Pallaval Veera Bramhachari *Editor*

Understanding the Microbiome Interactions in Agriculture and the Environment

Springer

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Foreword

The microbial world is largely invisible to the human eye, but it is almost beyond imagination. There are hundreds of thousands of different kinds of bacteria (leaving aside other kinds of microbes: archaea, viruses, fungi, and protists) living in every possible environment including the deep seabed, high in the clouds, and in the boiling hot springs. Multicellular organisms created an entirely new set of habitats, in and on all those animals and plants.

Research data suggests that during the last two decades, extensive research has been carried out on endophytic fungi and several biologically active compounds have been isolated from endophytic fungi. This book makes all the reader generally conversant in the language of microbiomes and metagenomics. It also provides excellent examples of how microbial communities affect health and cure diseases and doles out typical practical examples of how medical interventions interact with the microbiome and change outcomes.

This volume, *Understanding the Microbiome Interactions in Agriculture and the Environment*, published by Springer Nature is important, and I strongly believe that it will attract readers working in the field. The present volume has 16 chapters contributed by several competent academicians and scientists working on microbiome research throughout the world. I congratulate the editor of the book Dr. P.Veera Bramhachari for bringing out this volume with excellent contributions from scientists working on the microbiome and their application in understanding the microbiome interactions in agriculture and the environment.

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K. B. Chandra Sekhar

Preface

Microbiomes are vital to major biogeochemical processes on which life on earth depends—therefore knowing the microbiomes will be crucial to understanding and tackling the problems of environmental and climatic changes. Soil, plant, animal, and marine ecosystem microbiomes are critical to environmental health and a food system that provides us with highly nutritious, inexpensive, safe, and sustainable food. Research and innovation on microbiomes in the food chain are continually advancing. Providing nutritious meals for everyone and transforming the present food system to one that is more environmentally friendly will be made possible thanks to advances in our knowledge of the microbiome in the food supply chain.

Many intricate problems surrounding the intimate connections between plants and microbiomes in agriculture and the environment have limited answers to these topics. Researchers in the field of the microbiome continue to make significant and exciting advances to our knowledge of the fundamental biology of agriculture and the environment. Notably, microbiomes play a crucial role in plant tolerance to harsh circumstances, such as salt, drought, and exposure to heavy metals. Plants' resilience to salty soil can be improved by the microbiome's synthesis of phytohormones, which lessens the harmful effects of excessive soil salinity. Plants are commonly used in remediation techniques to eradicate the negative effects of these toxins, and their efficacy is ascribed to the microbiomes associated with them. These microbiomes in the consortium are capable of decomposing and stabilizing pollutants.

Several pieces of research have been dedicated to exploring the structure and function of the microbiome for plants and have discovered that plants give habitats and nutrients to the microbiota, while the microbiota supports plant development, nutrition, and defense against diseases. The microbiome dynamically interacts with the plant host to generate synergistic interactions, which, in turn, alter the host's physiology. Several investigations are motivated to examine how the microbiome is generated and what are the driving variables that alter its dynamics to shape plant performance in the ecosystem.

Microbiome research in agriculture can lead to inoculants or manipulations of the microbiome to choose more efficient bacteria groups for plant growth. Besides that, decreasing the usage of pesticides and chemical fertilizers based on an awareness of the potential of the plant microbiome is of crucial significance for promoting

sustainable farming methods. As a result, taking an all-encompassing look at the microbial communities that are connected to plants and the environment is unquestionably a novel approach. The relevance of research on plant and environment microbiomes can be seen with the high number of publications on this topic, with countless studies considering different plant compartments from the soil to plant continuum, research indicating modifications in the microbiome influenced by environmental factors and its potential benefits for agriculture. Conversely, sustainable technologies have been gathering traction in global agriculture and are considered the most devoted to conserving plant microbiomes. In this context, various research is being carried out to understand the influence of sustainable practices on the structure of the plant's microbial population.

Healthy global ecosystems benefit from the contributions of microbiomes in many different ways. Microorganisms in the water for example aid in storing carbon and they create half of the oxygen we breathe. In the soil, certain bacteria help plant development by fixing nutrients and digesting organic materials. Furthermore, microbes can contribute to energy generation by creating biogas and they are also utilized in the treatment of wastewater and the restoration of contaminated locations. Extremophiles and bioremediation microbiomes, as well as environmental microbiomes (such as mangroves, marine sponges, and corals), are critical to the global ecosystem's functionality, services, and existence. All of the significant global geochemical cycles, for instance, the crucial biogeocycles, would collapse if certain microbiomes, which are responsible for keystone phases within the cycle, were disrupted in such a manner that they fail to serve their evolved functions. Using a microbiome-led approach will help us identify where we need to concentrate our efforts to guarantee that these environmental cycles continue to uphold an ecosystem suitable for life.

Yet the practical translational applications of this fascinating and enthralling area of science are outstanding. The book also discusses that research on microbiomes provides a more comprehensive view of their interactions in agriculture and the environment. For the beginner and microbiome enthusiasts, this book may be an essential reading of its importance with existing applications in Agriculture, Environment, and Climate changes. With these aims in mind, the material of this textbook has been structured from basic to more advanced topics in a sequential progression. Finally, this book also reviews advancements from fundamental research to relationships between plant and agriculture microbiomes and the environment.

We hope that your creativity is inspired by this book and wish you luck in your experiments. This book illustrates astonishingly the urgency with which the numerous scientific brains are committed to the welfare of the scientific world. I am immensely grateful to the contributors for consistently paying attention to my request and expressing confidence in my skills. I will still be forever highly obliged to all the contributors. The worthlessness of their efforts cannot be explained by these terms.

Because of the heartfelt interest and painstaking effort of many other well-wishers whose names are not listed, but are already in our hearts, we have effectively

compiled our innovative and reflective research work. So, the reward for their sacrifices is worth it. I want to dedicate this book to my mum, S. Jayaprada (late). From the bottom of our souls, I and the contributing authors hope this book will be a good guide and guidance for scientific studies to understand the host-microbiome relationships in agriculture and the environment.

Machilipatnam, Andhra Pradesh, India Pallaval Veera Bramhachari

Acknowledgments

My sincere thanks are extended to all the academicians and scientists who have contributed chapters and happily agreed to share their work on *Understanding the Microbiome Interactions in Agriculture and the Environment* in this volume.

This book is a stunning reflection of the seriousness with which several scientific minds are dedicated to the research community. I am extremely thankful to the contributors for paying continuous attention to my requests and bestowing faith and confidence in my competencies and capabilities. I shall always remain highly grateful to all contributors forever. These words cannot justify the worthiness of their quintessential efforts. We appreciate the excellent work of the authors and coauthors who were invited to contribute chapters to this book. The credit for making this book a reality goes to them. The editor and the review team of the book especially appreciate sharing expertise with the contributors. Each chapter is pretty informative and written as stand-alone contributions from several research institutes so that the reader can begin reading anywhere in the book depending upon his/her interests and needs.

At the same time, I also express my deepest gratitude to my family members, especially my wife (Ramadevi Ramaswamy) and my kids (Ruthvik and Jayati) for their kind support which has prompted me to complete this assignment on time. I am also thankful to Krishna University administrative officials, and my colleagues in the Department of Biotechnology, Krishna University, for their intellectual support. I am equally thankful to the Springer Nature Publishing Group for their full cooperation during the peer review and production of the volume.

I am thankful to my beloved teachers and mentors for their constant support and motivation at all stages of my progress.

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About the Editor

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He was awarded a Travel Scholarship from QIMR-2007, Australia, for attending the 4th Indo-Australian Biotechnology Conference in Brisbane, Australia, and was awarded with a young scientist travel fellowship (2007) from the DST, Govt. of India, for attending XVII Lancefield International Symposium at Porto Heli, Greece-2008. He was conferred with various prestigious awards, notably Science Education Research Board (SERB), Government of India-Young Scientist award (2011) with a research project and was nominated as Associate Fellow of Andhra Pradesh Academy of Sciences (APAS)-2016, MASTER TRAINER: Andhra Pradesh English Communications Skills Project. British Council & APSCHE 2017, Andhra Pradesh State Best Scientist award-2017, and Dr. V. Ramalingaswamy Memorial award for Biomedical Sciences-2019. He also obtained two Indian patents in 2017. He has more than 13 years of teaching and research experience at the university level.

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Abbreviations

ACC	1-aminocyclopropane-1-carboxylic acid
AHLs	*N*-acyl homoserine lactones
AI-1	Autoinducer-1
alkB	Alkane monooxygenase
AlmA	Flavin-binding monooxygenase
AMGs	Auxiliary metabolic genes
amoA	Ammonia monooxygenase subunit A
AmpC-βL	AmpC-β-lactamase genes
AMR	Antimicrobial resistance
APX	Ascorbate peroxidase
ARGs	AMR genes
ASD	Autism spectrum disorder
BMC	Beneficial Microorganism of Corals
BSC	Biological soil crusts
CA	Citric acid
CAESAR	Central Asian and Eastern European Surveillance of AMR
CAT	Catalase
CAZyme	Cellulosome enzymes and their domains
CBM	Carbohydrate-binding modules
CBM10	Cellulosome-bound genes
CCR	Cinnamoyl-CoA reductase
CD-F	Electrospun cyclodextrin fiber
CDPK	Calcium-dependent protein kinases
CE	Carboxylesterases
CMV	Cucumber mosaic virus
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats
Cytochrome P_{450}	Cytochrome P_{450}, monooxygenase
DAPG	2,4-diacetylphloroglucinol
DAPG	4-diacetylphloroglucinol
DDR	The Distance Decay Relationship
DDS	Direct disease suppression
DNASIP	DNA stable isotope probing
DOC	Dissolved Organic Carbon

