Vipin Chandra Kalia · Yogesh Shouche
Hemant J. Purohit · Praveen Rahi
Editors

Mining of Microbial Wealth and MetaGenomics

Springer

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Dedicated to our mentors

Preface

Microbes, the tiny little "invisible" organisms, exist along with all living beings. Though difficult to be seen by the naked eye, their effects provide strong evidences of their existence. Microbe–human associations are viewed as dangerous, leading to unhealthy scenes. However, the best part of the living world is that each of them is susceptible to attack by others. Hence, all of them have developed a strong defense mechanism to protect themselves. All living beings have unique genetic background and almost perfect metabolic pathways. The realization that human beings carry microbes on their skin and gut throughout their lives has provoked us to look deeper into these relationships. Recent studies have revealed that microbes produce metabolites which are essential for our health status. The research works of the last century have seen the rise and fall of antibacterials, the most important secondary metabolites. The process of elucidating the identity of organisms has gathered momentum by the advent of novel molecular biology techniques, the latest being the next-generation sequencing technologies. It has led to the discovery of the presence of organisms including those which are extremely low in abundance. Oceans, rivers, mountains, and the gut are unique ecosystems with great potential for harvesting secondary metabolites, i.e., natural products and bioactive molecules, which can find applications in the fields such as agriculture, food, medicine, water, bioremediation, etc. Oceans including ecological habitats such as coral reefs, hydrothermal vents, sponge reefs, sea grass beds, mangroves, and soft sediments are the largest reservoir of unknown living beings. Quite a few organisms are difficult to cultivate under laboratory conditions especially because of variations in temperature and pressure at different depths of the ocean. Metagenomics allows elucidation of the maximum biodiversity within an ecosystem, without the need to actually grow and culture the organisms. Microbiomes are associated with plants (leaves and roots), animals including humans (skin and gut), and marine metazoans (especially sponges). These multidimensional interactions need to be understood for developing sustainable ecosystems, agriculture, and healthy human beings.

Society strongly supports scientific adventures, which are constantly striving hard to improve human lives. In order to ensure that there is strong interest and continuity in scientific pursuits, it is almost imperative that the next generation is prepared to meet the future challenges. We need to train the young minds and impart scientific skills in them. We wish to present the status of the diverse possibilities and our views and opinions to finally provide mankind with novel, innovative, and

long-lasting strategies, in the book entitled: *Mining of Microbial Wealth and Metagenomics*. This book has reached this stage only because of the fact that dedicated and academically accomplished members of the scientific community had agreed to share their vision and wisdom, which can be acquired only through decades of sincere and dedicated efforts put in to better understand the living world. This book has been presented in a manner such that all human beings can take advantage of the latent features of the world around us. Our sincere thanks are to all those whose invaluable contributions enabled us to bring out this book. We are indebted to all of them. This acknowledgment may not be sufficient to justify the worthiness of their efforts.

New Delhi Vipin Chandra Kalia

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Yogesh Shouche is a scientist at the National Centre for Microbial Resource, National Centre for Cell Science, Pune. After working on different aspects of the microbial, especially bacterial, diversity of various ecological niches, his research is now focused on understanding the succession of microbial communities in the human gut during development and their role in health and diseases. He has worked for 25 years in the fields of microbial ecology, microbial molecular taxonomy, and biodiversity; published more than 200 publications in journals of relevant area; and reviewed publications in international journals like *FEMS Ecology*, the *International Journal of Systematic and Evolutionary Microbiology*, and *Microbial Ecology*. His works have been cited 5662 times with an h-index of 37 and an i10-index of 147. He is also on the editorial board of prestigious journals such as *Current Science*, the *European Journal of Soil Biology*, *PLOS ONE*, and *Scientific Reports*. In 2009, he was given responsibility for the establishment of the National Centre for Microbial Resource (NCMR). Dr. Yogesh now heads a large group of researchers at NCMR including 30 scientists and technicians.

