Chapter 6
Bacterial Community Composition Dynamics in Rice Rhizosphere: A Metagenomic Approaches

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Abstract The rhizosphere area of plant root surface shows bountiful diversity of microorganisms. Microbial community within rhizosphere inhabiting the rice field ecosystem have been studied previously. It is not possible to isolate the whole microbial genome by traditional culture dependent method. Metagenomic covers entire genome of all microbial community irrespective of any habitat without in vitro culturing. Present review has been aimed to summarize the past practices and recent issues of metagenomic analysis of paddy field bacterial communities within rhizosphere from different geographic locations. So, this chapter deals with the recent tools, platform, pipelines and software of metagenomics used with other techniques (e.g., 16S rRNA gene sequencing with V3-V4 hypervariable region, Pyrosequencing, Metaproteomic, etc.) for the study of bacterial composition from different regions such as rhizosphere, phyllosphere, bulk soil, wetland region of soil, irrigated soil, flooded and non-flooded soil, high prevalence of salt soil and high incidence of rice blast fungus contaminated soil. The findings from this review helps to enhance the crop production, improve soil quality by more use of biofertilizers and also helps in disease management with biocontrol agent.

Keywords Metagenomic · Pyrosequencing · Metaproteomic · Hypervariable region · Biocontrol agent

6.1 Introduction

Rice is widely consumed staple food for 50% of population of world (up to three billion people) especially in Asia and Africa. Interestingly, rice plants represent a habitat for a varied microbial population that colonizes the rhizosphere, a restricted

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zone of the plant roots' surface (Kowalchuk et al. 2010). The root growth of the majority of plants in soil has altered the spatial structure (Angers and Caron 1998). According to Curtis et al. (2002) a soil can have up to 4×10^6 different types of microbial taxa, and 1 g of soil can have more than one million distinct microbial genomes which ultimately shows an enormous microbial diversity remains within the soil especially in rhizosphere, predicted by Gans (2005). Since, majority of microorganisms cannot be cultured by culture dependent or conventional method, is intrigued by unraveling soil microbial community structure as well as functionality remains as an attractive challenge for enhancing the plant health and crop production (Yang et al. 2019). About 20–50% of the plant photosynthate is transported below the ground level and it is totally depending upon the different plant species (Kuzyakov and Domanski 2000) and about 18% of plant photosynthate is discharged into the soil environment on average (Jones et al. 2009). The favorable impacts of the rhizosphere microbial population on rice plants, including the production of plant growth-promoting bacteria (PGPR) have been thoroughly investigated (Subhashini and Singh 2014; Majeed et al. 2015), phosphate solubilization (Elias et al. 2016), nitrogen fixation process, mycorrhizal fungi, also acts as biocontrol agents for management of various plants diseases (Massart et al. 2015) with a high level of stress tolerance (Tsurumaru et al. 2015).

Rice differs from most crops in that it is typically cultivated in flooded soil, which results in the formation of oxic and anoxic zones within the rice rhizosphere area of soil, which select for specific physiological groups of microbial community with either aerobic, anaerobic, or facultative metabolism (Brune et al. 2000). The structure of the microbial population in the rhizosphere of the rice field environment has previously been characterized. The majority of research has concentrated on isolating, identifying, and characterization of rice rhizospheric bacteria from various locales and types (Vacheron et al. 2013). The bacteria in the rhizosphere had been studied widely (Zhang et al. 2016; Prajakta et al. 2019; Yang et al. 2020a, b; Maheshwari et al. 2021). They also influence the rhizosphere microbiota's chemical composition and offer crucial microbial growth substrates through rhizodeposition (Lynch and Whipps 1990). The decomposition of organic matter in soil is also largely attributed to the microbial population (Kuzyakov 2002; Yang et al. 2020a). Recently developed technologists, provide relatively quick and prompt sequencing of metagenomic DNA samples at very moderate cost in short time (Subhashini et al. 2017; Yang et al. 2020b), metagenomic DNA sequencing, however completely sequenced whole genome sequencing, depends on the DNA extracted (Gautam et al. 2019).

