UM et al (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:172

Miller RW, Donahue RL (1995) Soils in our environment, 7th edn. Prudence Hall, Englewood Cliffs, NJ

Mithöfer A, Schulze B, Boland W (2004) Biotic and heavy metal stress response in plants: evidence for common signals. FEBS Lett 566:1–5. https://doi.org/10.1016/j.febslet.2004.04.011

Mitra S, Pramanik K, Ghosh PK et al (2018) Characterization of Cd-resistant Klebsiella michiganensis MCC3089 and its potential for rice seedling growth promotion under Cd stress. Microbiol Res 210:12–25. https://doi.org/10.1016/j.micres.2018.03.003

Morgan JW, Forster CF, Evison L (1990) A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. Water Res 24:743–750. https://doi.org/10.1016/0043-1354(90)90030-A

Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbiol 53:1141–1149. https://doi.org/10.1139/W07-081

Nadeem SM, Ahmad M, Zahir ZA et al (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol Adv 32:429–448

Naseem H, Bano A (2014) Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. J Plant Interact 9:689–701. https://doi.org/10.1080/17429145.2014.902125

Naveed M, Mitter B, Reichenauer TG et al (2014) Increased drought stress resilience of maize through endophytic colonization by Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. Environ Exp Bot 97:30–39. https://doi.org/10.1016/j.envexpbot.2013.09.014

Nazarian A, Mousawi M (2005) Study of bacterial resistance to organophosphorous. Iran J Environ Heal Sci Eng 2:207–211

Neumann PM (2008) Coping mechanisms for crop plants in drought-prone environments. Ann Bot 101:901–907. https://doi.org/10.1093/aob/mcn018

Nielsen PH, Jahn A (1999) Extraction of EPS. In: Microbial extracellular polymeric substances. Springer, Berlin, Heidelberg, pp 49–72

Noumavo PA, Kochoni E, Didagbé YO et al (2013) Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. Am J Plant Sci 4:1013–1021. https://doi.org/10.4236/ajps.2013.45125

Omar MNA, Osman MEH, Kasim WA, Abd El-Daim IA (2009) Improvement of salt tolerance mechanisms of barley cultivated under salt stress using Azospirillum brasilense. Springer, Dordrecht, pp 133–147

Pandey S, Ghosh PK, Ghosh S et al (2013) Role of heavy metal resistant Ochrobactrum sp. and Bacillus spp. strains in bioremediation of a rice cultivar and their PGPR like activities. J Microbiol 51:11–17. https://doi.org/10.1007/s12275-013-2330-7

Parent B, Hachez C, Redondo E et al (2009) Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach1[w][OA]. Plant Physiol 149:2000–2012. https://doi.org/10.1104/pp.108.130682

Passariello B, Giuliano V, Quaresima S et al (2002) Evaluation of the environmental contamination at an abandoned mining site. Microchem J 73(1–2):245–250

Pearce RS (2001) Plant freezing and damage. Ann Bot 87:417–424. https://doi.org/10.1006/anbo.2000.1352

Pieterse CMJ, Zamioudis C, Berendsen RL et al (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Pineda A, Dicke M, Pieterse CMJ, Pozo MJ (2013) Beneficial microbes in a changing environment: are they always helping plants to deal with insects? Funct Ecol 27:574–586

Pramanik K, Ghosh PK, Ghosh A et al (2016) Characterization of PGP traits of a hexavalent chromium resistant Raoultella sp. isolated from the rice field near industrial sewage of Burdwan district, WB, India. Soil Sediment Contam 25:313–331. https://doi.org/10.1080/15320383.2016.1137861

Pramanik K, Mitra S, Sarkar A et al (2017) Characterization of cadmium-resistant Klebsiella pneumoniae MCC 3091 promoted rice seedling growth by alleviating phytotoxicity of cadmium. Environ Sci Pollut Res 24:24419–24437. https://doi.org/10.1007/s11356-017-0033-z

Pramanik K, Mitra S, Sarkar A et al (2018a) Characterization of a Cd2+-resistant plant growth promoting rhizobacterium (Enterobacter sp.) and its effects on rice seedling growth promotion under Cd2+-stress in vitro. Agric Nat Resour 52:215–221. https://doi.org/10.1016/j.anres.2018.09.007