Mining Metagenomes for Novel Bioactive Molecules

1

Vipin Chandra Kalia

Abstract

Living organisms especially a wide range of microbes and plants produce secondary metabolites, which prove beneficial in improving the efficiency of metabolic processes. These metabolites with unique properties are categorized as bioactive molecules (BAMs). The utility of these BAMs has found its way to almost all biological processes, such that there has been a dramatic surge in their demand. The unique characteristics can be assigned to the chemical structures and associated groups. Conventional methods for searching novel BAMs are proving counterproductive. Modern molecular biological techniques in association with bioinformatic tools have provided the much necessary boost to the morale of the scientific community. The most effective genomic tool for searching these novel BAMs are (1) sequencing of whole genomes and (2) culture-independent (metagenomic) analysis. These approaches allow generation of huge amount of information, which can be easily analyzed through bioinformatic tools.

Keywords

Bioactive molecules • Metagenomics • Antibacterials • Therapeutics • Anticancer

1.1 Introduction

A wide range of prokaryotic and eukaryotic organisms produce secondary metabolites. These have been quite valuable and categorized as bioactive molecules (BAMs) (Debbab et al. 2010; Guaadaoui et al. 2014; Bandyopadhyay et al. 2015; Trindade

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et al. 2015; Hernández-Saldaña et al. 2016; Kalia et al. 2016). However, the rate of discovering novel BAMs has declined dramatically. The basic reasons are (1) the conventional methods have been proving quite inefficient and uneconomical, (2) poor access to high-throughput techniques, and (3) inability to predict whether the organisms are genetically and metabolically active to yield the final product. With the advent of molecular techniques coupled with bioinformatics, there has been a dramatic rise in the possibilities of predicting the presence of genes for novel BAMs (Kalia 2013; Karumuri et al. 2015; Yin et al. 2015; Ambardar et al. 2016; Jeyanthi and Velusamy 2016; Parmar et al. 2017; Sharma and Lal 2017). Here, the exploitation of whole genome sequences either of a single organism or a mixture of related or unrelated organisms ranging from prokaryotes to eukaryotes is the most lucrative (Charlop-Powers et al. 2014). The most advantageous feature of the gene/genome sequencing technique is its extrapolation to metagenomics, which eliminates the need to culture the organisms (Daniel 2004). This culture-independent technique allows preparation of gene-based libraries and screening at least a few million genes in a single stroke. These genetic and metabolic reservoirs can be finally exploited for isolating novel BAMs. Metagenomics is motivating enough to substantially influence industrial production of BAMs and their applications in daily life (Torsvik et al. 2002; Handelsman 2004).

The most commonly looked for BAMs are industrial enzymes (detergents, food applications, agriculture, textile processing, leather industry, etc.), antibiotics, and related pharmaceutical products (Singh et al. 2009, 2013, 2015; Arasu et al. 2015; Azman et al. 2017). In spite of high intensity of efforts, the culture-dependent techniques have not proved very productive in the last few decades. The primary reason is that we are not able to provide the culture conditions to the microbes and 99.9% of the genetic potential remains untapped. The alternative approach to out-beat this limitation was to use replace nutrient-rich media with oligotrophic media, which allows slow-growing and nutrient-selective organisms to grow. Other approaches employed were simulated environments, cell encapsulation, etc. (Janssen et al. 2002; Joseph et al. 2003). Though quite impressive, these techniques may not hold good for all the organisms, which are yet to be explored (Daniel 2004).

The course of biological research took a dramatic turn through the development of a few innovative genomic approaches, toward the end of the last century. These opened the ways for dramatic and rapid progress. These genomic tools include (1) genome sequencing, (2) cultivation-independent protocols, and (3) bioinformatics. (Courtois et al. 2003; Robbel et al. 2010; Kalia 2013; Yin et al. 2015; Pooja et al. 2015; Ambardar et al. 2016; Parmar et al. 2017; Sharma and Lal 2017).