Without in vitro culturing, prior individual identification, or gene amplification, a metagenomics study covers the entire genome of any microbial community inhabiting any habitat such as soil and water (Abulencia et al. 2006; Kunin et al. 2008). Metagenomic analysis in terms of the functions that they drive and regulate, analysis involves isolating DNA from an environmental sample, cloning the DNA into a suitable vector, transforming the clones into a host bacterium, and screening the resulting transformants (Zhang et al. 2019). Recent technological development has gradually increased our knowledge about the global ecological distribution of

microbiota across the space and time and have furnished evidence for the contribution to ecosystem function (Chu et al. 2020). The use of metagenome sequencing techniques, such as Next Generation Sequencing technologies, has yielded enormous amounts of data, resulting in remarkable developments. To obtain detailed information on the diversity and ecology of microbial forms, the method involved isolating metagenomics DNA directly from an environmental niche (e.g., soil and water), fragmentation, generation of a sequence clone library, taxonomy and gene family community profiling, and high-throughput sequencing (Spence et al. 2014). The overall Metagenomics steps is illustrated in Fig. 6.1. The progress of metagenomics is totally dependent on high-throughput techniques for processing DNA from various environments and analyzing their sequence after running on high-end sequencers. Furthermore, examining millions or trillions of reads and putting them together to create a full genome is a difficult operation (Aguiar-Pulido et al. 2016a, b). Metagenomic analysis data provide the functional properties of a complex below-ground soil microbial community, such as intra and inter interactions, and so assist in the understanding their evolutionary aspect of microbial ecosystems as genetic and metabolic networks (Filippo et al. 2012; Ponomarova and Patil 2015).

This chapter article explains the current understanding of comparative metagenomic analysis of microbial diversity of paddy rhizospheric compartments and makes comparison of rhizospheric bacterial community structure among the different locations. A 16S rRNA gene profiling and shotgun metagenome analysis were used by Metagenomics. PCR will be used to amplify the V3-V4 region of 16S rRNA genes, which will then be sequenced on the Illumina Platform. Metagenomic library will be made and analyzed by different software. After then, a taxonomic analysis of a representative sequence from each OUT would be carried out to determine species distribution. The results will be represented in two-dimensional PCOA plots. The findings will be extremely useful since they may aid in the process of increasing rice output, improving crop quality, and reducing environmental impact owing to the usage of chemical fertilizers.

In this study, we focused on a variety of high-throughput sequencing investigations, collecting taxonomic data on bacterial communities at the genus level in the paddy rhizosphere and comparing them at the phylum level between rice plants from various places (Cox et al. 2010). Furthermore, this study explores metagenomic techniques to rhizospheric microbiomes and reports on the bacterial community composition in paddy rhizosphere (Mendes et al. 2013).

6.2 Approaches for Communities Structure Dynamics

Rhizospheric soil microbial communities play variety of roles in the function of soil by including enhancing organic and inorganic nutrient availability and nutrient cycling by boosting organic matter breakdown (Singh et al. 2019). The rhizospheric