DSF	Lipid-based diffusible signal factors
EARS-Net	European AMR Surveillance Network
EBM	Ecosystem-based management
ECO-SENS	European Centre for Disease Prevention and Control
EPS	Exopolysaccharide
ESβL	Extended spectrum β-lactamase genes
ET	Ethylene signaling pathway
FDD	Fungal dockerin domain
GH	Glycoside hydrolase genes
GHG	Greenhouse gas emissions
GISAID	Global Initiative on Sharing All Influenza Data
GWAS	Genome-wide association studies
HAE	Human-assisted evolution
HCN	Hydrogen cyanide
HGT	Horizontal gene transfer
IAA	Indole-3-acetic acid
ICTV	International Committee on the Taxonomy of the Virus
IMMs	Interpolated Markov models
INPs	Ice nucleation proteins
IPCC	Intergovernmental Panel on Climate Change
ISR	Induced systemic resistance
ITS	The internal transcribed spacer region
JA	Jasmonic acid
LadA	Monooxygenase long-chain alkane
LO	Lipoxygenase
LPS	Lipopolysaccharide
LRR-RLK	Leucine-rich repeat receptor-like kinase
MAMPs	Microbe-associated molecular patterns
MAPK	Mitogen-activated protein kinases
MDR	Multidrug resistant
MHC	Major histocompatibility complex
MRSA	Methicillin-resistant *Staphylococcus aureus*
MTI	MAMP-triggered immunity
MYB72	Root-specific transcription factor
NCDDs	Noncatalytic dockerin domains
nCoV-2019	Novel coronavirus-2019
NGPs	Next-generation proteomics
NGS	Next generation sequencing
NOx	Nitrous oxide
OA	Oxalic acid
OTU	Operational Taxonomic Unit
PAH	Polycyclic aromatic hydrocarbon
PAHs	Polycyclic aromatic hydrocarbons
PAL	Phenylalanine ammonia-lyase
PCBs	Polychlorinated biphenyls

PGP	Plant growth promoter
PGPR	Plant growth-promoting rhizobacteria
PHZ	Phenazines
PL	Polysaccharide lyases
PLT	Pyoluteorin
POD	Peroxidase
POPs	Persistent organic pollutants
PPO	Polyphenol oxidase
PR	Pathogenesis-related genes
PRRs	Pattern recognition receptors
PSB	Phosphate-dissolving bacteria
PSI	Plant-soil interface
QS	Quorum sensing
QTL	Quantitative trait loci mapping
RA	Rosmarinic acid
RLKs	Receptor-like kinases
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
SLH	S-layer homology
SOD	Superoxide dismutase
SOS system	DNA-repair systems
SRA	NCBI Sequence Read Archive
SST	Sea surface temperature
SynComs	Synthetic communities
VLP	Virus-like particles
VOCs	Volatile organic compounds
VRE	Vancomycin-resistant Enterococci
WGS	Whole-genome shotgun sequencing
WHO	World Health Organization
WWTPs	Wastewater treatment plants

Unravelling the Microbiome Interactions in the Environment and Agriculture in the Era of Metagenomics

1

Pallaval Veera Bramhachari

Abstract

Global food security is threatened by serious agricultural difficulties. To enhance agricultural practices and the environment, microbiomes have the potential to deliver sustainable and economically advantageous solutions. To learn more about the dynamics of microbial populations and their interactions with plants and the environment, we also give information on basic microbiome research. It is now possible to link genotypes and phenotypes in complex interactions involving plants, microbes, and the environment. Additionally, we take into account the genotype of the plant, interactions amongst microbial taxa, the impact of agricultural methods, and environmental conditions that can influence the development and enrichment of microorganisms that are advantageous to plant health and growth. Ultimately, we intend to illustrate how microbial communities may be integrated into agricultural and environmental clean-up systems of the current day in order to provide a more tailored and long-term utilization of scarce resources. This review attempts to discuss the advantages and variables that modify the composition of the plant and environment microbiomes. Ultimately, a paradigm that transfers this information towards biotechnological applications will indeed be highlighted.

Keywords

Agricultural practices · Environment · Microbiomes · Biotechnological applications

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1.1 Introduction

There is growing recognition that microbiomes are pervasive, hidden entities that can have a significant impact on plant and animal functioning, community assembly, biodiversity maintenance, and ecosystem function, health, and stability. Microbiomes can also effect (Cho and Blaser 2012). Because the microbiome's impacts on ecological and evolutionary processes are context-dependent, this will be a major issue in incorporating microbiome effects into ecological and evolutionary processes. A plant's "microbiome" consists of a symbiotic relationship with a variety of microorganisms. Multiple interactions between the plant's microbiome and its cells occur; certain microorganisms are helpful and aid plant growth, whereas diseases harm plants and diminish agricultural yields. Net zero carbon emission sustainable farming systems, food and energy security, and reducing the effects of climate change and land degradation may all be accomplished by utilizing the microbiomes.

The importance of microbiota in the health of all organisms and ecosystems has led to a great deal of research into the issue. Data from the microbiome must still be analysed and converted into biologically relevant findings because of the difficulty. Microbiome research can benefit from the use of network-based analytical tools, which can assist detangle of the intricate polymicrobial interactions between microbes and their hosts, as well as the microbes' interactions with the human body. A microbiological community is a group of organisms that interact with one other in a contiguous habitat, such as an ecosystem (Konopka 2009).

There has been a great upsurge in microbiome research in the last several years, spurred by technological breakthroughs and huge decreases in the cost of testing. As a result of this research, scientists have gained a plethora of information on how the microbial communities interact with each other as well as how they affect the environment around them. As more is learned about the microbiome and how it interacts with its host, as well as with other microbes, it becomes possible to develop new techniques and strategies that could be applied across a wide range of disciplines, from ecology to agriculture to medicine and from forensic science to exobiology. Microbiome research has changed our understanding of the dynamics and organization of microbial communities during the last 10 years or more. We're only just beginning to grasp the intricate webs of interdependence that these societies weave with one another and with the natural world, both biotic and abiotic. Our knowledge of the processes occurring inside microbiomes and their interactions with hosts has improved because of advances in experimental methodologies and new technologies (Goodrich et al. 2017). In this review, we explore emerging tools and methodologies in microbiome research to provide an overview of the present status of the agriculture and environment fields. To learn more about the dynamics of microbial populations and their interactions with plants and the environment, we also present information on basic microbiome research. There are several potential uses for microbial communities in modern agricultural and environment systems that might be explored in order to make the most efficient and long-term use of scarce resources possible.

1.2 From Functional Predictions to the Phenotype

Since the high-throughput study of many genomes and metagenomes provides effective tools for tackling the functional potential of individual microbes as well as communities in their natural habitat, these new possibilities have changed microbial ecology (Knight et al. 2018). Methods for researching microbiomes, or multiomics, include high-throughput isolation (culturomics), microscopy, metabarcoding of functional genes, and metagenomics, which focuses on microbial activity and metabolism (metatranscriptomics, metaproteomics, metabolomics). In recent years, the first metagenome-assembled genomes were rebuilt from environmental materials (Anantharaman et al. 2016); however, millions of bacterial genomes were thrown away without growing the organisms behind. For instance, a recent study rebuilt 154,723 microbial genomes from 9428 metagenomes of the worldwide human microbiome (Pasolli et al. 2019). In the last several years, there has been a tremendous amount of study on the microbiome and its involvement in agriculture and the environment. The importance of environmental stimuli on microbiome composition and function, and its development through time, has been shown by studies of environmental-microbe interactions, such as those that investigate microbiome diversity within communities. Many different domains, including ecology, environmental sciences and engineering, biotechnology, and computational sciences, might benefit from research into the microbiome, which can lead to the development of new tools and methodologies that could ultimately change current paradigms. Microbiome research has the potential to tackle some of humanity's greatest global concerns, such as climate change, in addition to advancing research in specific sectors. As a result of such developments, the area of microbiome study will be able to continue to thrive for future decades. As a result, bioengineers and inventors will have the tools they need to build new and better applications, unlocking the maximum potential of the microbiome.

Many of the advances now under development will be hampered by the ineffectiveness of various microbiome technologies non-real-world contexts. It is possible to apply plant microbiome technologies in conjunction with smart agriculture, synthetic biology, satellite, big data, and genomic approaches to reach their full potential in the agricultural and environmental sectors. One day, modern agriculture may incorporate the use of microbiome therapies to enhance plant productivity while simultaneously minimizing the environmental effect once these challenges are solved. If the main (core and hub) microbiota of a crop species protecting or promoting immunity against pathogens can be identified, it is possible to construct an effective intervention (e.g. microbial cocktails, probiotics, microbial transplant) to mitigate infection rates and thus enhance farm production. Plant health and performance can be improved by adjusting microbiome therapy in context-specific circumstances after this concept has been widely accepted.

1.3 Microbiome Innovation in Agriculture

By 2050, global food consumption is expected to rise by 70%, and farmers will have to contend with a changing climate, reduced soil nutrients, polluted soils, and scarcity of water (Singh and Trivedi 2017). Plant microbiomes, for example, are the most sustainable option in this situation. Microbiome research has progressed, but more has to be done to expand molecular techniques, such as the collection of DNA, extraction and amplification, the decrease in sequencing costs, and the advancement of bioinformatics, among other things. Traditional methodologies must also be supplemented with these aspects in order to gain a deeper understanding of plant microbiomes. These microorganisms can be used to create novel-mixed bioinoculants that can boost the plant's functionalities. We still need to learn more about the structure of plant microbiomes, the advantages they provide to plants, and the interactions between bioinoculants and the resident microbiota.

1.4 Microbiomes for Future Farming

Microbiomes, which are collections of many different types of microbes, have a mutually beneficial connection with plants, providing them with food and a plentiful supply of life. Plants gain greatly from these natural relationships and interactions, minimizing the need for external chemical inputs. Because of this, the soil is naturally fertilized as a result of these organisms. This robust and self-sustaining ecosystem is the result of a close and dynamic interplay between microbiomes, plants, and the surrounding environment. Non-crop plants and experimental species in controlled environments have provided most of our information concerning microbiomes and their role in protecting the environment and promoting plant development. A growing number of stakeholders are interested in working together to develop profitable bio-based solutions that will lead the way for ecologically friendly crop production paradigms, thanks to the growing interest in microbiomes. A thorough knowledge of crop-specific microbiomes is required to meet this objective. The first step towards reaching this objective is to catalogue crop-specific microbial genomes, an essential research priority. Transforming genetic information into solutions and sustainable farming management techniques would necessitate integrative and cross-disciplinary research efforts (Bandla et al. 2020).

Pesticide resistance and environmental and health concerns are spurring interest in new techniques of controlling insect pests, but there is also a rising demand for less agricultural chemical use. For decades, the function of microbes in pest management has been primarily restricted to employing entomopathogens, with just a few of microbial species being turned into natural pesticides. New technologies like high-throughput sequencing and functional omics, as well as gene editing and gene editing techniques like CRISPR/cas9, are changing the way we think about the role of microbes in complex ecosystems and how they interact with one other. Many insect features are shaped by the presence of beneficial bacteria, according to overwhelming evidence. The word "microbiome" refers to a grouping of bacteria

(both biotic and abiotic) and their genetic material. Research into the microbiomes of insects, plants, and other natural resources might help in insect pest control. It's an exciting time to learn about new bacterial or microbiome capabilities that might be put to use in the fight against insect pests. Bioactive substances may be extracted from uncultured bacteria using cutting-edge gene editing and microbial engineering techniques, as well as nanotechnology. These methods will be extremely beneficial for agricultural advancements. Despite this, research on the microbiome in less agrochemically intensive production methods including conventional farming and protected cultivation is sparse, but it has the potential to contribute significantly to the development of more agriculture practices.