Pramanik K, Mitra S, Sarkar A, Maiti TK (2018b) Alleviation of phytotoxic effects of cadmium on rice seedlings by cadmium resistant PGPR strain Enterobacter aerogenes MCC 3092. J Hazard Mater 351:317–329. https://doi.org/10.1016/j.jhazmat.2018.03.009

Qadir S, Qureshi MI, Javed S, Abdin MZ (2004) Genotypic variation in phytoremediation potential of Brassica juncea cultivars exposed to cd stress. Plant Sci 167:1171–1181. https://doi.org/10.1016/j.plantsci.2004.06.018

Quartacci MF, Argilla A, Baker AJM, Navari-Izzo F (2006) Phytoextraction of metals from a multiply contaminated soil by Indian mustard. Chemosphere 63:918–925. https://doi.org/10.1016/j.chemosphere.2005.09.051

Qurashi AW, Sabri AN (2012) Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. Braz J Microbiol 43:1183. https://doi.org/10.1590/S1517-838220120003000046

Ramgareeb S, Cooke JA, Paula Watt M (2004) Responses of meristematic callus cells of two Cynodon dactylon genotypes to aluminium. J Plant Physiol 161:1245–1258. https://doi.org/10.1016/j.jplph.2004.03.004

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21(1):30

Sahoo RK, Ansari MW, Dangar TK et al (2013) Phenotypic and molecular characterisation of efficient nitrogen-fixing Azotobacter strains from rice fields for crop improvement. Protoplasma 251:511–523. https://doi.org/10.1007/s00709-013-0547-2

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635–648

Saravanakumar D, Kavino M, Raguchander T et al (2011) Plant growth promoting bacteria enhance water stress resistance in green gram plants. Acta Physiol Plant 33:203–209. https://doi.org/10.1007/s11738-010-0539-1

Sarma RK, Saikia R (2014) Alleviation of drought stress in mung bean by strain Pseudomonas aeruginosa GGRJ21. Plant Soil 377:111–126. https://doi.org/10.1007/s11104-013-1981-9

Sen S, Chandrasekhar CN (2014) Effect of PGPR on growth promotion of rice (Oryza sativa L.) under salt stress. Pelagia Res Libr Asian J Plant Sci Res 4:62–67

Serraj R, Sinclair TR (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ 25:333–341. https://doi.org/10.1046/j.1365-3040.2002.00754.x

Shaharoona B, Naveed M, Arshad M, Zahir ZA (2008) Fertilizer-dependent efficiency of pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (Triticum aestivum L.). Appl Microbiol Biotechnol 79:147–155. https://doi.org/10.1007/s00253-008-1419-0

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2:587. https://doi.org/10.1186/2193-1801-2-587

Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123–131

Silvente S, Sobolev AP, Lara M (2012) Metabolite adjustments in drought tolerant and sensitive soybean genotypes in response to water stress. PLoS One 7:e38554. https://doi.org/10.1371/journal.pone.0038554

Singh DP, Prabha R, Yandigeri MS, Arora DK (2011a) Cyanobacteria-mediated phenylpropanoids and phytohormones in rice (Oryza sativa) enhance plant growth and stress tolerance. Antonie van Leeuwenhoek 100:557–568. https://doi.org/10.1007/s10482-011-9611-0

Singh R, Gautam N, Mishra A, Gupta R (2011b) Heavy metals and living systems: an overview. Indian J Pharmacol 43:246–253

Skirycz A, Inzé D (2010) More from less: plant growth under limited water. Curr Opin Biotechnol 21:197–203

Solomon S, Qin D, Manning M et al (2007) Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, p 996

Sorty AM, Meena KK, Choudhary K et al (2016) Effect of plant growth promoting bacteria associated with halophytic weed (Psoralea corylifolia L) on germination and seedling growth of wheat under saline conditions. Appl Biochem Biotechnol 180:872–882. https://doi.org/10.1007/s12010-016-2139-z

