Chapter 7
Application of Soil Microorganisms for Agricultural and Environmental Sustainability: A Review

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Abstract Microorganisms play a significant role in the edaphic ecosystem. Distribution and diversity of soil microorganisms such as bacteria, fungi, actinomycetes, algae, protozoans, and viruses are important to understand their functional significance at a given site of soil. In the edaphic ecosystem, microbial processes determine the exchange of matter and flow of energy between plant and soil which affect productivity and ecosystem stabilization. Thus, soil microorganisms show precise contributions to sustainable biosphere. They are also extremely important sources of food, feed, medicines, enzymes, and antimicrobial substances. More recently, their potential to serve in human and animal health applications, genetic engineering technology, environmental protection measures, agricultural biotechnology, and management of agricultural and municipal wastes has taken them in the category of "jewels of the environment." Their significance toward a prosperous environment helps them to be "jewels." Nowadays, genetically modified organisms are being used for applications in agriculture, bioremediation, industries, and human health. Many new methods and technologies have been added to understand the relationship between microbial diversity and its function in soil processes. Now, with technical improvements and focused researches, we can hypothesize the results from micro-scale to large-scale processes for the prediction of climate changes.

Keywords Biodiversity · Bioinformatics · Biopesticides · Bioremediation · Metagenomics · Metaproteomics

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7.1 Introduction

The microbial world comprises miscellaneous existing organisms in any ecosystem, and different organisms are discovered regularly. It is the largest unexplored reservoir of biodiversity on the earth. Microorganisms play a specific role in the maintenance and functioning of the ecosystems for preparing a sustainable biosphere (Nannipieri et al. 2002). Microbes are divided into six groups according to their distinct characteristics: prions, virus, bacteria, protozoan, unicellular algae, and fungi. Huge diversity is found within these groups. Soil is considered to be a complex and dynamic ecosystem as it is difficult to determine the microbial community composition in soil. Soil is a structured, heterogeneous, and discontinuous system and also a medium pulsating with life in the environment. Being a perfect culture medium for the growth and development of microbial communities, the soil is known as a complex microhabitat by having several unique properties (Nannipieri and Badalucco 2003).

Microbial diversity encompasses genetic as well as ecological diversity. Genetic diversity refers to the amount and distribution of genetic information within microbial communities, while ecological diversity portrays the structural variations in the communities, interaction complexities, number of trophic levels, and number of ecological guilds. Soil possesses several different groups of microorganisms among which bacteria are the most abundant in comparison to the other microorganisms. In the soil, microbes are found maximum in the upper portion (i.e., horizon A/topsoil) and decrease with the depth. Different soil organisms play a significant role in specific change/transformation occurring in the soil. The major role of microorganisms in the soil is to make the soil an excellent medium for the proper growth and development of higher plants. A huge diversity of microbes is observed not only in pristine soils but also in polluted soils and in most environments under extreme conditions (Guimaraes et al. 2010). Therefore, such contaminated soils should be conserved for their unique biopotentials and microbial diversity.

Soil microbial diversity is very crucial for life on earth. Various phenomena occurring above the ground are determined directly or indirectly by microbial processes in the soil (Wardle 2002; Bardgett and Bowman 2005). Structure and function of various organisms are regulated directly by soil microorganisms through the stimulation or inhibition of their growth and development. On the other hand, soil microorganisms play an important role in the regulation of aboveground communities indirectly by altering the nutrient dynamics (Van Der Putten 2003; Wardle et al. 2004).

Human health issues have provoked the awareness regarding soil ecosystem and geochemistry, while soil and water conservation problems are already becoming hot cakes in several parts of the world (Sparks 2001; Ward and Pulido-Velazquez 2008; Nowak 2013). Soil influences human health through contact with pathogens (Burras et al. 2013). The study of soil ecosystem is significant for global change and biodiversity preservation. The other facet of this study is that the impact of human activities on soil and water resources is increasing continuously with the growing

population resulting in the loss of organic matter, fertility, erosion, pollution, losses of soil microbial diversity, and losses of soil functions. The present review could be helpful to open more opportunities for soil scientists, soil microbiologists, professionals of other related disciplines, and industrialists for obtaining a more comprehensive perceptiveness of the environment and sustainable development in the future.

7.2 Microbial Functions in the Soil

Microbial processes occurring in the soil are responsible for the structure and functioning of aboveground world. Soil microbes play a significant role in plant nutrition by organic material decomposition and increasing nutrient availability to the plants. Through nitrogen fixation, plants are benefitted by using an infinite source of nitrogen from the atmosphere, and this procedure concurrently increases soil fertility as dead plant root remains add some of the biologically available nitrogen to the soil. Some soil microorganisms act as determinants for the mineralogical properties of most soils and sediments. Microorganisms play an important role in weathering process which liberates many essential elements (C, S, N, P) from the lithospheric resources within which they are generally unavailable to many living organisms (Douglas and Beveridge 1998). Another important role of the microbes is biomineralization which supports soil structural characteristics (Wardle et al. 2004).