1.2 The Potential BAMs

1.2.1 From Terrestrial Microbes

Microbes have been used as active platforms for obtaining BAMs (Lorenz and Eck 2005; Peña-Yam et al. 2016; Radivojevic et al. 2016; Pessione et al. 2017; Saini and

Keum 2017; Sanchart et al. 2017). Microbes isolated from soil have resulted in extraction of BAMs, which act as antifungal and anticancer and can inhibit aminopeptidase activity (Go et al. 2015; Begum et al. 2016; Varsha et al. 2016; Thakur et al. 2017).

In contrast to culturing techniques, and designing the microbes for a mediocre molecule, screening metagenomes for BAMs has been found to hold more promises (Lorenz and Eck 2005). Metagenomic studies have been done largely by employing *Escherichia coli* as host. In order to improve the possibility of finding novel genes, other host organisms such as *Streptomyces*, *Bacillus*, and *Pseudomonas* spp. were employed (Wang et al. 2000; Courtois et al. 2003; Lorenz and Eck 2005). DNA libraries screened for genes encoding the enzyme alcohol oxidoreductases were based on polyol-fermenting microorganisms. For this purpose, 1.2 and 2.1 million clones from enriched and non-enriched samples, respectively, allowed recovery of 20 positive clones (Knietsch et al. 2003a, b). Antimicrobial metabolites from metagenomic libraries (bacterial artificial chromosome, fosmid and cosmid types) yielded positive hits from 1 to 10 at the rate of 1 hit per 100–900 Mb (Lorenz and Eck 2005). Metagenomic soil libraries have been found to yield interesting results on industrial enzymes and BAMs: agarases, amidase, antibacterials, amylases, biotin production, DNase, dehydratases, dehydrogenases, lipases, β-lactamase, indirubin, oxidation of polyols, oxidoreductases, lipase, polyketide synthase, terragine, violacein, turbomycin, and proteases (Richardson et al. 2002; Knietsch et al. 2003c; Daniel 2004; Lorenz and Eck 2005).

1.2.2 From Marine Microbes

Most of the BAMs available so far have been obtained from terrestrial sources. It was realized that the bulk of natural products obtained from marine sources were unique. The ecological niches in the oceans are home to more microbes than in any other environment (Montaser and Luesch 2011; Balakrishnan et al. 2015; Blunt et al. 2015; Tao et al. 2015). The efficiency of marine products is expected to be very high since they get diluted on release. Among marine microbes, algae, sponges, and soft corals have been the focus for quite some time; however, there has been a shift toward bacteria and fungi (Teasdale et al. 2009; Shiva Krishna et al. 2015). Marine sources are rich in antimicrobial, antitumor, antifungal, antiparasitic, anti-nematodal, anti-pathogenic, and anti-inflammatory molecules (Solanki et al. 2008; Teasdale et al. 2009; Trindade et al. 2015).

Marine ecological niches as a source for searching BAMs through metagenomic approaches are yet to make significant contributions to the pharmaceutical industry (Trindade et al. 2015). In spite of these limitations, metagenomic screenings based on prior knowledge on chemical structure and biological function have yielded a few BAMs: (1) bryostatins (type I polyketide) show cytotoxicity against carcinomas and are being tested for its activity as anti-Alzheimer's drug, which were linked to "*Candidatus* Endobugula sertula" (Sudek et al. 2007); (2) ecteinascidin 743 (ET-743) with anticancer activity was shown through metagenomic strategy to be linked

to "*Candidatus* Endoecteinascidia frumentensis" (Rath et al. 2011; Schofield et al. 2015); (3) patellazoles-polyketides with antifungal potential and cytotoxicity were proposed to be produced by "*Candidatus* Endolissoclinum faulkneri" (Kwan et al. 2012; Schmidt et al. 2012); (4) psymberin, an antitumor polyketide (Fisch et al. 2009); and (5) polytheonamides, toxins like Calyculin A (Freeman et al. 2012).