Furthermore, it is impossible to articulate the many chemicals involved in communication networks between plants and microorganisms using present technologies because of the huge range of molecules involved (Singh and Trivedi 2017; Nesme et al. 2016). Molecular methods like as metatranscriptomics and metabolomics, as well as improved sensitivity of current instruments such as spectroscopies, are thus required for the identification of microbiome signalling molecules (Singh and Trivedi 2017). The isolation of microorganisms contributes to the consolidation of knowledge gained from current procedures in unique biotechnological products, such as bio-inoculants. Despite this, molecular tools are more commonly utilized in microbiome investigations (Singh and Trivedi 2017). Multidisciplinary techniques that combine cutting-edge technology with conventional methods are essential for developing plant microbiome research. As an example, the microbiota that occupies the plant's many niches may be identified using these ways.

1.5 Impact of Microbiomes on Climate-Smart Agricultural Practices

All ecological functions solely rely on microbes for their survival. Research on the use of beneficial microbes, such as plant growth-promoting fungi, endophytic microbes, to improve agricultural yields, as well as the role of climate and soil microbiomes in promoting innovative sustainable agricultural alternatives, are the focus of the latest biotechnological interventions. Climate change has a direct impact on the agricultural ecosystem, affecting both the quantity and quality of agricultural products. Microbes' physiology can be negatively or positively affected by changes in biomass, diversity, and composition due to changes in the microbial metabolism. Increasingly, understanding the impact on native microbiomes, particularly the distribution of methanogens and methanotrophs, nutritional content, and microbial biomass, is necessary to build resilience against climate change. Because of this, soil microbes play an imperative role in a variety of biogeochemical cycles and agroecosystem resilience functions, such as decreasing greenhouse gas (GHG) emissions and preventing organic matter degradation. Recent studies have demonstrated that agriculture and accompanying land-use change continue to be a significant source of biogenic GHGs, such as carbon dioxide (CO_2), methane, and

nitrous oxide (NOx). It was discussed how microorganisms can impact crop output, soil carbon sequestration, GHG reduction, and adaptation to climate change in climate-smart agricultural management techniques (Ajala et al. 2022).

1.6 Mitigation of Biotic and Abiotic Challenges by Microbiomes

The ability of microbes to mitigate a variety of biotic and abiotic challenges, as well as to acquire nutrients, may be enormous. This might be a low-cost, high-efficiency, and long-term input in an agro-production ecosystem's process. Agroecosystems are being overrun by synthetic inputs created in factories, resulting in falling productivity and returns for agricultural factors. As a result, the role of microorganisms in various production systems and ecologies must be investigated in order to make it a crucial input in long-term production. New production ecologies including conservation agriculture-based production systems protected cultivation lack knowledge on microorganisms. High crop performance in these conditions, on the other hand, is clearly attributable to a distinct microbial community (Lau et al. 2022). According to these ecologies' claims, their superior yields and product quality may be attributable to bacteria or consortia that have evolved in these environments. These organisms should be studied and used as agricultural inputs. The increased proportionate impact of helpful microorganisms on plant performance in more stressful settings can be used to quantify the amount of microbial stress amelioration. These ecologies are also employed to determine microbiomes or consortia that play a key role in coping with stress, such as cold and dry deserts and salinity/alkalinity/acid-prone places. Resources are required for the isolation, purification, identification, and characterization of culturable microorganisms. The capacity of microorganisms to function in a variety of agricultural environments and be stored is also a constraint on their usage in agriculture. A key drawback of using such microbiomes on a big scale is their application approach (Porter et al. 2020).

1.7 Utilization of Beneficial Microbiome to Boost Agriculture Productivity

There has been an increase in the study of beneficial bacteria as a means of enhancing plant development and alleviating stress, thanks to increased understanding of the microbiome's role in these processes. Microbiome utilization in agriculture begins with an investigation of the many "spheres" of a healthy crop-growing environment, such as soil and water. In the pre-genomics period, microbial variety was explored by isolating microorganisms in different culture mediums and examining their phenotypic diversity. Metagenomics, a culture-independent sequencing technology that can identify all the microorganisms in an environment, has become increasingly popular in recent years. Bacteria can be used in agriculture; however, cultivability of target microbes is still a determining factor. It is possible to sell

microbiota that have been economically mass produced in order to improve resource efficiency, promote plant growth, alleviate stress, or combat insect pests (Qadri et al. 2020).

1.8 The Environmental Microbiome in a Changing World

According to environmental microbiome studies, microbial communities play an important role in forming complex ecosystem processes that have an influence on human and environmental health. Microbes are being investigated in a variety of environments, including agricultural soils, biocrusts, coral reefs, sponges, and geological settings all around the world. Researchers are now looking at how environmental microbiomes respond to climate and ecological changes and how these alterations may be utilized to address present climate change challenges. Syntrophic interactions between bacteria, plants, and animals have long been studied, but researchers have lately begun to shed light on the various syntrophic interactions that take place in environmental systems including bacteriophages, protists, and other organisms. Microorganisms' seasonal, temporal, and geographic changes may now be compared, thanks to the advent of metagenomic research. Nevertheless, the flexibility, resilience, and development of microbial ecosystems can be better understood in other ecosystems.

As a result, phenotypic plasticity can be assessed much more easily by interpreting the microbiome communities of varied habitats, which may help provide significant information about the drivers and effects of stoichiometric trait distribution in agriculture and the environment. Nutrient-organism interactions are still largely a mystery, as is how microbiome composition affects ecosystems.

1.9 Ecology and the Environment-Microbiome Nexus

The function of microbiomes in soil, marine, and human habitats is becoming more and more apparent. It is now possible to screen and identify the microbial community in environmental samples using next-generation sequencing (NGS). Deciphering the microbiomes' genomes and comparing them to the genomes of other creatures in the environment helps us understand more about microbial diversity and evolutionary relationships. Few researchers have become increasingly interested in modelling the ambient microbiome for both pollution bioremediation and human health consequences. Intricate webs of interdependence connect all living organisms, much more of which has yet to be discovered.

The microbiomes may also tend to maintain a relationship between emerging diseases and climate change. In a new model of animal sickness, the microbiome is considered. According to their results, climate warming may lead to the emergence of new infectious diseases. Changes in the microbiomes of animals, such as those caused by climate change, might lead to the emergence of novel infectious diseases. Nonetheless, the microbiomes influence whether or not an animal is infected with a

virus when it is exposed to it. Bacterial interactions in the microbiome have a role in antimicrobial resistance in agriculture and the environment. Research here will undeniably focus on how microbe interact with each other in their environment and the evolution of antibiotic resistance, as well as the various methods in which antibiotic resistance can be passed from one strain or species to another. A healthy microbiome is essential in today's fast-paced world. However, the microbiome may be changed by changes in the environment. An organism's immune system is aided by the microbiome, which strives to maintain an even population of various bacterial species.

1.10 The Way Forward

Microbial communities with specific activities can be used to improve sustainable agricultural output by enhancing crop health, combating plant diseases, and minimizing the need of fertilizer. As a means of accomplishing this aim, it is necessary to have a better knowledge of how soil and environmental microbial communities evolve through time and how they respond to environmental changes, as well as the interactions between microbes and plants within those communities. In addition, because individual bacteria are critical to the stability and structure of microbial communities, more extensive studies employing these microorganisms and associated soil and environmental microbial communities would indeed be beneficial to the area of research. As a result of this research, we can better understand how these microorganisms affect agricultural yields and disease resistance, and we can also learn how to manipulate the microbiome.

As we learn more about metagenomics and the complexity of microbiomes, this book focuses on microbiome interactions (whether within themselves or with agricultural and environmental systems) and microbial ecosystems' resilience. Short communications that would provide significant insight into the various aspects of the agriculture and environmental microbiomes: metagenomic analysis of microbiomes of novel or extreme environments, experimental research on microbial resilience or temporal fluctuations, studies on symbiosis and coevolution of the microbial community, novel microbial interactions, and the recycling of nutrients in agriculture and environmental microbiomes. Microbiome interactions in agriculture and the environment are the primary focus of this book. An ecosystem's nutrient cycle depends heavily on the microbiome of the soil, air, and water. Microbiomes in different ecosystems and their functional dynamics are covered in the following chapters, which give up-to-date information on current trends. Bioremediation, microbiomes in space, geomicrobiomes, coral microbiomes, antibiotic resistomes, and marine microbiomes are just a few of the many subjects covered in the book. Syntrophic relationships between bacteria, protists, plants, and some animals in agricultural and environmental systems are also examined in the book proposal.

This book also offers a unique perspective on how microbial ecosystems adapt, recover, and evolve. Essential subjects linked to metagenomic microbiome study of novel or severe settings, investigations on microbial resilience or temporal

variations, symbiosis and coevolution of the microbiome, and novel microbial interactions in agriculture and the environment were addressed in the chapter. Plant-agriculture microbiomes and their contribution to the sustainability of agriculture, microbiota populating the phyllosphere, endosphere, rhizosphere, and their usage might be a sustainable crop production strategy is also discussed in the book. Finally, the book reveals the enormous potential of plant and environmental microbiome structural and functional diversity through a thorough but representative description.

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Antimicrobial Resistance in Environmental Microbiome: An Overview

2

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Abstract

Antimicrobial resistance (AMR) is a great concern worldwide distressing the health of humans and animals directly or indirectly, which is truly problematic for the clinicians in disease control. This chapter highlighted the manifestation and spread of AMR and AMR genes (ARGs) in the environment that resulted from the human intrusions. Natural environments were less reported for the outbreak of pathogenic microbes, but with the human interventions such as the effluents of hospital wastes, human and animal wastes, etc. converted them into hotspots for antimicrobial genes by providing suitable medium for the exchange of ARGs among the organisms. Moreover, they can also serve as vehicle for the transfer of pathogenic microbes between human and animals that resulted in a wider epidemiological issue. Therefore, proper surveillance of microbiological risks should be there to maintain a healthy microbiome. It is a huge task to functionally characterize environmental microbiomes by conventional isolation method, with advances in high-throughput sequencing and computational biology today permit researchers the exploration of even un-culturable microbes by using metagenomics approach, which have been used effectively not only in determining the diversity of microbes but also in the characterization of pathogenic and antibiotic resistance microorganisms that can be a great help in this regard.