Some microbes develop mutual beneficial relationships with the plants. These microbes colonize plant roots and obtain nutrients from the soil. Soil microbes protect roots from pests and pathogens and also provide a greater root area for nutrient uptake. Along with the beneficial microbes, pathogenic microorganisms are also present in the soils which are involved in the pathogenesis in host plants. These pathogenic microorganisms infect the plant and kill living tissue, creating a weakened and diseased plant. High biodiversity in soil suppresses soil-borne pathogens and diseases. In suppression mechanisms, native microorganisms outcompete the pathogenic organisms, physically protect the roots, and provide better nutrition to the plants. Thus, soil microbiota performs various modification and biotransformation in the soil and provides better nutrition to the plants for proper growth and development in the soil. Some microbes execute important soil functions like nutrient cycling, disease suppression, and soil and water dynamics, all of which promote plants to become healthy, disease resistant, and vigorous.

7.3 Applications of Soil Microbes

The exceptionalities and biosynthetic capabilities of the soil microbes have made them the most desired *organisms* for overcoming some major *problems in the life sciences* and other relevant fields. The pivotal role of microorganisms in several

areas such as genetic engineering, advanced medical technology, human and animal health, pharmaceutical drugs, enzyme *technology,* food processing, food safety and quality, environmental protection, agricultural biotechnology, and agricultural and municipal waste management has provided a most remarkable achievement. Major applications of soil microorganisms such as enhanced symbiotic or associative N_2 fixation (Alexander 1984; Stacey and Upchurch 1984), plant growth promotion (Burr and Caesar 1984; Gaskins et al. 1985), biological control of soil-borne plant pathogens (Watrud et al. 1985), degradation of xenobiotic compounds (Brunner et al. 1985), and exploitation of industrially important enzymes on commercial scale (Nigam and Singh 1995; Nigam 2013; Prasad et al. 2013) are deciphering their potentials. Nowadays, enormous scopes in the beneficial application of soil microorganisms and the potential for developing specific strains through genetic engineering and molecular techniques have definitely contributed to various fields.

7.3.1 Application of Soil Microbes in Agriculture

The farmers generally use synthetic chemical methods for increasing agricultural production, and these practices definitely enhance the crop yield. In turn, the random application of agrochemicals has resulted in environmental pollution and poor human and animal health. Thus, the alternative methods are needed in place of chemical-based conventional agriculture to reduce these problems. Soil-borne microbes are becoming very popular and beneficial as an additive to chemical fertilizers in improving the quality and yield of crops and are now applied in a wide variety of agricultural systems for better productivity and integrated pest management (Antoun and Prevost 2005). Regarding this, plant growth-promoting microorganisms (PGPM) are found as potential contributors in sustainable crop production (Shoebitz et al. 2009).

7.3.1.1 Soil Microbes as Biofertilizers

Rhizospheric soil of plants possesses *several* beneficial microorganisms (Kathiresan and Selvam 2006). Application of these beneficial soil microorganisms for improving the plant growth and productivity as "biofertilizer" has been intensively studied (Artursson et al. 2006; Berg 2009). An extensive variety of bacterial species are applied as biofertilizers in the plants. These bacteria include strains of *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, and *Rhizobium* (Lagos et al. 2015) and are termed as plant growth-promoting rhizobacteria (PGPR) and act as biofertilizers (Burr and Caesar 1984; Podile and Kishore 2007). The *Bacilli* and *Pseudomonas* are the predominant genera among the diverse bacteria (Podile and Kishore 2007). These rhizobacteria improve plant growth by increasing photosynthetic capacity (Xie et al. 2009); synthesizing precursors of phytohormones (Ahmad et al. 2008), antibiotics, enzymes, vitamins,

and siderophores (Burd et al. 2000); and inhibiting ethylene synthesis (Khan et al. 2009). In addition, the rhizobacterial strains can solubilize inorganic P (Khan et al. 2007), mineralize organic P (Ponmurugan and Gopi 2006), improve plant tolerance to salt and drought stress (Xie et al. 2009; Zhang et al. 2010), improve plant growth and plant nutrition, and provide *plant resistance* to *phytopathogenic organisms* (Avis et al. 2008; Hayat et al. 2010; Pii et al. 2015). Dai et al. (2016) conducted an experiment to show that pyrogenic organic matter addition in soil induced the root growth and several soil parameters more in rhizospheric soils in comparison to bulk soil. Thus, PGPR application as eco-friendly biofertilizer may facilitate in reducing the environmental problems caused by the excessive use and high production costs of fertilizers. The application of PGPR also improves the physicochemical properties of the soil which facilitate the growth and efficiency of symbiotic soil microbes such as nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi.

Some rhizospheric fungi are also capable of promoting plant growth through root colonization like PGPR and are known as plant growth-promoting fungi (PGPF) such as *Trichoderma*, *Penicillium*, *Fusarium*, and *Phoma* (Hyakumachi 1994). Some species of PGPF are found to induce systemic resistance against several *pathogens* in cucumber plants (Shoresh et al. 2005). Being non-pathogenic soil-inhabiting saprophytes, PGPF have been reported as beneficial microbes for several crop plants with the properties of growth promotion and protection from several diseases (Shivanna et al. 1994). Among PGPF, some isolates of *Penicillium simplicissimum* and *Phoma* sp. were found effective against cucumber anthracnose caused by *Colletotrichum orbiculare* through the activation of systemic resistance (Koike et al. 2001). Generally, P-solubilizing ability of PGPF is greater than PGPR. Some PGPF genera like *Aspergillus*, *Penicillium*, and *Trichoderma* have been reported as efficient P-solubilizers (Altomare et al. 1999; Babana and Antoun 2005).