1.2.3 From Plant Endophytes

BAMs of plant origin have great potential. Among these endophytes have been recognized as the most promising (Kusari and Spiteller 2011). During the last few years, the following secondary metabolites have been identified as BAMs: paclitaxel, podophyllotoxin, hypericin, camptothecin, and emodin. Their production and utilization of a large scale has yet not been achieved. Whole genome sequencing and metagenomics hold the potential to fish out novel BAMs. Mining of genome sequence of *Streptomyces coelicolor* has revealed *cch* cluster encoding for natural product biosynthetic systems: nonribosomal peptide synthetases (NRPSs). The BAM had a potential role in ferric iron acquisition. Similarly, mining of genomes resulted in BAMs, (1) new peptides: *Pseudomonas fluorescens* yielded orfamide A, a NRP antibiotic where as *Stigmatella aurantiaca* and Myxococcus virescens also produced novel peptides, and (2) terpenoid from *Arabidopsis thaliana* (Van Lanen and Shen 2006; Challis 2008).

Fungi are well known to produce BAMs. Although metagenomic techniques have seen a surge, however, metagenomic analysis of fungal genetic material has been quite scarce. Endophytic fungal DNA extracted from the leaves of *Rhododendron tomentosum* has the potential to produce antibacterial and antioxidant metabolites (Tejesvi et al. 2011). However, metagenomic analysis did not reveal any clear-cut information with respect to fungal genomes. Mining of genomes of *Aspergillus nidulans* has resulted in identification of genes encoding for anthranilate synthases (Scherlach and Hertweck 2006).

1.2.4 From Human Microbiome

Association of microbes with human beings has been reported from their skin and gut. These microbes have been shown to greatly influence human health. The ability of these microbes to produce secondary metabolites can be easily envisaged; however, the current knowledge on these aspects is very limited. Metagenomic studies of human microbiome have revealed a diversity of secondary metabolites (Donia et al. 2014; Joice et al. 2014; Sharon et al. 2014; Donia and Fischbach 2015; Koppel and Balskus 2016). In spite of the enormous quantum of effort, little is known about their identity and functions (Wilson et al. 2017). A few BAMs revealed through metagenomic studies include (1) antibiotic, lactocillin from *Lactobacillus gasseri* (Wieland Brown et al. 2009; Milshteyn et al. 2014); (2) commendamide (Cohen et al. 2015), a signaling molecule exhibiting activities such as antibacterial, activation of calcium channel and agonist; and (3) colibactin (Wilson et al. 2017).

1.3 The Future Prospects

The scope to use BAMs for biotechnological applications is widening with time (Kumar et al. 2014, 2015a, b; Ray and Kalia 2017a, b, c). This potential usage in health-related areas is sufficient to fuel the urge for searching novel and more efficient BAMs. The areas which make this search lucrative are cancer, therapeutics, nutrition, etc. (Milshteyn et al. 2014; Bose and Chatterjee 2015; Dobrucka and Długaszewska 2015; Park et al. 2015; Szweda et al. 2015; Wadhwani et al. 2016; Ahiwale et al. 2017).

1.4 Opinion

The present status of bioactive compounds is quite encouraging in terms of their applications. A large number of such molecules have been elucidated. However, we need to take advantage of recent discoveries and exploit them for accelerating the rate of discovery and development. Expression of genes in multiple host systems and prescreening strategies should be developed. This obviously also demands improved and innovative techniques to detect novel and beneficial BAMs.