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Environmental microbiome · AR · ARGs

2.1 Introduction

Antimicrobial agents have been successfully used for the past many years to treat and reduce infectious microbial diseases. But in time, overuse and misuse of antibiotic in the past few decades has tremendously increased the number of resistant bacteria which accounts for thousands of human deaths every year (Jørgensen et al. 2017). It has been well reported that different microorganisms including bacteria, parasites, viruses, and fungus are well capable of developing resistance which infers that the antimicrobial agents have become less effective to that particular microorganisms (Alexander et al. 2013; Mediavilla et al. 2016).

Antimicrobial resistance (AMR) is when an antimicrobial agent becomes less effective to certain microbial pathogens by losing the ability to inhibit or kill the pathogens which were previously susceptible; this leads to the persistence of the particular disease in the body. This also increases the risk of spreading to others. Infections caused by AMR bacteria may lead to morality, prolong the hospital stay, etc. (de Kraker et al. 2011). AMR is a global concern as it is known as the major problem to human and animal health with significant impact on the economy (O'Neill 2017).

The occurrence of AMR is mainly due to the overuse and misuse of the antimicrobial agents for disease control, treatment, and prevention. Antimicrobials used for growth regulators in animals and the misuse of prescribed human medicine have highly contributed to the AMR (Brinkac et al. 2017). As the usages of antimicrobials have increased, the complexities on which the bacterial pathogens exhibit the resistance mechanisms have also increased. Scientists are struggling to have control against infections, but the development of new antimicrobial agent is not manageable to cope up with the rate of increasing resistance since microorganisms evolve to have better resistance mechanism (Krause 1992).

The genes encoded for their AMR are capable of moving to other microbes through vertical and horizontal gene transfer and further incorporated into the normal microbiota of human beings, animals, and environment which includes food, sewage, soil, and water. This underlying forces and development of AMR depend on the communication linkages connecting all these ecological, biological, and genetic entities (Baquero et al. 2019).

The transmission of AMR to human is well documented, and the natural environment serves as a major passage by which transmission has occur (Davies and Davies 2010). The level at which this transmission may occur remains uncertain. It is comparatively important to understand the role of environment for the transmission of antimicrobial bacteria to humans rather than the transmission through animal carriers, food, or the flow of AMR in healthcare and community settings (Huijbers et al. 2015).

AMR bacteria are most commonly found in infirmary settings which possibly reached to different environments such as wastewater treatment plants (WWTPs) via hospital wastes and the wastewater associated with this environment (Hocquet et al. 2016). The further route for these AMR bacteria is unknown that no trace in WWTPs (Flach et al. 2018) while a number of reports have been found on AMR bacteria conceivably of hospital origin which survive the treatment process and thereby are released into recipient waters (Rizzo et al. 2013).

There are certain monitoring surveillance program setup by different networks to monitor AMR bacteria which has increased the knowledge of dissemination of resistant bacteria, for example, the European AMR Surveillance Network (EARS-Net) (European Centre for Disease Prevention and Control 2017), ECO-SENS (Kahlmeter and Poulsen 2012), and Central Asian and Eastern European Surveillance of AMR (CAESAR) (World Health Organization 2015). This chapter highlighted an overview on the manifestation and spread of AMR and ARGs in the environment that resulted from the human interventions.

2.2 Antimicrobial

Antimicrobials remain the most significant pharmaceutical products in the management of bacterial infections, both humans and animals globally (WHO); this has become a global concern with the evolution of new pathogens. Moreover, it has played a key role in the development of sustainable livestock production by giving them healthy life besides serving as food preservatives and growth regulators; they also help in the control and management of the possible risks associated with infectious diseases that are zoonotic.

2.3 Antimicrobial Resistance (AMR)

The World Health Organization defined AMR as the resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it. The overuse and misuse of antimicrobials has caused the occurrence of resistant microorganisms and therefore gave birth to the term AMR (Davies and Davies 2010). Microorganisms are extremely adaptive organisms even under unfavourable conditions; they can undergo mutations and are able to survive in several environmental stresses. Therefore, the rise in AMR is not a surprise rather it was much predictable with the invention of the first antimicrobials. The first antimicrobial-resistant strain *Staphylococcus aureus* was isolated from a patient in British hospital in the year 1948 which was found to be resistant against Penicillin (Barber and Rozwadowska-Dowzenko 1948). Later in the same year, *Mycobacterium tuberculosis* was observed to be resistant against streptomycin (Crofton and Mitchison 1948). After that in 1950s, a bunch of pathogenic bacteria such as *Escherichia coli*, *Shigella* spp., and *Salmonella enteric* showed AMR (Watanabe 1963; Olarte 1983; Cantas et al. 2013). Later on VRE (vancomycin-resistant

Enterococci), MRSA (methicillin-resistant *Staphylococcus aureus*) were found in the 1960s which leads to the idea of multidrug-resistant bacteria where the microbes are resistant to at least three antimicrobials (Marshall and Levy 2011).

2.4 Origin of AMR in the Environment

Different sources where the AMR is believed to be originated such as hospitals, waste water, animal farm, and agriculture (Fig. 2.1) have been reviewed and highlighted as described below. *Enterococci* was found to be the most dominant AMR organisms in the selected site followed by *S. aureus* and *E. coli.* Vancomycin resistance was found to be the most dominant antimicrobials in the selected study sites (Table 2.1).

2.4.1 Hospitals

Hospital-acquired infection which is also termed as nosocomial infections are of a serious concern with regard to AMR (Monnet et al. 1998). The normal bacteria usually commensal that becomes pathogenic when they multiply in normal sterile sites, such as the lower respiratory tract or the blood, are usually the type of bacteria that are responsible for nosocomial infection (Bonten and Weinstein 1996). For limiting nosocomial infection especially to reduce antibiotic-resistant bacteria, several measures have been taken up such as maintaining proper sanitation like frequently washing hands and barrier precautions within the hospital (Slaughter et al. 1996). The main transmission from one hospital to another hospital takes place when one hospital refers patients for various reasons to another hospital; in this way, the patient may transfer hospital-acquired pathogens between healthcare institutions.

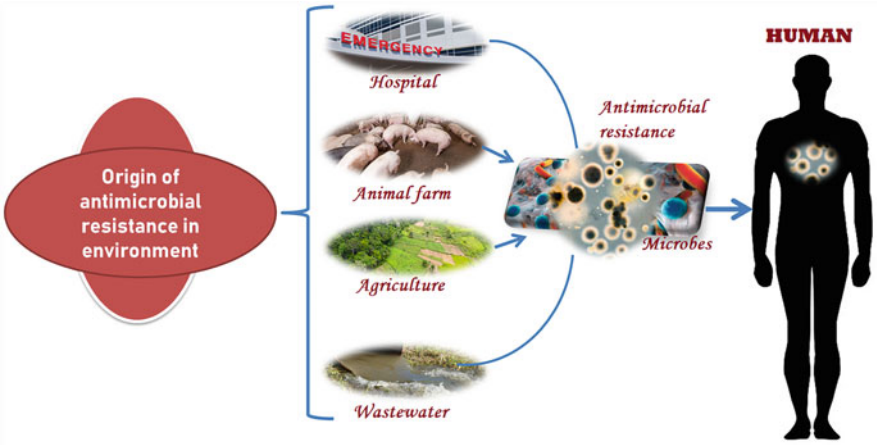

Fig. 2.1 Origin of AMR and their gene flow

Table 2.1 Selected common resistance microorganisms with antimicrobials and their origin

S. no	Origin of antimicrobial resistance	Microorganisms	Antimicrobials	References
1.	Animal farm (pig farm)	*E. coli*	Cephalosporin	Agersø and Aarestrup (2013)
2.	Animal farm (pig farm)	*E. faecium* and *E. faecalis*	Tylosin	Agersø and Aarestrup (2013)
3.	Animal farm (broiler farm)	*E. faecium*	Avilamycin	Aarestrup et al. (2001)
4.	Animal farm (chicken farm)	*E. coli* and *Salmonella enterica serovar Heidelberg*	Ceftiofur (a third-generation cephalosporin)	Dutil et al. (2014)
5.	Wastewater (hospital effluent)	*Enterococci*	Amoxicillin	Leclercq et al. (2013)
6.	Wastewater (hospital and community effluent)	*Enterococci*	Vancomycin	Caplin et al. (2008)
7.	Wastewater (sludge)	*Enterococci*	Vancomycin	Bates et al. (1994)
8.	Animal farm (poultry manure)	*Enterococci*	ESBL	Blaak et al. (2014)
9.	Soil (manure-amended)	*Staphylococcus aureus*	Methicillin	Huijbers et al. (2015)
10.	Soil (agricultural soil)	*Enterococci*	Vancomycin	Huijbers et al. (2015)
11.	Hospital (patient)	*Klebsiella pneumoniae*	Carbapenem	Zhao et al. (2019)
12.	Hospital (patient)	*Staphylococcus aureus*	Methicillin and vancomycin	Heinze et al. (2018)

Through the shared patients, different hospitals become connected (Donker et al. 2010) which eventually lead to the transmission of MDR (multidrug-resistant) pathogens like MRSA (methicillin-resistant *Staphylococcus aureus*), VRSA (vancomycin-resistant *Staphylococcus aureus*), etc. On the other hand, wrong or unnecessary prescription of antibiotics to patients by doctors is the major cause of AMR in hospitals. In 2010, India was the largest consumer of antibiotics when assessing total tonnage; however, their per capita usage (7.5 units per capita) was comparatively low as compared to Australia and New Zealand which recorded among the highest usage rates of 87 and 70 units per capita, respectively (Van Boeckel et al. 2014).

2.4.2 Wastewater

Wastewater coming from different sources could be a favourable habitat for resistant bacteria and resistance genes (Munir et al. 2011; Reinthaler et al. 2013). Resistant bacteria may reach the wastewater treatment plants (WWTPs) from hospital water as discussed above, since resistant bacteria are abundantly present in hospitals (Hocquet et al. 2016). Some studies have suggested the correlation between resistances rates among bacteria present in wastewater with corresponding to human population in that particular area. Therefore, basic research on the resistance rate of the indicator bacteria such as *E. coli* in wastewater is an important tool to observe the changes in the resistance pattern of the normal human intestinal microbiota (Blanch et al. 2006). For instance, *Enterococcus faecium*, i.e. a Swedish clone that carries ampicillin and fluoroquinolone resistance, could be traced from its hospital origin (Torell et al. 2003) to wastewater coming out from hospital (Iversen et al. 2002). The clone was further found in untreated water and the samples were still further found in receiving waters (Iversen et al. 2002), which is likely a source for hospital origin, antibiotic resistance bacteria colonization in human. In addition to this, a high bacterial population are found in biofilms from wastewater system especially from activated sludge of sewage treatment plants. Biofilms are also generated in surface water and drinking water distribution systems (Schwartz et al. 2003).