7.3.1.2 Soil Microbes as Biocontrol Agents

The extensive use of rhizobacteria and PGPF application for overcoming the soil-borne diseases is replacing the chemical pesticides, which is a major concern in inducing the environmental pollution and health hazards (Walsh et al. 2001). The most commonly used soil microorganisms as biopesticides include biofungicides (*Trichoderma* sp.), bioherbicides (*Phytophthora* sp.), and bioinsecticides (*Bacillus thuringiensis* and *B. sphaericus*). Several bacteria, particularly *Pseudomonas* and *Bacillus* strains, are capable of controlling the growth of various fungi. *Burkholderia* sp. was found to suppress the virulence factors (that normally activate immune response in several plants) by forming a biofilm at the root surface (Paungfoo-Lonhienne et al. 2016). Rhizobacteria act as biocontrol agent and protect the root surface from soil-borne pathogens. Being rhizosphere competent, they have the capacity to rapidly colonize the root surface and spread down the root after single seed treatment or drench application in the soil (Rangarajan et al. 2003). The biocontrol potential of *Bacillus* spp. was assessed in many crops including chickpea

and found it as an important agent to resist root and soil-borne pathogens (Landa et al. 1997). The antagonistic actions of *Pseudomonas fluorescens* have been studied extensively against several plant pathogens (Saravanakumar and Samiyappan 2007) and also in diseases of crops grown in saline agricultural soils (Paul and Nair 2008). There are several PGPR that suppress diseases by releasing antimicrobial or antifungal compounds that prevent plant pathogens (Weller et al. 2002). Members of the genus *Trichoderma* were found very effective biocontrol agents against several soil-borne plant pathogens (Benitez et al. 2004). *Glomus fasciculatum* and *Gigaspora margarita* have been reported to suppress root rot diseases of asparagus caused by *Fusarium oxysporum* f. sp. asparagi (Matsubara et al. 2001) and *Glomus clarum* against root necrosis caused by *Rhizoctonia solani* in cowpea (Abdel-Fattah and Shabana 2002). The arbuscular mycorrhizal fungus *Glomus mosseae* was found to suppress "take-all" disease caused by *Gaeumannomyces graminis* var. tritici in barley (Al-Askar and Rashad 2010).

7.3.1.3 Soil Microbes in Saline Agricultural Soils

Soil salinity is a serious problem affecting the vegetables and crops causing growth inhibition particularly in plants of arid and semiarid areas (Parida and Das 2005). It has been reported that plant growth under salt stress can be improved by inoculation of PGPR and PGPF (Cho et al. 2006) and application of mycorrhizal fungi which promotes abiotic stress tolerance in host plants and plays a significant role in plant survival under different stress conditions (Rodriguez et al. 2009). Thus, selected PGPR, PGPF, and other microbes, particularly, AM fungi, could serve as a potential tool for alleviating salinity stress in salt-sensitive crop plants.

7.3.2 Applications of Microbes in Industries

Microorganisms are progressively more important to industry, where they are used in large-scale processes ranging from food production to soil/water treatment. The development of recombinant DNA technology brought many changes to industrial applications of microorganisms.

7.3.2.1 Enzyme Production

Majority of the industrial enzymes are of microbial origin. Enzymes from soil microorganisms are of *great* significance in various *industries* such as pharmaceutical, food, dairy, textile, leather, detergent, paper and pulp, animal feed, biosurfactants, bioplastics, natural bioproducts, cosmetics, etc., and their range of applications is gradually increasing. Soil microbes are used in the production of several enzymes such as cellulase, lipase, amylase, proteases, and pectinases.

Cellulase is produced by several fungi (such as *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Chaetomium*, and *Phoma*), aerobic bacteria (such as *Bacillus*, *Acidothermus*, *Pseudomonas*, *Cellvibrio*, *Staphylococcus*, *Streptomyces*, and *Xanthomonas*), and anaerobic bacteria (such as *Acetivibrio*, *Bacteroides*, *Butyrivibrio*, *Clostridium*, *Erwinia*, *Eubacterium*, *Caldocellum*, *Pseudonocardia*, *Ruminococcus*, and *Thermoanaerobacter*) (Zhang et al. 2006). Crude enzymes produced by these microorganisms are commercially available for agricultural and industrial use. Commercial lipases are produced from *Rhizopus*, *Geotrichum*, *Rhizomucor*, *Aspergillus*, *Burkholderia cepacia*, *Candida antarctica*, *Candida rugosa*, *Pseudomonas alcaligenes*, and *Pseudomonas mendocina* (Jaeger and Reetz 1998). However, α-amylase-producing species are *Aspergillus niger*, *A. fumigatus*, *A. foetidus*, *A. terreus*, and *Rhizopus delemar* (Pandey et al. 2005). Proteases are produced by *Aspergillus niger*, *A. oryzae*, *Bacillus amyloliquefaciens*, *B. stearothermophilus*, *M. pusillus*, and *Mucor miehei*. However, pectinase producers are *Aspergillus*, *Bacillus*, *Trichoderma*, *Rhizopus*, *Pseudomonas*, *Penicillium*, *Fusarium*, *Kluyveromyces*, and *Erwinia* (De Gregorio et al. 2002). The fungi synthesizing pectinolytic enzymes such as *Aspergillus niger*, *Aspergillus carbonarius*, and *Lentinus edodes* are mostly preferred in industries. Specificity, thermostability, and pH response of the microbial enzymes are critical properties for the growing interest in soil microbial enzymes compared to chemical processes for their industrial use. This led to the search of new strains of soil microorganism, which can be used in the development of processes for producing such microbial enzymes on a commercial scale.