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Rhizosphere Microbiome Metagenomics: Elucidating the Abditive Microflora

2

Asifa Mushtaq and Seema Rawat

Abstract

The rhizosphere is a zone of biological activity between plant roots and soil harboring a plethora of microorganisms. The key interactions among a multitude of microorganisms in the rhizosphere have a direct or indirect effect on the plant. Being versatile and intriguingly complex, a comprehension regarding the elementary principles of microbial ecology and functioning is significant to enhance the plant productivity and agroecosystem working. The interplay between plant roots and the associated microbes is regulated by profound chemical signaling. Most of the known facts about these interactions till recently have been derived through the studies based on culturing the microbes; however, it is an established fact that majority of the microbes are uncultivable. Novel insights into enhancing our ability to unravel the quintessential factors determining the rhizosphere microbiome could offer the progress towards the development of sustainable agriculture. We now have the opportunity to utilize the advanced culture independent techniques to have an insight into the intriguing plant-microbe interplay. Metagenomic studies present a strong mandate to understand the enormous richness and diversity of rhizosphere microbiome as well as the key biological processes.

Keywords

Rhizosphere • Microbiome • Interplay • Signaling • Metagenomics

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2.1 Introduction

The rhizosphere, a microecological zone of soil surrounded and influenced by root system, is a dynamic site for diverse organisms (Dobbelaere et al. 2003; Hartmann et al. 2008; Prashar et al. 2014). This zone, in comparison to the bulk soil, is rich in nutrients due to the accumulation of various plant exudates such as amino acids, aromatic compounds, and sugars, providing a nutrient-rich environment for the colonization of diverse microbes (Gray and Smith 2005; Beneduzi et al. 2012). This zone of influence withholds higher microbial population densities, approximately 10- to 100-fold greater, as compared to bulk soil (Berg et al. 2006; Costa et al. 2006; Hein et al. 2008) suggestive of intense race between microorganisms for the availability of nutrients and for the sustenance of species showing functional diversity and metabolic versatility (Dube and Yeole 1999; Sinha et al. 2001). The rhizosphere itself can be differentiated into three zones: (a) endorhizosphere, the internal root area extending generally to the cortical region which harbors diverse population of microlife with versatile functions; (b) rhizoplane, zone adjacent to the root; and (c) ectorhizosphere, extends from the rhizoplane out to what is called bulk soil (reviewed by Johri et al. 2003). The significance of the rhizosphere as an environment copious in varied microbial populations playing an imperative role in plant health and soil fertility has already been perceived during the late nineteenth century (Bais et al. 2006; Nautiyal et al. 2008).

Rhizosphere microbiota, directly or indirectly, promote plant productivity by a range of varied mechanisms (Buckling et al. 2007; Lugtenberg and Kamilova 2009; Hider and Kong 2010; Leveau et al. 2010; Mapelli et al. 2012; Pii et al. 2015). The diversity of microbes present has a pivotal role in maintaining the soil fertility and, in turn, the plant growth because these microorganisms are involved in many significant biological processes such as soil formation, facilitating the uptake of specific nutrients by the plants, toxin removal, biogeochemical cycling, and many more (Nautiyal et al. 2010). The interplay between plants and the associated microorganisms promotes a physicochemical heterogeneity in the local soil microhabitat making a major contribution to the biotic components of soil, one of the most intricate ecosystems on Earth. Advancement in agricultural sustainability can be achieved by making optimal and systematic use of biotic and abiotic components of soil both of which rely on soil biodiversity and biological processes.

Analyzing bacterial communities traditionally began with culturing microorganisms from an ecological niche. This technique has limitations as the largest proportion of soil microbes cannot be cultured efficiently in the laboratory, due to the complexity of their growth conditions and the presence of cells which are in a viable but noncultivable state. As a result, only 1% of the microbial diversity present has been explored until now using classical cultivation techniques. Various molecular techniques have been put into e to assess microbial communities from diverse environments (Table 2.1). Over the past decade, molecular genetics has opened our eyes to several of the complex host-microbe interactions. One of the rapidly growing techniques used for elucidating the uncultured microorganisms, their functions, cooperation, and evolution from various environments is metagenomics.