2.4.3 Animal Farm

Animal husbandry has extremely increased over the past five decades worldwide. According to FAO, 2018 global meat production has almost increased in fourfold, from 84 million tons in 1965 to about 335 million tons in 2018, and it is assumed that this is likely to be continued. Antimicrobials are used extensively in farm animals for the treatment of certain diseases as well as for growth regulators. Therefore, animal farms are zoonotic pathogen reservoirs, as well as sources of veterinary antimicrobials and ARGs. This kind of case happens in countries that produce antimicrobials in large amount without any essential regulation (Wellington et al. 2013). Stokstad and Jukes were the first to report the used of antimicrobials in farm animals after noticing a small doses of penicillin and tetracycline could enhance growth (Stokstad and Jukes 1950). After that, the use of antimicrobial agent in farm animals has increased. For instance, in China, antimicrobials have been used as low-dose feed additives for livestock and poultry since the mid-1970s; since then, China is currently the leading country in the production and consumption of antimicrobials for animals worldwide (Zhu et al. 2013). In many underdeveloped and developing countries, where the resources do not meet the requirement of the people, the use of antimicrobials is increasing rapidly due to the high demand for animal protein, shifting animal husbandry into large-scale industry. For example, BRICS countries were estimated to consume 99% increase of antimicrobials from 2010 to 2030 (Van Boeckel et al. 2019). In addition to this, the manure produced by

these farm animals was found to constitute a great number of antimicrobial-resistant bacteria, where the genes associated with antibiotic resistance, ribosomal protection, and enzyme inactivation mechanisms were commonly detected in such manure and the soils where applied (Cadena et al. 2018).

2.4.4 Agriculture

The extensive use of antimicrobials in animal farm has increased the number of AMR as well as antimicrobial genes inside the animal body. Reports have shown that manures collected from such farms to fertilize the soil of agricultural land has introduced novel ARGs to the soil as well as enriched the naturally present ARGs (Yu et al. 2017). Even though, samples collected and analysed from an isolated soil have suggested that ARGs also occurs naturally in the soil (Miteva et al. 2004; Bhullar et al. 2012). But ARGs are more abundant as compared to the isolated native soil, which suggested the enrichment of agricultural soil with ARGs with the application of manures (Davies and Davies 2010). From agricultural land, it will be eventually transported through the waterways and will contaminate the water quality.

2.5 Resistance Transmission

The flow of AMR and the genes associated have been observed from microorganisms to microorganisms, animal to human, and environment to human.

2.5.1 Microorganisms to Microorganisms

Occurrence of one mutation to cause resistance on microorganism is well known. In addition to that, the new mutated genetic material can be exchanged between one microorganism to another which in turn may lead to the host cell and its progeny to have new AMR genes, following different mechanisms mostly through plasmid transmission (Walsh et al. 2011; Unemo et al. 2012). The emergence of AMR is influence by antimicrobials using a selective pressure, also by inducing transfer of resistance determinants between microbes (Beaber et al. 2004).

2.5.2 Human to Human

Transmission of resistance microbes may occur between human to human contact, and this type of transmission is one of the most common ways of transmission. In the community, faecal–oral transmission is the most common route of transmission which is often due to poor sanitation. This type of transmission plays an important part especially in the transmission of resistant Enterobacteriaceae (Wellington et al.

2013). In addition, sexual encounters may also lead to the transmission of resistant bacteria, for instance, *Neisseria gonorrhoeae*, which leads to a widespread distribution of resistant clones (Lewis 2013). Hospital or healthcare-associated infections also play vital role in the transmission, if proper sanitation is neglected. For example, healthcare workers' hand is an important mode of transmission of resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) if proper sanitation is not maintained (Chamchod and Ruan 2012).

2.5.3 Animals to Human

The transmission of antimicrobial-resistant microbes from animals to human beings, which is due to the use of antimicrobial growth promoters in farm animals, was first recognised in the 1960s (Anderson and Lewis 1965). Bacteria and mobile genetic elements consulting resistance may remain on animal skin and in faeces, and by any means, the mobile genetic elements may be transferred to bacteria, and eventually the bacteria will however direct to humans (Kruse and Sørum 1994). This intertwining of animal and human microbial population includes both commensals and opportunistic pathogens, which may include *E. coli*, *Enterococci*, and *Staphylococcus aureus*. There are certain evidences to support the transmission of resistant bacteria from animals to human beings. For instance, ESβL and AmpC-β-lactamase genes on plasmids and of *E. coli* possibly through food chain clones have been reported (Kluytmans et al. 2013).

2.5.4 Environment to Human

Environment such as soil, water, drainage system, etc. may also transmit resistant bacteria to human beings. The contribution of the environment to AMR transmission is a global concern. This existed to be confirmed by the isolation of AMR microbes in several sewage systems (Kristiansson et al. 2011). These opportunistic AMR pathogens have enormous chance of transmission to human beings. These indirect transmissions are not well studied and are a great area of research interests with the advancement of high-throughput metagenomic approaches.

2.6 Mechanism for the Development of AMR

AMR could be either innate or acquired in microorganisms. In some species of bacteria, resistance to any one class of antimicrobial agents are innate. In such cases, all the strains of that particular bacterial species are resistant to all the members of that antibacterial class. Since innate resistance is a naturally occurring process, it is of a lesser concern. The more serious concern is acquired resistance where microbes which were previously susceptible to a particular antimicrobial agent developed resistance, which means that antimicrobial agent has lesser effect on that microbe.

These resistant bacteria will proliferate and spread under the selective pressure of use of that agent. The mechanism includes the following: First step is the attainment of the genes encoding enzymes such as β-lactamases, capable of destroying antimicrobial agent before having an effect. Second step is the removal of antimicrobial agents out from the cell where the bacteria may acquire efflux pumps for forcing the antibacterial agent terminating its effect before reaching the site of action. Third step is the alteration of bacterial cell wall by acquiring several genes for a metabolic pathway modifying the binding site of that particular antimicrobial agent. There is also a possibility that bacteria may hamper the entry of antimicrobial agents inside the target cell via downregulation of porin genes by mutation. In this way, susceptible bacterial populations may attain resistance to antimicrobial agents through mutation and selection or by acquiring the resistant gene from other bacteria. Resistance to multiple classes of antimicrobial agents mainly occur due to the exchange of genetic information from one bacterium to other bacteria. Such kind of bacteria that shows resistant to at least three classes of antimicrobial agents are termed as MDR (multidrug-resistant) and have become the most critical issues and challenges, mostly in hospitals and other healthcare institutions where they tend to occur most commonly. Although a single mutation may not be enough to acquire resistance, it could reduce the susceptibility and may be the key to acquire additional mutations or additional genetic information resulting in full resistance to the antibacterial agent (McManus 1997).

2.7 Future Perspectives and Conclusions

The development of AMR is a great concern worldwide. This leads to search for alternative sources for drugs having potential to inhibit MDR pathogens. The major issue in the development of AMR is the misuse and overuse of antimicrobials. There is a need to understand the proper use of antimicrobials to avoid further development of AMR.

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Mechanistic Adaptation of Microbiomes in Extreme Environments

3

K. Viswadeepika and Pallaval Veera Bramhachari

Abstract

Extreme environments are referred to as ecosystems with a constant or fluctuating exposure to one or more environmental factors such as high and low temperatures, salinity, osmolarity, UV radiation, barometric pressure, and pH. Microbiomes inhabiting these ecosystems have vast and flexible metabolic diversity combined with extraordinary physiological abilities to colonize harsh environmental conditions. Extremophilic microbes offer a variety of adaptation strategies that include structural, physiological, and metabolic changes primarily in the cell membrane, DNA, RNA, protein, and enzymes. Adaptive strategies inevitably incorporate biological and geological processes such as pigment production, cell membrane changes, or movement into solid rock layers and geological modifications. In addition, the synthesis and accumulation of small molecules in the cytoplasm, surface modifications on proteins, for instance, acidification or increase in the stable amino acid content, molecular chaperones, polyphosphates, and mobile genetic elements also lead to better survival in hostile environments. Furthermore, characterisation of cell signalling systems in these populations, horizontal gene transfer, and transcriptomic and proteomic studies along with metabolomics may be especially useful in the analysis of the possibility of adaptations at group level. Owing to the enormous potential of commercial exploitation of extremophiles in biotechnology, understanding the processes underlying the adaptation of microbes to extreme environments from both evolutionary and ecological perspectives is of fundamental importance.

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Keywords

Extreme environments · Extremophilic microbes · Adaptive strategies · Transcriptomic and proteomic studies

3.1 Introduction

Extremophiles live in severe physical and geochemical environments that threaten life's physicochemical limitations such as high salinity, temperature, radiation, pH, desiccation, etc. This kind of harsh environmental conditions might be regarded as innate or induced factors which compel most living systems tough to survive and grow (Rothschild and Mancinelli 2001). Most of the genetic, bio, and physicochemical techniques that extremophilic microbes use are not fully explored so far. Apparently, nutritional requirements must be adapted to the availability at the particular extreme environment. Adaptation to physiological requirements may be complicated and diverse (Rampelotto 2013). Nevertheless, it was reported that some biological molecules and unusual biochemical strategies enable extremophiles to thrive, which garner great attention in the fields of biotechnology and other industrial processes.

Communication mechanisms (quorum sensing) used by these microorganisms in extreme environments are important for the survival of microorganisms (de Oliveira et al. 2015; Pérez-Rodríguez et al. 2015). Cell signalling controls many essential activities in microorganisms and can perform crucial tasks in managing diversity levels of microbes in extreme environments as well as ecological balance. Moreover, for synchronized expression of genes at elevated cell density, most bacteria rely on quorum sensing (QS) which is focused on the synthesis and recognition of autoinducer signalling molecules (Miller and Bassler 2001). Characterising cell signalling networks in these populations can present distinct ways to decipher the microbial communication associated with existence and functioning in intense climatic conditions. The most commonly reported signalling system is autoinducer-1 (AI-1) apart from thermophilic organisms since autoinducer-2 is present in them. Although peptide-based system was not common in this kind of microorganisms. Extremophiles use quorum sensing for processes such as cold adaptation, reduction of the freezing point and development of biofilms, tolerance to oxidative damage, and persistent cell development. Model organisms for all extremophile groups include *Leptospirillum ferriphilum* (acidophile) (Christel et al. 2018), *Sulfolobus solfataricus* (thermoacidophile) (Quehenberger et al. 2017), *Natronomonas pharaonis* (haloalkaliphile) (Falb et al. 2005), *Bacillus halodurans* (halophile) (Van-Thuoc et al. 2013), *Haloferax volcanii* DS2 (halophile) (Hartman et al. 2010), *Halobacterium* sp. NRC-1 (halo radiophile) (Berquist et al. 2007), *Deinococcus radiodurans* (radiophile) (Pavlopoulou et al. 2016), *Thermococcus barophilus* (piezophile) (Birien et al. 2018), *Halorubrum lacusprofundi* (psychrohalophile) (Liao et al. 2016), *Pseudoalteromonas haloplanktis* (psychrophile) (Parrilli et al. 2019), *Thermococcus kodakarensis*

(thermophile) (Atomi and Reeve 2019), *Thermus thermophilus* (thermophile) (Miyazaki and Tomariguchi 2019), and *Cronobacter sakazakii* SP291 (xerophile) (Srikumar et al. 2019).