7.3.2.2 Triacylglycerol Production

The members of actinomycetes such as *Streptomyces*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Dietzia*, and *Gordonia* produce the triacylglycerols efficiently. They produce variable amounts of neutral lipids on culture media containing different carbon sources. Eukaryotic microorganisms such as fungi and yeast also accumulate TAG during metabolic stress (Lemann 1997).

7.3.2.3 Biosurfactants

Microbial biosurfactants are useful biotechnological products with a broad range of applications in various industries (Mulligan 2009). Biosurfactants are an assorted group of surface active chemical compounds produced by a variety of soil microbes. These include bacteria, yeasts, and filamentous fungi (Mulligan 2005). Bacterial surfactant-producing members include *Pseudomonas aeruginosa* (mono- and di-rhamnolipid); *Corynebacterium*, *Rhodococcus*, and *Nocardia* (phospholipids, trehalose dimycolates/dicorynomycolates, glycolipids, etc.); *Arthrobacter paraffineus* (trehalose and sucrose lipids); *Bacillus subtilis* (surfactin); and *Bacillus licheniformis* (lipopeptide similar to surfactin). Fungi involved in surfactant

production include yeasts such as *Candida* spp. (liposan, phospholipids) and *Torulopsis* spp. (sophorolipids). Several researches have demonstrated the increase in pollutant desorption and availability by application of biosurfactants (Oberbremer et al. 1990; Volkering et al. 1995). It was observed that a biosurfactant-producing species of *Burkholderia* isolated from oil-contaminated soil could be used for the bioremediation of various pesticide-contaminated sites (Wattanaphon et al. 2008). Hermane et al. (1995) suggested the application of biosurfactants in controlling the bioavailability of toxicants in soils and other environment due to their biodegradability. *Pseudomonas* produces biosurfactants which solubilize and degrade the polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene (Burd and Ward 1996). Noordman et al. (2002) observed the effect of biosurfactant produced from *Pseudomonas aeruginosa* on hexadecane degradation. The biosurfactants are used extensively in agriculture for improvement of soil quality, plant growth promotion, enhanced biodegradation of pollutants, and protection from plant pathogens because they show antimicrobial activity and increase plant-microbe interactions which are beneficial to the crop plants (Dhara and Swaranjit 2013).

7.3.2.4 Food Industry

Application of soil microbes in the food industry has been used widely in the production of several commercially important foods such as yoghurt, cheese, pickles, brewing, winemaking industries, etc. *Saccharomyces cerevisiae* is extensively used in food industries. The microorganisms involved in the food biopreservation are especially lactic acid bacteria and some yeast such as *Acetobacter*, *Brevibacterium*, *Corynebacterium*, *Gluconobacter*, *Pseudomonas*, and *Erwinia* (Sugisawa et al. 1990; Sauer et al. 2004; Bremus et al. 2006). Several microbes are extensively used for vitamin production in food industry, for example, vitamin B12 is produced on an industrial scale by *Propionibacterium shermanii* or *Pseudomonas denitrificans* (Bremus et al. 2006). Microbial enzymes produced by microbial systems have extended application in food industries.

7.3.3 Pharmaceutical Applications

Soil microorganisms are also infinite source of some novel chemicals with various potential therapeutic applications. The members of *Actinomycetes* group isolated from soil serve as potential sources of antiinfection, antitumor, and antidiabetic compounds and also agents for the treatment of various neurodegenerative diseases (Thomashow et al. 1997). Antibiotics are one of the commercially exploited secondary metabolites produced by several microorganisms like bacteria and fungi. Approximately 80% of the world's antibiotics are known to be produced from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora*. The

genus *Streptomyces* produces amphotericin, erythromycin, streptomycin, tetracycline, and rifamycin (Thomashow et al. 1997).

7.3.4 Environmental Applications

Some soil microorganism-based bioremediation techniques for controlling the environmental pollution have been developed in the recent years to utilize the potential of certain taxa to degrade and detoxify *the* contaminants (Lee et al. 1983; Guengerich 1990). Soil microorganisms have the potential to degrade various environmental pollutants without producing toxic and harmful compounds as byproducts (Kothe et al. 2005) and evolved multifaceted mechanisms to neutralize the toxic effects of pollutants (Silver and Phung 1996). These microbial systems are more cost-effective and help in the development of appropriate techniques for cleaning up soil-contaminated environments for environmental restoration and protection. Nowadays, several soil microbes are isolated from contaminated sites and are extensively used for the bioremediation of numerous environmental pollutants (Machado et al. 2008; Ray and Ray 2009; Ruta et al. 2010).