Table 2.1 Molecular techniques applied to analyze microbial diversity

Techniques	Applications	Shortcomings	References
FISH (fluorescent in situ hybridization)	Direct visualization of bacteria in the environment Detects active cells by targeting rRNA	Can mislabel cells when probe is not universal Visualization can become difficult with background fluorescence Smaller fraction of the community can be hard to detect	Zwirglmaier (2005), Li et al. (2008), Caracciolo et al. (2010), Lundberg et al. (2012), Schmidt and Eickhorst (2014)
PCR based: DGGE, RFLP, RISA, sequencing of amplified genes, etc.	Easy to implement Detailed picture of rhizosphere diversity Amenable to high throughput analysis	Subject to PCR bias Labor intensive More than one microbial species may be represented by a single band	Acinas et al. (2005), Spiegelman et al. (2005), Thompson et al. (2005), Hong et al. (2006), Bentley et al. (2008), Rawat and Johri (2014), Pascual et al. (2016)
Metagenomics	Whole-community-level genome characterization Characterization of genomes of unculturable microbes	High cost Data analysis is challenging and time consuming Can miss lesser abundant members in the microbial community	Daniel (2005), Uroz et al. (2010), Myrold and Nannipieri (2014), Pascual et al. (2016)
Metatranscriptomics	Gene expression Identification of active community members, correlating them with their metabolic activities	Short mRNA half-life Limiting RNA amounts Presence of enzyme activity inhibitors in soil	Jones and Dangl (2006), Bastida et al. (2012), De Vleesschauwer and Hofte (2009), Simon and Daniel (2011), Carvalhais et al. (2012)
Metaproteomics	Gene activities Metabolic functions Gives an understanding of microbial interactions	Niche discipline restricted Superior separation and measurement protocols Nonannotated and unassembled metagenomic data can be a major hurdle	Wang et al. (2011), Becher et al. (2013), Bao et al. (2014)

Metagenomics aids in evaluating the richness, distribution, and activity of microbial communities in any environmental sample even without the need of culturing. Furthermore, it provides an easy access to the diverse microorganisms present in an environment thus aiding in the discovery of new groups of microbes (Amorim et al. 2008). In this chapter, we will try to explain the potential of metagenomic approach towards the understanding of diverse root-associated microbes.

2.2 Plant-Microbe Interactions

Plants develop an intriguing relationship with the microbial diversity present within the surrounding soil. There are diverse microbial communities present in the soil, and these microbial communities, so far, are considered to be an epitome of biological diversity present on Earth (Gams 2007; Buee et al. 2009; Singh et al. 2009). Rhizosphere, a narrow zone of soil, is home to an overwhelming population of microbes and can harbor up to 10^9 prokaryotic cells per gram (Wegley et al. 2006; Egamberdieva et al. 2008) containing more than 30,000 species (Mendes et al. 2011). As the collective genome of the inhabiting microbial community is much larger than that of the plant itself, it is usually ascribed as plant's second genome (Berendsen et al. 2012). Subsequently, the significance of root microbiome, consisting of the entire rhizosphere-associated microbes, their genetic elements, and their interactions, in determining the plant health (Berendsen et al. 2012), has been demonstrated by various in-depth studies from different parts of the world.

Microbial colonization in the rhizosphere is governed by various biotic and abiotic factors. The heterogeneous physicochemical characteristics of soil are paramount in shaping the rhizosphere microbiome as they affect root exudation patterns by affecting the plant physiology. Moreover, these root exudates are known to vary in accordance with plant species and environmental conditions (Hogberg et al. 2006; Lesuffleur et al. 2007; Micallef et al. 2009) influencing the microbial diversity in rhizosphere (Grayston et al. 1997; Kuklinsky-Sobral et al. 2004; Salles et al. 2004; Somers et al. 2004). The majority of the root exudates are believed to be comprised of amino acids, fatty acids, hormones, organic acids, sugars, and vitamins as well as antimicrobial compounds (Bertin et al. 2003; Jones et al. 2004; Badri and Vivanco 2009). Plant roots exert pronounced effect on the surrounding soil through these "rhizodepositions" (sloughing of root-cap cells along with mucilage secretion and controlled root exudate dispersion), thus render appropriate ecological niche for microbial colonization (Bais et al. 2006). The rhizosphere microbes establish a synergistic relationship with the host plant facilitating nutrient uptake as well as suppressing soilborne phytopathogens, thus helping in improving the plant productivity (Berendsen et al. 2012). (Fig. 2.1).