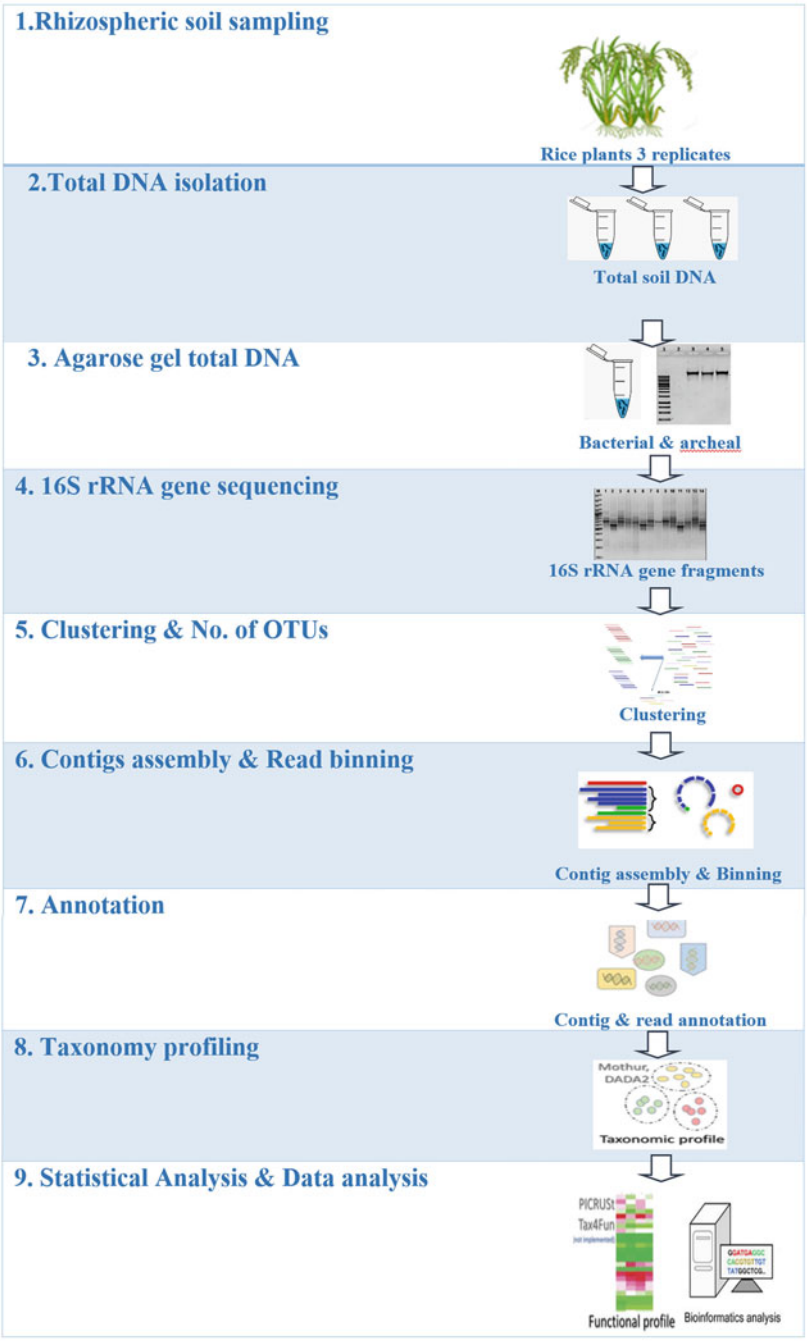

Fig. 6.1 Stepwise illustration of metagenomics

soil bacterial population is typically dominated by Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi (Hussain et al. 2012).

In one of the studies in this research field, Arjun (2011), 16S rRNA sequencing retrieved from database found total 12 representative clones from the paddy field rhizosphere soil in Kuttanand, Kerala. About 600 bp were viewed and compiled and aligned using BioEdit version 5.0.6 software (Hall 2001) and generated phylogenetic tree by neighbor joining method with 1000 resampling bootstrap analysis by using Mega v.4 software (Tamura et al. 2007). The dominant taxa in the library were found to be Proteobacteria (7/12) followed by Firmicutes (2/12), Bacteriodetes (2/12) and Acidobacteria (1/12) (Table 6.1). These four phylotypes are also thought to describe the bacterial community structure in rice rhizospheric soil in previous investigations, and Proteobacteria are the largest and most metabolically diverse group of soil bacteria (Lu et al. 2006).

Knief et al. (2012) obtained 749,569 and 1,340,274 Rhizospheric and Phyllospheric soil sequences from paddy fields at the International Rice Research Institute in Los Banos, Philippines after a year. In the rhizospheric soil samples were found most abundantly Alphaproteobacteria and Deltaproteobacteria. Further more significant taxa such as Firmicutes, Actinobacteria, Gammaproteobacteria and Deinococcus—Thermus. Most abundant phyla include Archaea in the rhizosphere than phyllosphere region was detected. In this research article, scientists also studied Metaproteogenome and they were found majority of proteins within Alphaproteobacteria (33%) in these samples, proteins assigned particularly *Azospirillum*, *Bradyrhizobium*, *Rhodopseudomonas*, *Methylobacterium*, *Magnetospirillum*, and *Methylosinus* (Table 6.1). Based on metagenome readings and clone library analyses, the Betaproteobacteria (Acidovorax, Dechloromonas, and Herbaspirillum) and Deltaproteobacteria (Anaeromyxobacter, Desulfovibrio, and Geobacter) genera dominated the bacterial population. Furthermore, Sinclair et al. (2015), were focused microbial community structure in rice producing areas of Guadalquivir marshes (Seville). In the months of July (tillering or vegetative stage) and September (between blooming or ripening and maturity stage), rice rhizospheric soil was examined (Marschner et al. 2001). Total 240 cfu were obtained. The soil samples were collected from four different regions in rice yielding areas of Guadalquivir marshes (Seville). These areas were: Puebla, Colinas, Calonge and Rincon. The soil in these locations has two major issues that have impacted rice production: increased salt levels in irrigation water and rice plants infected with the rice blast fungus *Magnaporthe oryzae*, 25 different bacterial genera were identified based on 16S rRNA gene sequencing analysis, although only eight were found at both sample times, July and September. From July to September, the Paenibacillus, Bacillus, and Pantoea communities grew in dominance, whereas the Enterobacter, Pseudomonas, and Exiguobacterium communities decreased. In July, there was a 21.34% increase in Exiguobacterium and a 20.21% increase in Enterobacter. Conversely Bacillus (37.33%) was more abundant in September. According to 16S rRNA sequencing of total DNA from four areas found that Proteobacteria, Acidobacteria and Anaerolineae were found to be more significantly in all areas. Proteobacteria (Betaproteobacteria) was most abundantly detected group followed by Bacteriodetes

Table 6.1 Different Bacterial taxa identified in the different geographic locations of rice rhizospheric soil composition