7.3.4.1 Bioremediation

Bioremediation is an eco-friendly technique which utilizes the microorganisms to reduce or neutralize pollutants present in the contaminated environments. Some soil microorganisms have the ability to decompose or transform the petroleum products. The bacterial groups such as *Arthrobacter*, *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Burkholderia*, *Nocardia*, and *Pseudomonas* sp. are used to degrade hydrocarbons in soil environments. The fungi and yeasts such as *Amorphotheca*, *Graphium*, *Neosartorya*, *Talaromyces*, *Candida*, *Yarrowia*, and *Pichia* isolated from petroleum-contaminated soil were found to be effective in hydrocarbon degradation (Chaillana et al. 2004). Singh (2006) also reported that *Aspergillus*, *Cephalosporium*, and *Penicillium* were potential degraders of crude oil hydrocarbons. The fungi *Rhodotorula*, *Sporobolomyces*, *Aspergillus*, and *Penicillium* possess biodegradation potential of oil. Applications of soil bacteria *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, and *Arthrobacter* sp. are known for toxic waste management in polluted sites (Brunner et al. 1985; Nicholas 1987). PGPR has also been reported as an efficient remediator of contaminated soils (Zhuang et al. 2007).

7.3.4.2 Phytoremediation

Phytoremediation is the technique of cleanup of contaminants using green plants, and its efficiency is affected by the activity of a variety of rhizospheric

microorganisms (Khan et al. 2009). Rhizospheric bacteria degrade and detoxify the toxic compounds (rhizodegradation) (Kuiper et al. 2004). The combined application of both plants and biodegradative bacteria is used to remove petroleum products (Alarcón et al. 2008), polycyclic hydrocarbons and other aromatic compounds (Daane et al. 2001), as well as a variety of halogenated compounds (Leigh et al. 2006) from contaminated soils. Rhizodegradation enhances the plants' yield in the polluted soils (Lucy et al. 2004), for example, the amendment of some PGPR (*Pseudomonas* and *Acinetobacter*) has been found to enhance the phytoremediation abilities of non-hyperaccumulating maize (*Zea mays* L.) plants by favoring their growth and biomass production (Lippmann et al. 1995).

7.3.5 *Applications of Soil Microbes as Genetically Modified Microorganisms*

Soil microbes are utilized in several aspects such as agriculture, human health, environmental protection, and industries (such as food, paper, pharmaceuticals, textiles, leather, etc.) after the development of molecular techniques and recombinant DNA technology. These modified microbes are termed as genetically modified microorganisms (GMMs). The applications of GMMs include enhancement of nitrogen fixation (Gerhold and Stacey 1990), fungal pathogen restriction (Howell 1990), insect pest control, or biodegradation of pesticide residues (Snow et al. 2005) and production of proteins (insulin, interferons, and interleukins) for therapeutic use. *Rhizobium* species have been genetically modified either to improve their nitrogen fixation efficiency (Cullen et al. 1998) or to enhance their survival by the application of marker genes (Mendum et al. 2001; Hirsch 2004). Genetic manipulation of phosphate-solubilizing bacteria has been made to enhance their ability to improve growth and productivity of plants (Rodríguez and Fraga 1999). Another important application of genetically modified microorganisms is as a sensor to assess biologically relevant concentrations of agrochemicals, petroleum products, heavy metals, and toxins in various environmental samples of contaminated sites (Belkin 2003).

7.4 Advances in Soil Microbial Ecology

The primary target of microbial ecology is to determine the position and number of microbes in the environment after the development of several modern molecular techniques (Brock 1996). Recent molecular methods have contributed in the knowledge of microbial diversity in soils and also the interactions between diversity and its function in soil processes. Recently, the interest toward the soil microbes and ecology has been increased after knowing their role in the maintenance of biosphere and environment. Now the world has started moving in task of preserving the

environment and maintaining the sustainable land and exploitation of genetic resources. The recent advancements in the field of soil microbial ecology are offering fresh perspectives in the under-appreciated microbial world.

7.4.1 DNA Extraction, PCR, Cloning, and Sequencing Techniques

Nucleic acid isolation and characterization of microbes has revolutionized the microbial ecology (Nesme et al. 2016). DNA isolation is the primary and most essential step in the molecular studies of microbial ecology in which firstly DNA is recovered from the soil. The important task in the extraction is to isolate a sufficient amount of DNA without contamination, which inhibits the amplification of nucleic acid during PCR (Macrae 2000). PCR amplification of 16S rRNA genes (16S rDNA) using specific bacterial primers and separation of the resultant PCR amplicons either by cloning or by denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE) and sequencing are the most popular molecular techniques for the determination of soil bacterial ecology (Muyzer and Smalla 1998). The numbers of rRNA gene copies are related to the life strategy of bacteria, and species with lesser copy numbers inhabit low nutrient environment (Větrovský and Baldrian 2013). In the past few years, these molecular studies have been carried out in various diverse environments (Rheims et al. 1996; Duinveld et al. 1998). These studies have developed the ribosome-based sequences, and the environmental sequences deposited online are used for the design and application of oligonucleotide probes for the isolation, identification, and screening of several bacterial species in diverse environments (Busse et al. 1996). Another advantage of the extensive use of 16S rDNA techniques is to study bacterial diversity in geographically distinct soils (Ludwig et al. 1997). Therefore, differentiation in 16S rRNA gene sequences of different bacterial species has enormously improved our understanding about the ecological diversity of bacterial communities in soil.