Rhizosphere microbes perform various ecological functions including maintenance of soil structure and water relationships, organic matter decomposition, biogeochemical cycling, and, above all, growth of the inhabited plant. Various biological processes that are carried out by the microbes in rhizosphere include symbiosis, nutrient uptake, plant protection as well as antibiotic production, geochemical

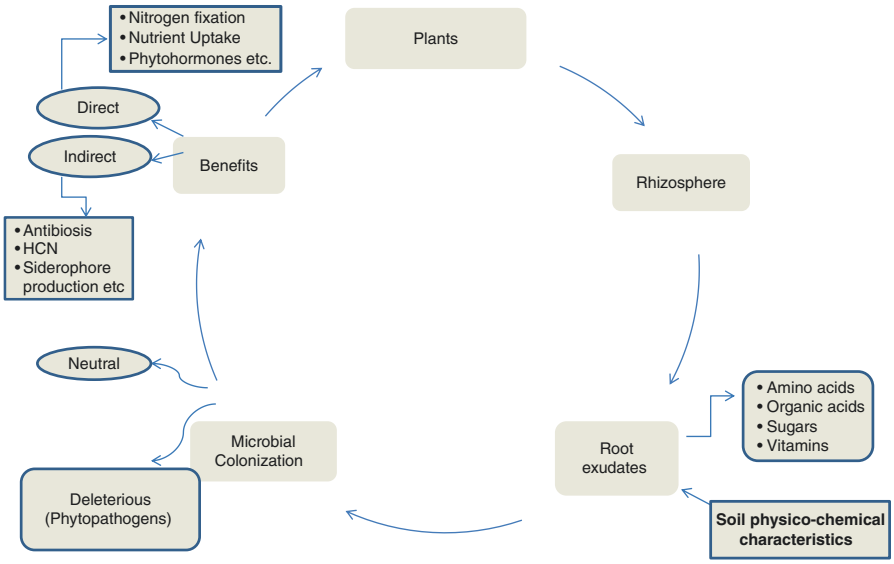

Fig. 2.1 Factors governing plant-microbe interactions (Mushtaq & Rawat, HNBGU)

cycling of minerals, etc. (Kent and Triplett 2002; Bulgarelli et al. 2013; Philippot et al. 2013; Gianfreda 2015). Even though substantial improvement has been made in our understanding of the microbial ecology, more comprehensive studies in soil microbiology must be undertaken to unravel plant-microbe interplay.

2.3 Diversity of Rhizosphere Microbiome

The diversity of root-associated microbes is immense, in the order of tens of thousands of different species. There is a clear-cut difference between the microbial populations with larger number of microbes harboring the rhizosphere as compared to those residing in the bulk soil. The enhanced microbial activity in rhizosphere as compared to bulk soil can be attributed to the availability of large amount of nutrients in the form of root exudates. In general, the organisms found in rhizosphere include bacteria, fungi, oomycetes, nematodes, protozoa, algae, viruses, archaea, and arthropods (Raaijmakers and Weller 2001; Nautiyal et al. 2008; Raaijmakers et al. 2009; Chaudhary et al. 2012). Various studies that have been undertaken to explore the rhizosphere-associated microbes of different plant species from different parts of the world suggest *Proteobacteria* and *Actinobacteria* to be the dominating populations of rhizobacteria (reviewed by Singh et al. 2007; Dokic et al. 2010; Lopes et al. 2016). Other major groups include *Firmicutes*, *Bacteroidetes*, *Planctomycetes*, *Verrucomicrobia,* and *Acidobacteria*. Although extensive studies have been done on decoding the diversity of root-associated microbes, we only know a minor portion of this diversity, the reason being that the majority of the