Extremophiles are crucial not only for their exceptional ability to survive extreme conditions but also for their wide range of uses in the areas of industrial and pharmaceutical biotechnology. Microbial communities were acclimatized to remarkable stress intensities in extreme conditions. These modifications play significant role in the improvement of remediation methods for certain polluted sites of hazardous waste and acid mine drainage sites. Novel enzymes isolated from extremophiles are tailored to extremes of temperature and pH. Lastly, they help in unravelling the evolutionary record as well as promising effects of climate in near future. Dynamic metabolic processes present in extremophiles were well explained by environmental transcriptomic and proteomic studies. Nonetheless, metabolic compounds explicitly associated with the microbial physiology are not fully explored. Due to the wide range of experimental complexities related to environmental matrix, metabolomic techniques fall behind other advanced technologies. The present chapter comprehensively discusses the basic microbial adaptations of various extremophiles for their survival.

3.2 Psychrophiles

At low temperatures, enzymes will be inactive, whereas solute concentrations are elevated and become lethal (Cavicchioli 2006). In addition, ice crystals can slice the cell membranes once the water is frozen, thereby damaging cell integrity (D'Amico et al. 2006). Psychrophilic membranes (*Shewanella putrefaciens*) contain increased amounts of unsaturated fatty acids, fatty acids with cyclopropane, and short-chain fatty acids that increase further with temperature reduction to modulate membrane fluidity (Feller and Gerday 2003; D'Amico et al. 2006; Gao et al. 2019) (Fig. 3.1). Psychrophilic microbes synthesize enzymes that are cold adapted and possess increased specific activities at cold conditions (Feller and Gerday 2003). Cold-tolerant enzymes can assist transcription and translation at very low temperatures. In addition, antifreeze proteins were found in microbes adapted to cold environments (Gilbert et al. 2004). AFPs have two major functions, namely thermal hysteresis and ice recrystallisation inhibition activity (Kawahara 2008). Such proteins can attach to ice crystals with a broad corresponding surface and thus avoid ice crystals from slicing the cell membranes.

Microorganisms have developed several physiological adaptations to balance the harmful impacts of cold environment such as producing temperature-related chaperones and antifreeze molecules, such as ice nucleation proteins (INPs), that shield the RNA and protein synthesis (De Maayer et al. 2014; Godin-Roulling et al. 2015). INPs arrest the extreme cooling of water due to ice crystallisation (Kawahara 2002; Muñoz et al. 2017). Psychrophiles control membrane fluidity by increasing the amount of branched-chain or unsaturated fatty acids or by reducing the stretch of fatty-acyl chains or both. Molecular chaperones help in protein refolding and have an

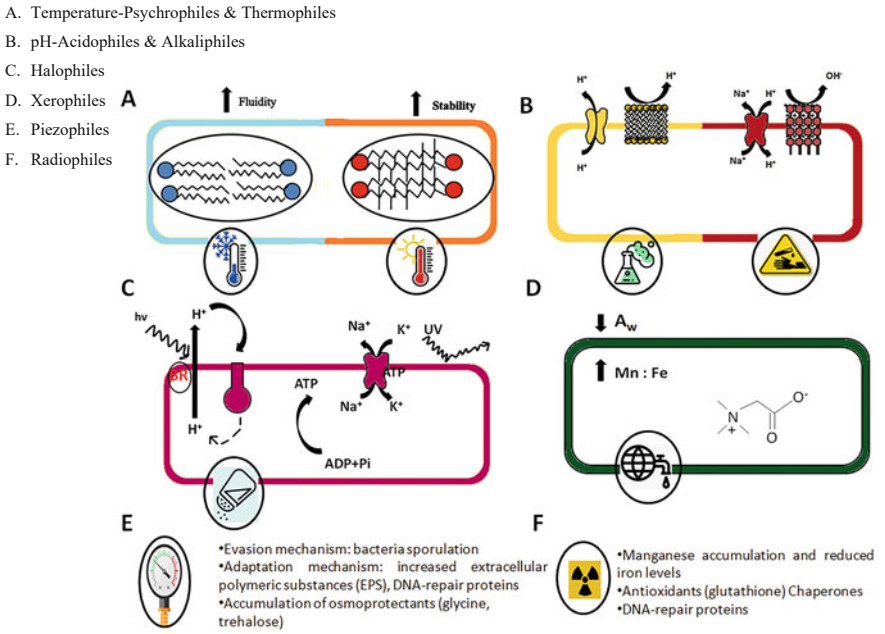

Fig. 3.1 Molecular mechanisms of extremophiles for their adaptation to extreme environmental conditions. (**a**) Temperature—psychrophiles and thermophiles. (**b**) pH—acidophiles and alkaliphiles. (**c**) Halophiles. (**d**) Xerophiles. (**e**) Piezophiles. (**f**) Radiophiles

effect on protein synthesis levels (Math et al. 2012). Prevention of UV damage to cells and reduction of cytoplasmic freezing point were achieved by aggregation of mannitol and other compatible solutes as cryoprotectants (Casanueva et al. 2010). In addition, they may probably stop protein assemblage/deterioration, stabilisation of membranes, and free radical scavenging in cold environment (Kandror et al. 2002) (Table 3.1).

The first bacterial AFP discovered from sea ice Gram-negative bacterium *Colwellia* strain SLW05 (Raymond et al. 2007). However, the earliest reported bacterial antifreeze characteristics were identified in soil bacterium *Rhodococcus erythropolis* and psychrophile *Micrococcus cryophilus* (Duman and Olsen 1993). Strikingly, *Pseudomonas fluorescens* KUAF-68 and *Pseudomonas borealis* DL7 have both ice nucleation and antifreeze activity (Kawahara et al. 2004; Wilson et al. 2006). Several types of ice nucleation proteins (i.e. InaK, InaQ, InaV, and InaZ) were reported in *Pseudomonas syringae* (Li et al. 2012). Temperature also affects structural proteins. To sustain their activities at low temperatures, enzymes have to surmount mainly two difficulties including cold distortion and slow reaction rates. Cold distortion takes place at freezing temperatures as they result in extra structured water molecules occupying the surface of protein resulting in less protein interaction and moving the system equilibrium towards the unfolded state (de Maayer et al. 2014). Other cold stress adaptations provided by EPS and cold shock proteins, in

Table. 3.1 Adaptations of extremophilic microorganisms under various climatic conditions

S. no	Type of extremophile	Microorganisms	Adaptation	Reference
1	Thermophiles	*Geobacillus* sp. TFV3	Chaperone protein DnaJ, chaperone protein DnaK, heat-shock protein GrpE, chaperone GroEL	Ching et al. (2020)
		Geobacillus, Parageobacillus	Heat-shock proteins (HSPs)	Wang et al. (2019)
		Geobacillus Thermodenitrificans ArzA-6, *Geobacillus toebii* ArzA-8 strains	EPS production	Panosyan et al. (2018)
		Thermolongibacillus, Aeribacillus, Geobacillus, Anoxybacillus	Biofilm formation	Cihan et al. (2017)
		Anoxybacillus sp. strain R4–33	EPS production	Zhao et al. (2014)
		Sulfolobus acidocaldarius, S. solfataricus, S. tokodaii	Biofilm formation	Koerdt et al. (2010)
2	Psychrophiles	*Paenisporosarcina Antarctica* CGMCC 1.6503^{T}	Fatty acid desaturases, dioxygenases, antifreeze proteins, and cold-shock proteins	Rong et al. (2020)
		Pseudoalteromonas sp. MER144	EPS production	Caruso et al. (2018)
		Colwellia psychrerythraea 34H	EPS production	Casillo et al. (2017)
		Pseudomonas mandelii	Alginate production, biofilm formation	Vásquez-Ponce et al. (2017)
		Flavobacterium frigoris PS1	Ice-binding protein (FfIBP)	Do et al. (2012)
		Pseudomonas syringae	Ice nucleation proteins (INPs) – Variant (InaQ)	Li et al. (2012)
		Sphingopyxis alaskensis	Polyhydroxyalkanoates (PHAs)	Ting et al. (2010)
		Pseudomonas putida GR12–2	Antifreeze protein (AfpA)	Muryoi et al. (2004)
		Colwellia strain SLW05	Antifreeze protein (AFP)	Raymond et al. (2007)
3	Halophiles	*Halomonas smyrnensis* K2	EPS production	Joulak et al. (2020)
		Alkalicoccus halolimnae BZ-SZ-XJ29^{T}	Ectoine biosynthesis gene cluster (ectA, ectB, and ectC)	Zhang et al. (2020a, b)

(continued)

Table. 3.1 (continued)

S. no	Type of extremophile	Microorganisms	Adaptation	Reference
		Nitriliruptoria species	K^+ influx and efflux, betaine and ectoine synthesis, and compatible solute transport	Chen et al. (2020a, b)
		Halomonas nitroreducens WB1	EPS production	Chikkanna et al. (2018)
		Salinibacter ruber	K^+ uptake via tropomyosin receptor kinase A	Oren (2002b)
		Methylarcula marina, M. terricola	Ectoine	Doronina et al. (2000)
		Halorhodospira Halochloris	Osmolyte glycine betaine	Galinski and Trüper (1982)
4	Acidophiles	*Acidithiobacillus caldus*	Ferric uptake regulator (AcFur)	Chen et al. (2020a, b)
		Acidithiobacillus ferrooxidans YNTRS-40	rus operon, res operon, petI, petII, sqr, doxDA, cydAB, and cyoABCD	Zhang et al. (2020a, b)
		Acidithiobacillus ferrooxidans	Proteins associated with inorganic sulphur compound (ISC) oxidation	Bellenberg et al. (2019)
		Acidithiobacillus ferrooxidans	Fumarate nitrate reduction transcription factor (FNR)-like protein (FNR_{AF})	Osorio et al. (2019)
		Acidithiobacillus	Squalene–hopene cyclase (SHC) sequences	Jones et al. (2012)
		Picrophilus torridus	Potassium-transporting ATPases and other cation transporter	Fütterer et al. (2004)
		Ferroplasma Type II, *Leptospirillum* group II (*L. ferriphilum*)	Proton efflux systems (H^+ ATPases, antiporters, and symporters)	Tyson et al. (2004)
		Ferroplasma acidarmanus	Tetraether-linked membrane monolayers	Macalady et al. (2004)
5	Alkaliphiles	*Bacillus* sp. AK13	EPS production	Jung et al. (2020)
		Alcaligenes sp., *Dietzia* sp.	Biofilm formation	Rout et al. (2018)
		Alishewanella, Dietzia spp.	Biofilm formation	Charles et al. (2017)
		Cronobacter sakazakii	EPS production	Jain et al. (2012)