Suarez C, Cardinale M, Ratering S et al (2015) Plant growth-promoting effects of Hartmannibacter diazotrophicus on summer barley (Hordeum vulgare L.) under salt stress. Appl Soil Ecol 95:23–30. https://doi.org/10.1016/j.apsoil.2015.04.017

Szabolcs I (1994) Soils and salinisation. In: Pessarakli M (ed) Handbook of plant and crop stress. Marcel Dekker, New York, pp 3–11

Timmusk S, Abd El-Daim IA, Copolovici L et al (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9:e96086. https://doi.org/10.1371/journal.pone.0096086

Tittabutr P, Piromyou P, Longtonglang A et al (2013) Alleviation of the effect of environmental stresses using co-inoculation of mungbean by Bradyrhizobium and rhizobacteria containing stress-induced ACC deaminase enzyme. Soil Sci Plant Nutr 59:559–571. https://doi.org/10.1080/00380768.2013.804391

Tuberosa R, Salvi S, Sanguineti MC et al (2003) Searching for quantitative trait loci controlling root traits in maize: a critical appraisal. Plant Soil 255(1):35–54

Tuberosa R, Salvi S, Giuliani S et al (2007) Genome-wide approaches to investigate and improve maize response to drought. Crop Sci 47:S-120–S-141. https://doi.org/10.2135/cropsci2007.04.0001IPBS

Vacheron J, Desbrosses G, Bouffaud ML et al (2013) Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci 4:356. https://doi.org/10.3389/fpls.2013.00356

Vardharajula S, Zulfikar Ali S, Grover M et al (2011) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6:1–14. https://doi.org/10.1080/17429145.2010.535178

Varshney RK, Pazhamala L, Kashiwagi J, Gaur PM, Krishnamurthy L, Hoisington D (2011) Genomics and physiological approaches for root trait breeding to improve drought tolerance in chickpea (Cicer arietinum L.). In: Costa de Oliveira A, Varshney RK (eds) Root genomics. Springer, Dordrecht, pp 233–250

Vejan P, Abdullah R, Khadiran T et al (2016) Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules 21:573. https://doi.org/10.3390/molecules21050573

Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotechnol 16:123–132

von Uexküll HR, Mutert E (1995) Global extent, development and economic impact of acid soils. Plant Soil 171:1–15. https://doi.org/10.1007/BF00009558

Wackett L, Sadowsky M, Martinez B, Shapir N (2002) Biodegradation of atrazine and related s-triazine compounds: from enzymes to field studies. Appl Microbiol Biotechnol 58:39–45

Waisel Y (1972) Biology of halophytes, 1st edn. Elsevier, Amsterdam

Wang C-J, Yang W, Wang C et al (2012) Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS One 7:e52565. https://doi.org/10.1371/journal.pone.0052565

Wang W, Chen Q, Hussain S et al (2016) Pre-sowing seed treatments in direct-seeded early rice: consequences for emergence, seedling growth and associated metabolic events under chilling stress. Sci Rep 6:1–10. https://doi.org/10.1038/srep19637

Wasson AP, Richards RA, Chatrath R et al (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J Exp Bot 63:3485–3498. https://doi.org/10.1093/jxb/ers111

Wilhite DA, Glantz MH (1985) Understanding: the drought phenomenon: the role of definitions. Water Int 10:111–120. https://doi.org/10.1080/02508068508686328

Witt M, Barfield BJ (1982) Environmental stress and plant productivity. In: Rechcigl M (ed) Handbook of agricultural productivity. CRC Press, Boca Raton, FL, pp 347–374

Yadav SK (2010a) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76:167–179

Yadav SK (2010b) Cold stress tolerance mechanisms in plants. A review. Agron Sustain Dev 30:515–527

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yu GR, Zhuang J, Nakayama K, Jin Y (2007) Root water uptake and profile soil water as affected by vertical root distribution. Plant Ecol 189:15–30. https://doi.org/10.1007/s11258-006-9163-y

Zaman M, Shahid SA, Heng L et al (2018) Introduction to soil salinity, sodicity and diagnostics techniques. In: Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques. Springer, Cham, pp 1–42