7.4.2 Fungal PCR Primers

The bacterial species are identified as variation in the 16S rRNA gene, whereas taxonomic identification of fungi is based on 18S rRNA which is more challenging with identification usually restricted to family or genus level. The highest 18S rRNA sequence variation was observed between species belonging to phylum *Glomeromycota* (Schüßler et al. 2001). Therefore, 18S rDNA primers are used more commonly for symbiotic arbuscular mycorrhizal fungi as there is significant variation in 18S rRNA gene sequences of different fungal species to differentiate isolates to species level and below (Vandenkoornhuyse et al. 2002). White et al.

(1990) designed the first fungal PCR primers for the amplification of fungal 18S rDNA and ITS regions of the fungal DNA. Although these primers were designed with limited reference gene sequence informations, they have been proved to be very useful and powerful tools in genetic studies of fungi. These primers were generally used to amplify as broad taxonomic range as possible, and some of them were also used to amplify plant DNA from the mixed DNA samples of plant and fungi (Gardes and Bruns 1993; White et al. 1990). Such lack of specificity for fungal templates limits their effectiveness in mixed DNA samples especially where the ratio of fungal DNA to non-fungal DNA is low. Later, Gardes and Bruns (1993) designed ITS1F and ITS4B primers for the specific amplification of basidiomycetous fungal DNA from mixed DNA samples extracted from the colonized ectomycorrhizal (ECM) plant root tips. Subsequently, these fungal primers have been extensively used in ECM fungal researches and have increased our knowledge about ECM fungal communities and their ecology. Furthermore, ITS1F primer has been used in association with ITS4A primer, specifically to amplify templates from mixed DNA samples of fungal communities (Chen and Cairney 2002; Dickie et al. 2002; Lord et al. 2002; White et al. 1990), and with the ITS reverse primer ITS4A, especially for ascomycete fungal DNA (Larena et al. 1999). Thus, different fungal primers were designed for specific fungi.

7.4.3 Metagenomics, Metaproteomics, and Metatranscriptomics

Metagenomics involves the construction of DNA library followed by sequencing and functional analysis. Phylogenetics (based on the 16S rRNA/DNA) revolutionized the field of microbial ecology (Woese 1987). 16S rRNA gene analysis is very helpful in studying diversity and evolution of microbial populations. It has been reported that microbes with identical 16S rDNA sequences may have different overall genomes and show remarkably different physiologies and growth patterns (Jasper and Overmann 2004; Hahn and Pöckl 2005). Due to 16S rRNA gene analysis, soil is known as the most abundant diverse habitat for prokaryotes in earth which was not investigated by the cultivation-based methods. Nowadays, the goal of microbial ecology is to concern the identities of various microbes to the processes carried out by them in that environment, and this could be achieved using the 16S rDNA to identify clones belonging to specific microorganism and gene sequencing to gain information about the physiology of the microorganism. Fluorescent in situ hybridization (FISH) is a classical microbial technique which has been developed for the need of metagenomic research, using fluorescent probes to detect 16S rRNA.

Metatranscriptomics deals with the characterization of mRNA which provides the knowledge of metabolic phenomena of the microbial communities (Simon and Daniel 2011; deMenezes et al. 2012). Therefore, metatranscriptomics has the ability

to find out novel genes and functions which allow the detection of active members in rhizospheric microbial communities correlated with their metabolic activities in soil (Kim et al. 2014).

Microbial functions generally refer to proteins, so the investigation of the microbial proteins is the most appropriate tool for confirming the potential activity of the microbial community (Myrold et al. 2013). Metatranscriptomics has certain limitations toward the study of indigenous microbial communities such as short half-life of RNA, differential transcriptional kinetics of similar genes present in different populations, and low correlation between RNA levels and corresponding protein synthesis (Hurt et al. 2001; Zhou and Thompson 2002), so these limitations have increased interest in metaproteomics. Wilmes and Bond (2004) studied the diversity in proteins of microbial communities present in activated sludges, and Schulze et al. (2004) characterized proteins from the samples taken from soil solutions, lake water, and soil particles by electrophoresis coupled with mass spectrometry (MS). Together with metagenomics and metatranscriptomics, there has been a steady evolution in the methodology for the extraction and analysis of proteins from soils (Bastida et al. 2009; Hettich et al. 2010; Siggins et al. 2012). In the last decades, the advances in proteomic technologies, in addition to the sequencing of various microorganisms, have enabled us to link phylogeny with the microbial functions. In this way, through metaproteomics study, several novel researches would be correlated with microbial ecology as a link between genetic and functional diversity in microbial communities and its relative contribution toward taxonomic and functional diversity for ecosystem stability.