S. No.	Geographic coordinates	Approach	Findings related to rhizospheric bacterial composition	References
1.	Kuttanand, Kerala, India.	16S rRNA Sequencing and Pyrosequencing	Bacterial Community in rice rhizosphere dominantly observed taxa were Proteobacteria, followed by Firmicutes, then Bacteroidetes and Acidobacteria.	Arjun (2011)
2.	Los Banos, Philippines	16S rRNA gene sequencing and Metaproteomic profiling.	Microbial community composition in rice rhizosphere includes Archaea (2.6%), Actinobacteria (8.5%), Chloroflexi (4.6%), Alphaproteobacteria (14%), Betaproteobacteria (16.6%), and Deltaproteobacteria (10.6%).	Knief et al. (2012)
3.	Guadalquivir marshes (Seville), Spain.	16S rRNA gene Sequencing	Most frequently present group was Proteobacteria Betaproteobacteria followed by Archaea, Bacteroidetes, Chloroflexi, Acidobacteria, Thermococci, Sphingobacteria, Vermicomicrobia, Bacillus, Enterobacteria, Exiguobacterium.	Lucas et al. (2013)
4.	South Korea, Philippine, Italy and China	16S rRNA, pmoA, and mcrA amplifications	16S rRNA gene sequencing, pmoA and mcrA amplification analysis observed that rice field methanogens mainly comprise Methanocella, Methanobacterium, and dominantly Methanosaeta all over the cultivation.	Hyo Jung Lee et al. (2014)
5.	Bogor, West Java and Indonesia.	16S rRNA gene sequencing and nif gene amplification	16S rRNA sequencing observed 5 genera of Actinomycetes including Geodermatophilus, Actinoplanes, Actinokineospora, Streptomyces, and Kocuria while nif gene amplification showed that strain member of species Rhizobium and Anaeromyxobacter.	Rusmana et al. (2015)
6.	Vercelli, Italy.	16S rRNA gene Pyrotag sequencing.	More abundance of Archaea and Acidobacteria in	Breidenbach et al. (2016)

(continued)

Table 6.1 (continued)

S. No.	Geographic coordinates	Approach	Findings related to rhizospheric bacterial composition	References
			rhizosphere observed. The rhizosphere also consists of higher relative abundances of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Cyanobacteria, Chloroflexi, Firmicutes, and Verrucomicrobia.	
7.	Venezuela.	16S rDNA taxonomic profiling.	Gammaproteobacteria was determined to be the most dominant phyla of Proteobacteria, followed by Betaproteobacteria and Alphaproteobacteria, Acidobacteria, Nitrospirae, Cyanobacteria, Firmicutes, Vermicomicrobia, Bacteriodetes, Caulobacter, and so on.	Venturi et al. (2018)
8.	Kerala, India	16S rRNA gene hypervariable V3-V4 region	In this sample, most detected phyla were Proteobacteria (26 ± 14%), Firmicutes (21 ± 9%), Actinobacteria (17 ± 6%), and Acidobacteria (14 ± 10%).	Imchen et al. (2018)
9.	Maritsa river and Zlato Pole wetland, Bulgaria.	V3-V5 hypervariable region of 16S rRNA amplicon sequencing.	Abundantly found phyla includes Proteobacteria (68%), Gammaproteobacteria (45%), Acinetobacter (54%), Alphaproteobacteria (21.4%), Actinobacteria (18.5%), Firmicutes (9.4%), and Bacteriodetes (8.3%).	Ivan et al. (2019)
10.	Faisalabad, Pakistan.	16S rRNA gene amplification.	Reports have been shown that dominant groups were Proteobacteria, Acidobacteria, Actinobacteria, Bacteriodetes, Chloroflexi, Firmicutes, Nitrospirae, Gaiella, Marmoricola, Clostridium.	Maria et al. (2020)

and Chloroflexi (Table 6.1). Thermococci archaea were identified in locations with high Magnaporthe oryzae frequency, while Sphingnobacteria archaea were discovered in areas with high salt occurrence. Conversely, Verrucomicrobiae class was only detected in control area.

Scientists performed rice field experiments at research farm located in Sacheon, South Korea (Lee et al. 2014). The rice field was ploughed and harrowed, and water was flooded up to 5 cm above the soil surface (Witt et al. 2000). Following that, 21-day-old Korean rice seeds (*Oryza sativa*, Japonica type) were planted. Every 30 days soil samples were collected in triplicate. For detecting the 16S rRNA gene copies Archaea and Bacteria and targeting the *pmo*A and *mcr*A gene (Breidenbach and Conrad 2015). 16S rRNA gene sequencing analysis obtained 80% of bacterial reads of four taxa including Proteobacteria, Acidobacteria, Chloroflexi and Actinobacteria during whole cultivation. 16S rRNA gene sequencing, *pmo*A and *mcr*A amplification analysis represents that the rice paddy methanogens mainly comprise Methanocella, Methanobacterium and dominantly Methanosaeta all over the cultivation (Table 6.1) (Vaksmaa et al. 2017; Wang et al. 2018).