(continued)

Table. 3.1 (continued)

S. no	Type of extremophile	Microorganisms	Adaptation	Reference
		Vagococcus carniphilus MCM B-1018	EPS production	Joshi and Kanekar (2011)
		Bacillus pseudofirmus OF4	Plasmids, cardiolipin synthase genes, sodium-coupled Npt type phosphate transporters, toxin-antitoxin genes mazE mazF	Janto et al. (2011)
		Thioalkalimicrobium aerophilum strain AL 3^T, *Thioalkalivibrio versutus* strain ALJ 15	Accumulation of unsaturated fatty acids, cyclopropane fatty acids, organic compatible solutes, pigments	Banciu et al. (2005)
6	Piezophiles	*Salinimonas sediminis* N102T	tesA (acyl-CoA thioesterase I), tesB (acyl-CoA thioesterase II), and yciA (acyl-CoA thioesterase YciA); polyhydroxyalkanoates, rRNA operons	Xue et al. (2020)
		Colwellia	More basic and hydrophobic proteome, archaeal methyltransferase for tRNA modification, NADH ubiquinone oxidoreductase (nuo) gene cluster	Peoples et al. (2020)
		Shewanella benthica DB21MT-2	Toxin-antitoxin (TA) system	Zhang et al. (2019)
		Thermococcus barophilus	Mannosyl-glycerate (MG)	Cario et al. (2016)
		Thermococcus piezophilus $CDGS^T$	Synthesis of compatible solutes, several hydrogenase gene clusters (hydrogenases and sulfhydrogenases)	Dalmasso et al. (2016)
		Photobacterium profundum SS9	Monounsaturated fatty acid accumulation	Allen et al. (1999)
7	Radiophiles	*Deinococcus radiodurans*	Single-stranded binding proteins (DdrB and SSB)	Lockhart and DeVeaux (2013)
		Rubrobacter xylanophilus, *Rubrobacter radiotolerans*	High intracellular concentration of trehalose, Mn^{2+}	Webb and DiRuggiero (2012)

(continued)

Table. 3.1 (continued)

S. no	Type of extremophile	Microorganisms	Adaptation	Reference
		Deinococcus radiodurans	Nucleotide excision repair pathway (uvrA1B), base excision repair pathway (ung and mutY), homologous recombination pathway (recA, ruvA, ddrA, and pprA)	Makarova et al. (2001)
8	Xerophiles	*Helicobacter pylori*	Serine protease HtrA	Zarzecka et al. (2019)
		Actinopolyspora, Nocardiopsis, Saccharomonospora, Streptomonospora, Saccharopolyspora	Polyketide synthetases and non-ribosomal peptide synthetases (NRPS)	Meklat et al. (2011)
		Nostoc commune	Water stress proteins (WSP)	Gao and Ye (2007)
		Caulobacter crescentus	Chaperone systems (DnaK/DnaJ and GroES/GroEL)	Susin et al. (2006)

addition to extensive microbial decomposition and nutrient reuse potential, are also documented for microbial mat communities from ice layers of Antarctica and the Canadian High Arctic (Varin et al. 2012).

3.3 Thermophiles

The molecular mechanisms of microbial adaptations to temperature extremes were thoroughly investigated in comparison with other conditions. Enzymes denature at elevated temperatures, become inactive, and, thus, hinder the metabolic activities. Besides, increase in the membrane fluidity takes place disrupting the cell. The thermophilic microbes possess a wide range of cell modifications to avoid cell disruption. Thermophilic membrane lipids include more saturated fatty acids and straight-chain fatty acids than mesophilic organisms (15–40 °C) (Reed et al. 2013) (Fig. 3.1). These features allow thermophiles to sustain elevated temperatures and maintain membrane integrity. Improved stability of proteins isolated from thermophiles was attributed to their more basic nature and small size (Kumar and Nussinov 2001).

In addition, monovalent and divalent salts improve nucleic acid stability since they conceal the negative charges. DNA will be protected from depuration and hydrolysis by the presence of phosphate groups, KCl and $MgCl_2$ (Hickey and Singer 2004). Another way of stabilising DNA is by using DNA-binding proteins and by compression of whole genome into chromatin (Marguet and Forterre 1998). A

common mode of thermophilic microbes to protect their cell machinery at extreme temperatures is the adaptation of these proteins by modifying the primary structure amino acid composition, thus enhancing their thermal stability (Xu et al. 2018). Proteins of thermophiles have a greater proportion of short length amino acids as well as α-helices containing amino acid residues (Urbieta et al. 2015; Xu et al. 2018). Another main strategy is the presence of heat-shock proteins (HSPs), like DnaK, GroEL, and GroES chaperones in protein folding. In a recent study by Wang et al. (2019), stress-tolerant HSP genes from thermophiles *Geobacillus* and *Parageobacillus* were isolated which contributed to the increased heat and osmotic tolerance. In addition, DNA damage is effectively handled by DNA-repair systems (SOS system). They use fatty acids arranged in branched chains and polyamines (spermidine) to stabilize the membranes. Another adaptation employs the suitable solutes to stabilize cellular components (Urbieta et al. 2015). Furthermore, proteins from the glycolysis pathway (pyruvate dehydrogenase complex) supply instant energy to survive the high-temperature stress conditions (Wang et al. 2015) (Table 3.1).

Additional hydrogen bond networks, reduced surface loop length, enhanced secondary structure tendency, increased core hydrophobic nature, improved Van der Waals interactions, ionic exchanges, and better packing density, on the whole, contributed to thermal stability of protein (Brininger et al. 2018). More recently, it has been shown that cells of archaea use a structure stabilisation strategy along with aforementioned adaptations, whereas bacterial cells utilize a sequence stabilisation strategy (Berezovsky and Shakhnovich 2005). The lipid composition of the thermophilic membranes is yet another well-known adaptation. Some species have novel/specific lipids, such as *Thermotoga maritima* (15,16-dimethyl-30-glycerylox-triacontanedioic acid) (Siliakus et al. 2017). In archaea, ether-based lipids were found to be hydrolysis-resistant at high temperatures. In contrast, cells of archaeal thermophiles comprise a monolayer consisting of "fused lipid bilayer", which was shown to be resistant for hydrolysis at higher temperatures (DasSarma et al. 2009).

In thermophiles, DNA shows thermal resistance by inserting positive supertwists by reverse gyrase (Jamroze et al. 2014). In addition, an increase in GC base pairs has been shown to stabilize DNA in specific regions (stem-loops). Thermophilic archaea contain histones directly correlated to the eukaryotic core histones (H2A/B, H3, and H4). Binding of these histones was demonstrated to enhance DNA melting temperature (Stetter 1999). Besides, specific microbial adaptations to improve protein stability at extreme temperatures comprise a greater number of disulphide bridges, improved aromatic peptide interactions, and enhanced peptide hydrogen bonding (Maier and Neilson 2015).

3.4 Acidophiles

Acidic pH conditions are a threat to cellular biochemistry, as extreme low pH contributes to protein degradation. Acidophilic microbes preserve their proteins by adding additional amino acids with neutral side groups and aggressively pumping

protons out of the cell to preserve steady intracellular pH conditions (Baker-Austin and Dopson 2007). They possess a complex of cell modifications to control pH within the cell. Many exoenzymes are reported to be efficient at very low pH than the pH of cytoplasm which is isolated from acidophiles. In addition to these enzymes, other significant biomolecules like plasmids, rusticynin, and maltose-binding proteins were isolated from acidophiles.

Acidophiles have many distinguishing structural and functional features for pH control (Golyshina et al. 2000; Crossman et al. 2004). Although these species are able to live under extremely acidic conditions, they do not withstand this kind of circumstances within the cell since DNA turns uncertain; hence, they have established strategies for pumping acid out of the cell to keep neutral to weak acidic environment (pH 5–7) within the cell (Matin 1999). Furthermore, some records of several other species with acidic internal pH (Van de Vossenberg et al. 1998; Macalady et al. 2004). Other proton flux systems include primary proton pumps (symporter) and secondary proton pumps (e.g. antiporter cation/HC), as well as proton-consuming reactions. *Leptospirillum ferriphilum* was shown to contain a carbonic anhydrase and amino acid decarboxylases which assist in pH equilibrium by overwhelming protons (Christel et al. 2018). Next strategy is a reduced cell membrane permeability which suppresses the cytoplasmic proton entry. The entry of protons is constrained by KC ions produced within positive membrane potential (Christel et al. 2018). In *Leptospirillum ferriphilum,* a broad range of genes associated with biosynthesis of cell membrane has been identified that can be related with acid tolerance.

Adaptations comprise a cell membrane that is relatively proton-impermeable (Konings et al. 2002). Another mechanism is the reduced pore size of membrane channel which was demonstrated for *Acidithiobacillus ferrooxidans* (Amaro et al. 1991). Acidophilic microbes comprise net positive charge within the cell which can offset the elevated H^+ ion concentration in their environment. They can use aggressive proton pumping, as reported in *Bacillus* and *Termoplasma* (Michels and Bakker 1985) (Fig. 3.1). Microbes should preserve a near-neutral cytoplasmic pH to allow cellular activities for their growth and metabolism (Krulwich et al. 2011; Jin and Kirk 2018). One of the first functions to evolve inside the earliest cells was possibly the balance of protons through various transporters, together with the ion-using ATP synthase (Lane and Martin 2012). Chemiosmosis is also a feature of both bacterial and archaeal cells (Lane et al. 2010).

Acidophiles may discharge organic metabolites like acetic acid and lactic acid in addition to intracellular pH, thereby modifying the nearby pH conditions (Zhang et al. 2016). Many of them consist of organic acid degradation pathways to avoid proton separation by organic acids (Baker-Austin and Dopson 2007). Archaeal members like *Ferroplasma acidiphilum* and *Sulfolobus solfataricus* were reported to contain tetrapetric lipids in the cell membrane which offer resistance to acidic pH. Advanced protein and DNA-repair systems were found in acidophiles compared to mesophiles. A pH shift from 3.5 to 1.5 externally persuades the proteins concerned with heat-shock reaction, for instance, chaperones were reported in *Acidithiobacillus ferrooxidans* (Amaro et al. 1991).