7.4.4 Community Profiling Techniques

Community fingerprinting techniques are generally used for investigating different bacterial communities and have extensively improved our knowledge about their role and diversity in the soil (Johnsen et al. 2001; Ranjard et al. 2003). However, these techniques include denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), single-strand conformation polymorphism (SSCP), terminal restriction fragment length polymorphism (T-RFLP), amplified rDNA restriction analysis (ARDRA), amplified ribosomal intergenic spacer analysis (ARISA), and cloning which have recently been adopted and used successfully for the community study of soil fungi.

7.4.4.1 DGGE and TGGE

Genetic diversity of a microbial community can be determined by fingerprinting techniques. In the early decades, electrophoretic separation technique was used, but later on, DGGE and TGGE were introduced which have the potential to separate DNA fragments of the same length but with different sequences (Riesner et al. 1991;

Muyzer and Smalla 1998). Now, DGGE is more widely used to investigate community complexity, community changes, isolation of bacteria, monitoring of the enrichment, and detection of microheterogeneity in rRNA encoding genes. One of the major limitations with these techniques is the separation of only relatively small fragments (up to 500 base pairs) which shortens the amount of sequence information for phylogenetic inferences as well as for probe designing (Myers et al. 1995).

7.4.4.2 T-RFLP Analysis of 16SrDNA for Characterization of Microbial Communities

Terminal restriction fragment length polymorphism (T-RFLP) analysis of PCR-amplified genes is a well-known fingerprinting technique for profiling of microbial community structure and dynamics in natural habitats (Schütte et al. 2008). This analysis depends upon the restriction endonuclease-mediated digestion of fluorescently end-labeled PCR products. The digested products are firstly mixed with a DNA size standard (already labeled with a distinct fluorescent dye) and then after fragments are separated by capillary or gel electrophoresis using an automated sequencer. After analysis, only the terminal end-labeled restriction fragments are detected and recorded. An electropherogram is prepared at the end which shows a profile of microbial community as a series of peaks of varying heights. This technique has been extensively used in the examination of complex microbial environments and in the ecological study of bacterial, archaeal, and eukaryal populations growing in natural habitats (Singh et al. 2006).

7.4.4.3 SSCP Analysis for Microbial Characterization

SSCP is used to identify and characterize specific microorganisms from the microbial communities in soil samples. In this technique, double-strand DNA of each microorganism is firstly transformed to single strand and separated by polyacrylamide gel electrophoresis. SSCP analysis has the ability to differentiate small variations within same-length DNA of different microorganisms due to the presence of differences in retention time, temperature, ionic strength, and electrophoretic mobility of single-stranded DNA (Bharathi et al. 2016).

7.4.4.4 ARDRA and ARISA

Amplified ribosomal DNA restriction analysis (ARDRA) is a commonly used technique to study microbial diversity which relies on DNA polymorphism (Deng et al. 2008). In this technique, amplicons containing 16S rDNA gene fragments are firstly amplified and then digested by restriction endonucleases, followed by separation of the resulting fragments through high-density acrylamide gel electrophoresis. Amplified ribosomal intergenic spacer analysis (ARISA) is used to amplify both

bacterial and fungal community in various soils. Various researches showed that ARISA is a high-resolution, high-reproducible, and vigorous technique to discriminate diverse microbial communities in soils (Ranjard et al. 2001).

7.4.5 Microarray Technology

Microarray is an extraordinary, precise, sophisticated, quantitative, and high-throughput technique used for the detection, identification, and characterization of microorganisms in the natural habitats. Due to swift advances in fingerprinting technology, microarrays contain hundreds to thousands of probes. Various studies have used microarray technology for investigating ecological problems. Some modern techniques such as PCR fingerprinting, real-time PCR, reverse transcriptase PCR, reporter genes, and fluorescence in situ hybridization (FISH) technology have developed to study the dynamics of simple microbial communities or small groups of dominant microbes in natural environments. Later, microarray technology has been predominantly developed to study gene expression profiling of pure cultures of diverse microorganisms; moreover, some major advances have been made regarding their efficient application to different environmental samples. Microarrays detect only the dominant populations of microorganisms in many environmental samples (Denef et al. 2003; Rhee et al. 2004). Different types of microarrays such as phylogenetic oligonucleotide arrays (POAs), functional gene arrays (FGAs), metagenomic arrays (MGAs), community genome arrays (CGAs), and whole-genome open reading frame arrays (WGAs) have been successfully used in microbial ecology research. These arrays are useful for functional genomic study of individual organisms and comparative genomic analyses and also for investigating the interactions of multiple organisms at the transcriptional level (Denef et al. 2003; Rhee et al. 2004).