Rusmana et al. (2015) collected rice rhizospheric soil samples from three different types of agroecosystems (irrigated rice, marshy tidal, and dry) in Indonesia while performing 16S rRNA gene and *nif*H gene amplification (Reichardt et al. 1997). 16S rRNA gene analysis found abundance of five genera of Actinomycetes mainly comprises *Actinokineospora*, *Actinoplans*, *Geodermatophilus*, *Kocuria* and *Streptomyces* (Nimnoi et al. 2010). Most abundantly found species within *Streptomyces* in almost all genera were *Streptomyces alboniger* and *Streptomyces acidiscabies* (Taj and Rajkumar 2016). The amplification of the *Nif* gene revealed a biological role that was closely related to Rhizobium and *Anaeromyxobacter* strains (Martina et al. 2008; Pereira et al. 2013).

6.3 Metagenomics Software as Bioinformatic Tools

Metagenomics is the study of genes in relation to their environment In addition, at the Rice Research Institute in Vercelli, Italy, a rhizospheric soil sample was taken from paddy fields. Rice plants were sampled at four different stages: Stage 1 (early vegetative or tillering), Stage 2 (late vegetative), Stage 3 (reproductive or flowering), and Stage 4 (maturity). Using the UPARSE workflow, 8685 OTUs with 97% identity were found from 16S rRNA Pyrotag sequence analysis (Edgar 2013). The Silva taxonomy and method were used to classify relative OTU sequences in MOTHUR version 1.31.2 (Schloss et al. 2009). Absolute abundance of Archaea was detected to be higher in rhizospheric soil than bulk soil sample. For Archaea, Methanosarcina and Methanosaeta were found more abundant in rhizospheric soil of Vercelli. Abundantly present genera such as Acidobacteria, Alphaproteobacteria, Deltaproteobacteria, Betaproteobacteria, Cyanobacteria, Chloroflexi, Firmicutes. Potential iron reducer (e.g., *Geobacter* and *Anaeromyxobacter*) (Conrad and Frenzel 2002; Hori et al. 2010). The VEGAN package version 2.2.1 was used to investigate OTU relative abundances (Oksanen et al. 2013). Fermenters (e.g., Clostridia and

Opitutus) and endophytic plant growth promoting bacteria (e.g., Herbaspirillum species) were reported to be more prevalent in rhizospheric soil (Andreesen and Schaupp 1973; Chin et al. 2001). Base pairs were viewed and compiled and aligned using BioEdit version 5.0.6 software (Hall 2001) and generated phylogenetic tree by neighbor joining method with 1000 resampling bootstrap analysis by using Mega v.4 software (Tamura et al. 2007). Multivariate analysis revealed considerable differences between the sites when comparing the taxonomic patterns of the bacterial communities. Ivan et al. (2019) studied V3-V5 hypervariable region of 16S rRNA amplicon sequencing using Miseq Illumina platform (Ebersberg, Germany). The gene expression of *Pmo*A and *mcr*A was studied using quantitative reverse transcriptase real-time PCR (qRT-PCR) (Lee et al. 2014). 16S rRNA gene sequencing, *pmo*A and *mcr*A amplification analysis perceived that rice paddy methanogens mainly consist of Methanocella, Methanobacterium and dominantly Methanosaeta all over the cultivation (Table 6.1) (Vaksmaa et al. 2017; Wang et al. 2018). The tools for deciphering the metagenome have been listed in Table 6.2.

6.4 Proteomics Analysis of Bacterial Community

Genomic, proteomic, metabolomic, metagenomic, and transcriptomic studies are all included in the term "omics." It refers to the joint characterization and measurement of biological molecule pools that translate into an organism's structure, function, and dynamics. Proteomics has enabled the identification of ever-increasing number of protein (Anderson and Anderson 1998; Blackstock and Weir 1990; Anwar et al. 2019). Recent research findings indicate that rhizosphere soil metagenomic analysis can provide a sketch of a protein domain's functional areas, which can be used for protein optimization and functional characterization (Jin et al. 2016). InterPro is a software used for access the information about Protein domains, protein activity, active site within the protein, protein families and function (Singh et al. 2016). Genes encoding dinitrogen reductase (*nifH*) and dinitrogenase (*nif*D and *nif*K) were often found in the phyllosphere and rhizosphere, according to metagenomic analysis in the current study (Zeng et al. 2005). In phyllosphere, the most abundant *nifH* sequence types were found to be Azorhizobium and Rhodopseudomonas while in rhizosphere, the *nifH* sequences was detected across diverse taxa such as Rhizobium, Methylococcus, Dechloromonas, Anaeromyxobacter, Syntrophobacter, and some methanogenic archaea (Knief et al. 2012; Singh et al. 2016). The metaproteomic analysis reveals that genus Methylobacterium were detected most dominant in phyllosphere community.