7.5 Future Prospects of Soil Microbial Ecology

Soil microbiology is a very fast-growing area of research with many relevant topics regarding the development of model ecosystems and sustainable environmental management. For maintaining and protecting the life-supporting natural resources and soil biodiversity, it is essential to develop and standardize the methodology and to specify overall data collection and quality assurance techniques. It is also important to understand the spatial and temporal variation of soil microbiological characteristics for the successful execution of monitoring programs. Another challenge for future research is to be efficient to generalize the results from microscale to large-scale processes even for the prediction of global climate changes. Although reliable techniques are crucial, the quality of research results does not depend solely on technical improvements. The advancement of knowledge/technology needs skillful

evaluation of the appropriate techniques and data analysis tools to be applied in each specific question regarding environmental sustainability. As per the perspectives of soil microbiologists, new molecular techniques offer new ways to explore community composition and processes of microbes on a microscale. In situ hybridization is able to explain where microorganisms exist and play an active role (Lübeck et al. 2000). The study of microbial hotspots occurring inside the guts of soil microfauna or around root surfaces is needed for future research. In microbial hotspots, various turnover processes occur, and microbial loops are formed (Clarholm 1994.). Sustainable management of soil ecosystem aims to establish desired microbial populations successfully. Such microorganisms may play an active role as degraders of xenobiotics, nitrogen fixers, or pathogen antagonists. In the future, alteration in a single key biological agent in the soil in a desired way would result in the alteration of soil functions for the benefit of human beings. Hopefully, such strategy would increase the agricultural sustainability and also help to remediate polluted soils and protect natural resources successfully.

Microarray technology and genome sequencing would have a major impact on our ecosystems. Microarray technology enables us to assess and analyze the community diversity in soils by directly expressing and hybridizing oligonucleotides fixed on specific membranes (Guschin et al. 1997; Ogram 2000). Another application of this technology to correlate community structure with community function by using mRNA and by combining with PCR amplification and/or rDNA would be possible (Gottschal et al. 1997). Using computational methods, it might be possible to describe a three-dimensional physical and functional model of a microbial niche. This goal of synthesis of complex information brings us closer to the computational sciences. Microbial model has many advantages over the macroecological models in our system. Many genes and traits will be potentially explorable at the genetic level by mutation. We will likely see the phylogenetic tree as a bush showing a continuum of many types of species.

Another emerging field is “bioinformatics” which is popular in almost all the branches of biological sciences. Bioinformatics converts complex biological information into the understandable model by using computer science and technology. Bioinformatics utilizes the integrated efficiency of the computational methods, simulation, analysis, and modelling to extract information and prediction of biological processes what exactly going on within a cell naturally (Altman and Klein 2002). Integration of genomic, proteomic, and metabolomic data sources will enable us to predict genetic mutations after the molecular analysis of disease symptoms and vice versa. The effects and outcomes of diseases and pests in agricultural systems can be predicted with the integration of GIS data like geographical mapping and weather systems with crop health and genotypic traits. Another challenging research area for bioinformatics is comparative genomic studies at large scale which could be achieved by the development of practical tools and techniques. The problems with digitization of phenotypic data such as complex behavior of microbes in diverse ecological niches correlated with crop or soil health offer future opportunities in the field of bioinformatics. Currently, there is quite a need to develop bioinformatics tools to open the hidden mystery of central dogma-based biological processes

occurring within the tiny and often unseen microbial life forms. Thus, bioinformatics technique will enable us to understand the complex biological processes of any organism through the integration of informations obtained from these key biological processes within the cells.

The current microbiological researches are focusing only on pathophysiological mechanisms behind microbial diseases in plants rather than their management measures. For this purpose, a recently emerging field of "omics" era called metabolomics has the potential to find out solutions along with bioinformatics capabilities toward data integration, analysis, and management in biological studies. In the coming years, targets of microbial research and development such as molecular taxonomy, microbial mapping, identification of different agroecological sites using culture-dependent or metagenomics approaches, searching of potential genes and gene products for the microbial management of disturbed agricultural soils, bioprospecting for novel metabolites, enhancement of biotic and abiotic stress tolerance in crop plants (Tiwari et al. 2011), microbe-associated soil fertility and crop improvement programs, enhanced bioremediation efficiency, enhanced biofermentation capability, and development of next-generation microbial inoculants as biofertilizers and biopesticides (Singh et al. 2011) could not be achieved without the applications of bioinformatics (Wollenweber et al. 2005). Recent emerging fields like interactome, which includes sets of protein-protein interactions, and localizome, which deals with the subcellular localizations of proteins, will certainly play a significant role in future molecular researches. In the future, the ultimate goal of microbial biotechnology will be the integration of genetic resources and biological databases which would result in the computational representation of any aspect of biology of living cells and microorganisms.

7.6 Conclusion

In summary, it can be concluded that soil microorganisms play a pivotal role in the functioning of the ecosystems for maintaining a sustainable environment and productivity. Soil microorganisms have precise contributions to the nutrient cycles and as sources of useful chemicals. Soil microbial diversity plays a key role in human survival and economic development and also provides a major reservoir of natural resources which can be utilized for the betterment of human lives. Thus, the future of soil microbial ecology is bright because many major challenges of society have their root in it. Soil microbiology is the rapidly growing area which would greatly benefit agriculture, industries, environment, and human health through the application of advanced technologies, development of suitable ecosystem models and ecological theories, sustainable soil management, and realization of ecosystem stabilization and global changes. For prosperous environment, we should save such "jewels" and use them wisely.

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