Table 6.2 Bioinformatics tools for metagenomic data analysis

S. No.	Software	Function of software	References
1.	MetaQUAST	For quality assessment of metagenomic assemblies.	Mikheenko et al. (2016)
2.	Mothur	Software for analysis of 16S rRNA gene sequencing	Schloss et al. (2009)
3.	MetaVelvet	Metagenomic de novo assembler	Namiki et al. (2012), Zerbino and Birney (2008)
4.	MG-RAST	Access to a number of tools for metagenomic analysis via a web-based platform.	Glass et al. (2010)
5.	IDBA-UD	For the building of contigs using a progressive cycle of rising k-mer values	Peng et al. (2012)
6.	Megahit	Useful in metagenomic analysis and uses similar approach to IDBA-UD	Li et al. (2015)
7.	UPSARSE	Pipeline for quality and length filtering of sequencing reads and OUT generation	Edgar (2013)
8.	MetAMOS	Ability to test multiple assembly tools and used for contigs length, contiguity, and error rates	Treangen et al. (2013)
9.	VEGAN	Software for diversity analysis and community ecology functions	Oksanen et al. (2013)
10.	InterPro	Software for access the information about protein domains, protein activity active site of protein, protein families and function	Mitchell et al. (2015)
11.	MegaGene Annotator	For high contig length and large number of predicted gene	Noguchi et al. (2008)
12.	RayMeta	Scalable software tool and assemblies are constructed on the basis of de Bruijin graphs	Boisvert et al. (2012), Pell et al. (2012)
13.	QUIIME	Quantitative insight into microbial ecology	Caporaso et al. (2010)
		Pipeline used for microbiome analysis from raw DNA sequencing data generated by Illumina platform	
14.	CONCOCT	Used to count the number of clusters and reconstruct pathogenic genomes (Shiga-toxin producing strain of *E. coli* outbreak in 2011)	Alneberg et al. (2014)
15.	CARMA	Used for Metagenomic analysis	Gerlach et al. (2009)
16.	Prokka	Pipeline used for annotation of bacterial genomes	Seemann 2014
17.	MEGAN	Software used for analysis of large metagenomic datasets	Huson and Weber (2013)
18.	Glimmer-MG	Software for gene prediction and provide accurate gene error-prone sequences than other method	Delcher et al. (2007)
19.	PICRUST	Used to connects taxonomic classifying metaprofiling results	Langille et al. (2013)

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Table 6.2 (continued)

S. No.	Software	Function of software	References
20.	MetaWatt	For metagenomic assembly, contig clustering or binning, and bin inspection for taxonomic signatures (through BLAST) and sequence coverage.	Strous et al. (2012)
21.	BioEdit	Software of biological sequence alignment editor	Hall (2001)
22.	FragGene Scan	Used to predicts fragments of gene from short reads	Rho et al. (2010)
23.	PIPITS	Used for processing of ITS amplicons	Gweon et al. (2015)
24.	EDGE	Software comprising QC, annotation, Assembly, binning, taxonomic profiling, and phylogenetic tree construction	Li et al. (2014)
25.	USEARCH	Open-source software	Edgar and Flyvbjerg (2015)
26.	VSEARCH	Open-source software	Rognes et al. (2016)
27.	EBI Metagenomics	Software used for data trimming and duplicates removal	Hunter et al. (2014)
28.	qRT-PCR	Real-time quantitative reverse transcriptase PCR	Lee et al. (2014)

6.5 Bacterial Community Structure at Different Level

There are several bacterial communities which present at different locations on geological areas of soil like some are associated with root endophytes, in phyllosphere, endorhizosphere, bulk soil, flooded and non-flooded soil, irrigated soil (Singh et al. 2020). Some bacterial communities are survived in high prevalence of *Magnaporthe oryzae* (Rice blast Fungus) and some in high incidence of salt.

6.5.1 Bacterial Community Composition Associated with Root Endophytes

Previously, research was conducted to investigate the microbial community structure of Indian rice root endophytes (Sengupta et al. 2017). Vittorio et al. used 16S rRNA taxonomy profiling of the rhizosphere and endorhizosphere of two high-yielding rice cultivars, Pionero 2010 FL and DANAC 6D 20A, which were cultivated intensively in Venezuela. Three Pionero 2010FL rhizosphere soil samples and three DANAC SD 20A rhizosphere soil samples were taken from Association of Certified Seed Producers of Western Plains paddy fields after 88 days of planting. After analyzing the complete rhizospheric and endorhizospheric bacterial community structure, they retrieved 326,496 bacterial readings. Proteobacteria accounted for 70–87% of all OTUs in the bacterial microbiota. Gammaproteobacteria was the most numerous

Proteobacteria class, followed by Alphaproteobacteria and Betaproteobacteria. Deltaproteobacteria and epsilonproteobacteria, on the other hand, were not found in the endorhizosphere. The colony of Acidobacteria and Nitrospirae was exclusively found in the rhizosphere. The phylum Cyanobacteria was also abundant in rhizospheric soil. Bacteriodetes and Verrucomicrobia abundant in Pionero 2010 FL. Caulobacter genus was significant and massively abundant in both rhizosphere and endorhizosphere soil sample.

Number of the researches have performed 16S rRNA gene amplification of hypervariable V3-V4 region and was amplified using primers set Pro341F and Pro805R (Takahashi et al. 2014; Merkel et al. 2019; Cichocki et al. 2020). They were collected rhizosphere and bulk soil sample from seven different areas of India. They obtained 28 phyla from all groups of bacteria. Among them the most dominant phyla were Proteobacteria (25.7 ± 14%) followed by Fermicutes (21 ± 8.7%) then Actinobacteria (16.7 ± 6%) and Acidobacteria (13 ± 10%). Candidatus koribacter (8 ± 19%) was most abundant genus in rhizosphere soil while Ktedonbacter (13%) most frequently detected in bulk soil sample. Furthermore, 18 methanogen genera were detected in all samples of rhizospheric and bulk soil (Lee et al. 2015). Most abundant genera of methanogen were detected Methanosaeta, followed by Methanobacterium and Methaocella (Rahalkar et al. 2016). Archaeal genera including type I and type II methanotrophs were significantly detected throughout the cultivation (Singh et al. 2016).

6.5.2 *Flooded and Non-flooded Located Bacterial Community*

Multivariate analysis revealed considerable differences between the sites when comparing the taxonomic patterns of the bacterial communities. Ivan et al. studied V3-V5 hypervariable region of 16S rRNA amplicon sequencing using Miseq Illumina platform (Ebersberg, Germany). At Zlato Pole, soil samples collected from flooded and non-flooded rice paddies, as well as sediments and non-flooded areas. Rice paddies are being located in wetlands along the Bulgarian side of the Maritza River, such as the Zlato Pole wetland and the Tsalapitsa paddy fields. After filtering of bacterial reads and OUT picking process, 181,328 sequences were obtained from flooded samples and 158,260 samples were obtained from non-flooded samples. Total 117 bacterial classes were identified among them 67 were detected in all soil samples. Proteobacteria (34%) in Plovdiv rice paddy sediments to (68%) in Zlato Pole sediments of all bacterial sequences. Alphaproteobacteria (21%) is the most common, followed by Gammaproteobacteria (13%), Betaproteobacteria (6.8%), and Deltaproteobacteria (4%). Moreover, abundant phyla were Actinobacteria (8–26%) and Acidobacteria (2–17%) detected the third most abundant phylum while Firmicutes (9%) and Bacteriodetes (8%) detected over Acidobacteria in Zlato pole sediments.

6.5.3 *Community Structure in Rhizosphere and Phyllosphere*

In Faisalabad, Pakistan, a comparison of 16S rRNA gene amplification studies of bacterial phyla in the rhizosphere and phyllosphere revealed that the rhizosphere had more diversity than the phyllosphere. According to reports, a total of 9383 16S rRNA sequences were retrieved from rhizospheric soil while 54,714 sequences were retrieved from Basmati rice phyllospheric soil (Yasmin et al. 2020). Eighteen different phyla detected from rhizosphere while seven phyla were from phyllosphere soil sample. Seven phyla were found in both compartments. Proteobacteria were most abundant phyla from both the compartments i.e., rhizosphere (37%) while phyllosphere (80%) followed by Firmicutes (10%), Bacteriodetes (9%), Chloroflexi (4%), Actinobacteria (1%) in phyllospheric soil sample. According to 16S rRNA gene amplification analysis was detected 208 different genera from rhizosphere while 24 genera from phyllosphere soil samples. In the bacterial community's rhizosphere and phyllosphere, 15 genera were determined to be common. Bacillariophyla (22%) was the most common genus in the phyllosphere, followed by Sphingomonas (9%), and Bradyrhizobium (7%). The most frequent genus in the rhizospheric soil sample was Thaurea (4%).

16S rRNA sequencing retrieved from database found total 12 representative clones from the paddy field rhizosphere soil in Kuttanand, Kerala (Arjun 2011). The dominant taxa in the library were found to be Proteobacteria (7/12) followed by Firmicutes (2/12), Bacteriodetes (2/12), and Acidobacteria (1/12). About 70–90% of total OTUs, Proteobacteria was dominated the bacterial microbiota. Gammaproteobacteria was the most important Proteobacteria phylum, followed by Alphaproteobacteria and Betaproteobacteria. In the endorhizosphere, deltaproteobacteria and epsilonproteobacteria were not found. Only the colony of Acidobacteria and Nitrospirae was found in the rhizosphere. Along with Cyanobacteria phylum was enriched in rhizospheric soil. Bacteriodetes and Verrucomicrobia abundant in Pionero 2010 FL. Caulobacter genus was significant and exclusively abundant rhizosphere as well as endorhizosphere (Sengupta et al. 2017).

6.5.4 *Bacterial Composition in Areas with High* **Magnaporthe oryzae** *Prevalence and High Salt Incidence*

Proteobacteria, Acidobacteria, and Anaerolineae were detected in all four areas, according to 16S rRNA sequencing of total DNA from the four regions. Proteobacteria (Betaproteobacteria) was most abundantly detected group followed by Bacteriodetes and Chloroflexi. Thermococci class archaea were identified in locations with high Magnaporthe oryzae incidence, while Sphingnobacteria class archaea were identified in areas of high salt incidence. The Verrucomicrobiae class,

on the other hand, was only found in the control region (Lucas et al. 2013). The rhizosphere has a higher absolute abundance of Archaea than the bulk soil sample. For Archaea, Methanosarcina and Methanosaeta were found more abundant in rhizospheric soil of Vercelli (Breidenbacht et al. 2016). Abundantly present genera such as Acidobacteria, Alphaproteobacteria, Betaproteobacteria, Cyanobacteria, Chloroflexi, Deltaproteobacteria, Firmicutes. Potential iron reducer (e.g., *Geobacter* and *Anaeromyxobacter*) (Conrad and Frenzel 2002; Hori et al. 2010). Fermenters (e.g., Clostridia and Opitutus) and endophytic plant growth promoting bacteria (e.g., *Herbaspirillum* species) are also more abundant in the rhizospheric soil (Andreesen and Schaupp 1973; Chin et al. 2001). Furthermore, 18 methanogen genera were detected in all samples of rhizospheric and bulk soil (Lee et al. 2015). Most abundant genera of methanogen were detected Methanosaeta, followed by Methanobacterium and Methaocella (Rahalkar et al. 2016). Archaeal genera belong to type I and type II methanotrophs were found in entire cultivation (Singh et al. 2016). Total 117 bacterial classes were identified among them 67 were detected in all soil samples. Proteobacteria (34.2%) in Plovdiv rice paddy sediments to (68%) in Zlato Pole sediments of all bacterial sequences. Alphaproteobacteria (21%) is the most common, followed by Gammaproteobacteria (13%), Betaproteobacteria (7%), and Deltaproteobacteria (4%). Moreover, abundant phyla were Actinobacteria (8–26%) and Acidobacteria (2–17%) detected the third most abundant phylum while Firmicutes (9%) and Bacteriodetes (8%) detected over Acidobacteria in Zlato pole sediments (Ivan et al. 2019).

6.6 Future Perspective

We're working hard to figure out which bacterial genera are invading the rice rhizosphere. From this review article, we conclude that among all bacterial community in different samples from different locations most abundant phyla were detected Proteobacteria in rhizosphere soil samples followed by Acidobacteria then Actinobacteria, followed by Choroflexi and Firmicutes. Methylobacterium was detected as most dominant genus from Methylotrophs. Archaea were predominantly found in rhizosphere bulk soil, flooded soil, and wetland soil samples. Methanogenic archaea are also found in some rhizospheric soil samples. Streptomyces were detected from agroecosystem (irrigated rice and swampy rice) of rice plants. Furthermore, analyzing the structure of microbial communities is required in order to investigate the individual functions of bacteria. This understanding and insights aid in the development of methods for greater crop production, improved soil quality, and disease-causing microorganism protection in order to preserve natural resources and, ultimately, to produce more sustainable agricultural production.

We may choose these succeeding strains for formulation of a suitable inoculant as a biocontrol agent for administration in the rhizosphere of rice and disease management of rice plants due to decreased efficacy of natural nutrients available in soil. Biocontrol presumes special connotation being an environmental-friendly and

cost-efficient strategy which can be used for effective rice disease management. Numerous microbial species are acts as a biocontrol agent against many plant pathogens. As a result, it is an inevitable step to gather as much microbial diversity as possible in order to provide a higher level of protection while retaining rice yields.

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