In a study conducted by Guazzaroni et al. (2013), novel acid resistance genes from the metagenome of the Rio Tinto River were isolated including ClpXP protease, the transcriptional repressor LexA, and nucleic acid-binding proteins such as an RNA-binding protein, HU, and Dps (Table 3.1).

3.5 Alkaliphiles

Under alkaline conditions, H^+ concentrations are particularly low and cells experience trouble using ATP synthase to generate energy and precipitation of other essential ions like Mg^{2+} and Ca^{2+} from water as salts will take place (Krulwich et al. 1998). Alkaliphiles overcome these complexities by vigorously pumping in these ions and by transporting others to preserve neutral conditions. Besides, the cell wall of alkaliphiles serves as a protective shield to harsh climatic conditions (Horikoshi 2006) (Table 3.1). Alkaliphilic microbes have evolved a cell wall with negative charge, lowering the environmental pH external to the cell. They also synthesize an additional acid cell wall consisting of teichurono-peptide and teichuronic acid or polyglutamic acid. All these acids absorb H^+ and resist OH^- and probably assist in generating the proton motive force required to stimulate the synthesis of ATP. The proton motive force for ATP synthesis is driven by Na^+ or K^+ antiporters in several alkaliphilic *Bacillus* species, which catalyses an electrogenic swapping of external ions (Na^+ or K^+) and high number of entries into H^+ ions (Preiss et al. 2015). In general, alkaliphiles can use these antiporters (Na^+/H^+ and K^+/H^+) (Krulwich et al. 2011) and also generate acids to lower the inner pH when metabolism is impaired due to elevated pH levels (Moran-Reyna and Coker 2014). The transporters are regulated, possibly through a transmembrane pH sensor signalling (Krulwich 1995) (Fig. 3.1).

3.6 Halophiles

Increased salt concentrations usually deprive protein water content leading to accumulation and precipitation due to exposed hydrophobic patches binding to one another. To neutralize this, these microbes have formed a proteome consisting mostly of acidic proteins (Brininger et al. 2018), and the acid remnants (aspartic & glutamic acid) are usually located on the protein surface. They help in arranging the water molecules (H^+ of water interacts with COO^- of acidic side chain) surrounding proteins building a "water cage" which guards the proteins from dehydration and precipitation (DasSarma and DasSarma 2015; DasSarma et al. 2009).

Many halophiles retain increased concentrations of various solutes in their cytoplasm in response to the salt to maintain their interiors in osmotic equilibrium with the external world. *Halophilic archaea* maintains exceptionally high KCl in its cells (Oren 2002a, b). Halophilic proteins must be properly folded and operative in heavy salt concentrations considerably similar to the hyperthermophilic proteins which stay functional around 100 °C (Michael et al. 1999). Halophiles achieve the necessary

osmotic balance by accumulating KC in the cytoplasm as a "salt-in" strategy and combined action on bacteriorhodopsin and ATP synthase (Margesin and Schinner 2001). Other strategy observed was the exclusion of salts through the synthesis of suitable organic solutes like polyols, amino acids, sugars, and betaines. The "salt-in" strategy has only been established in a small number of halophilic microbes (e.g. *Salinibacter* and *Halanaerobiales*) that need KCl to form active proteins (Fig. 3.1). On the contrary, various halophiles using salt omission approach can withstand a variety of salt concentrations because of the synthesis of organic solutes to counteract the high salt content in the surroundings (Oren 2013). Many microorganisms need to adapt to low water activity in saline environments. It was established that freezing point of water can be considerably decreased by salts; however, solutions containing saturated salts show very low water activity. Apart from pH and salinity, water activity is the only variable that certain microbes are able to control using their metabolites capable of accumulating or captivating water (e.g. EPS proteins and polysaccharides) (Frösler et al. 2017).

Halobacillus halophilus, isolated from a salt marsh on the North Sea coast of Germany, can withstand high levels of salt content up to 3.0 M NaCl with an optimum survival rate of 38% (Roeßler and Müller 1998). *H. halophilus* adopts a hybrid osmoadaptation approach by collecting together molar chloride concentrations and suitable solutes (glutamate, glutamine, proline, ectoine, N-acetyl ornithine, and N-acetyl lysine) (Saum et al. 2013; Saum and Müller 2008). More recently, *Halomonas socia* strain CKY01 developed polyhydroxybutyrate (PHB) with genes responsible for the absorption, synthesis, and transportation of osmolytes such as betaine, choline, ectoine, carnitine, and proline as a strategy for survival (Park et al. 2020) (Table 3.1).

3.7 Xero-Tolerant Extremophiles and Oxidative Stress

Xerophiles are able to sustain in dry climatic conditions with water activity <0.75 (Connon et al. 2007; Lebre et al. 2017). Additional parameters like hot and cold temperatures, poor water activity, increased salt concentrations, poor organic carbon content, and extreme radiation, in addition to low rainfall, intensify xeric conditions, limiting existence of microbes (Dose et al. 2001; Crits-Christoph et al. 2013). Xerophiles have evolved few survival mechanisms in dry environments including environmental stress avoidance and adaptive mechanisms (Table 3.1). Avoidance of dry environment requires alteration of cells into non-replicative viable state by development of spores (Crits-Christoph et al. 2013). Adaptive strategies are related to the prevention of water loss and improved water preservation by amassing of osmoprotectants (trehalose, L-glutamate, glycine betaine), synthesis of EPS, cell membrane alterations to maintain intracellular water, DNA repair, and protein synthesis (Dose et al. 2001; Lebre et al. 2017) (Fig. 3.1). Under oxidative stress, proteins undergo conformational changes that contribute to the unfolding and aggregation of proteins. The key drivers of protein folding are DnaK and GroEL

chaperones along with other cochaperones that stabilize proteins by promoting adequate folding and preventing their self-association (Susin et al. 2006).

Desiccation tolerance is distinctive in the midst of other extreme conditions faced by microbes since the cells will not proliferate under desiccation and most of their lifespan might be used up in desiccated condition. Therefore, desiccation cycles tend to persuade survival mechanisms for the cells instead of the capability to survive under harsh conditions. As stated by Maier and Neilson (2015), well-established survival mechanisms consist of DNA protection and repair ability when exposed to UV radiation, maintaining protein stability during desiccation and preserving membrane integrity. The main survival strategy of cyanobacteria is the synthesis of EPS which controls water absorption and loss, acts as a matrix for the immobilisation of cell contents formed by cell in reaction to dehydration, and possibly shields cell walls during shrinking and swelling (Potts 1999). EPS facilitates the formation of biofilms and may be essential components of water loss prevention mechanism that seals the cell (Ortega-Morales et al. 2001). When exposed to desiccation and UV stress, the cell produces multiple molecules. They were also reported in EPS and contain UV captivating molecules such as mycosporine-like amino acids and scytonemin, carotenoids, and detoxifying enzymes or radical quenchers that defend against harsh radicals, oxygen species, and water stress proteins (WSP) (Gao and Ye 2007). WSP were found to be highly stable, and up to 70% of the soluble proteins are present in *Nostoc commune*. Additionally *N. commune* cells contain trehalose and sucrose, which are capable of stabilising proteins and maintain membrane integrity during desiccation (Maier and Neilson 2015). In pathogens like *Helicobacter pylori*, serine protease HtrA plays key role in survival under various stress conditions (thermal, osmotic and acidic) (Zarzecka et al. 2019) (Table 3.1).

3.8 Piezophiles

As the pressure increases, membranes lose fluidity and permeability since lipids arrange themselves more compactly and reach a thickening process similar to what occurs at extremely low temperatures (Bartlett 2002). Organisms avoid this problem by increasing the proportion of polyunsaturated and monounsaturated fatty acids or phosphatidylglycerol and phosphatidylcholine in their membranes, rather than phosphatidylethanolamine (Usui et al. 2012; Siliakus et al. 2017) (Table 3.1). Protein-protein interactions are responsive to high pressure leading to the dissociation of enzymes (Sharma et al. 2002). Pressure is known to modify gene expression (Nakasone et al. 1998). Further modifications may contain chaperone-encoding genes for upregulation, respiratory chain alteration, porin expression, and development of osmolytes (Oger and Jebbar 2010; Jebbar et al. 2015) (Fig. 3.1).

3.9 Radiophiles

When bacteria are exposed to perils of environmental stress as ionising (gamma) radiation and UV radiation, a series of signals were expected to trigger physiological responses. Ionising radiation is primarily accountable for double-stranded breaks in microbial genome. On the other hand, both proteins and lipids will also get damaged, and constant oxidative stress was induced upon exposure (Slade and Radman 2011). Consequently, ionising radioresistant microbes developed unique strategies like new and robust DNA repair mechanisms, antioxidant and enzymatic defence systems, and a condensed nucleoid. Rapid and effective genome repair is important for sustaining ionising radiation doses. This was demonstrated by the use of the nucleotide excision repair pathway (uvrA1B), base excision repair pathway (ung and mutY), and homologous recombination pathway (recA, ruvA, ddrA, and pprA) in *Deinococcus radiodurans* (Makarova et al. 2001). In *Halobacterium* sp. NRC-1 (genes close to Rfa) (Berquist et al. 2007) and *D. radiodurans* (DdrB and SSB) single-stranded binding proteins were reported (Cox et al. 2010; Lockhart and DeVeaux 2013; Pavlopoulou et al. 2016). The cells of *D. radiodurans* consist of many of the following mechanisms for preventing oxidative stress and resistance. The cell cleans up by removing oxidized macromolecules, selective protein defence versus oxidative injuries, and the inhibition of reactive oxygen output. It has also been shown that a condensed nucleoid facilitates the efficiency/accuracy of DNA repair (Minsky et al. 2006) and restricts the diffusion of radiation-generated DNA fragments (Daly et al. 2007).

UV radiation enhances reactive oxygen species (ROS) production, with two distinct outcomes. Initially, ROS acts as cell signals encouraging cells to defend themselves against these stressors (Caldwell et al. 2007), and later when ROS levels exceed the cell's defence mechanisms, significant cell damage and apoptosis may occur. Biofilm formation is one of the techniques formed by microorganisms to colonize areas with high levels of UV radiation. Biofilms are layers of planktonic bacteria attached to each other, forming an intricate, growing, three-dimensional structure at their surfaces. Bacteria need to communicate with each other (quorum sensing) to develop this three-dimensional colony, and polyP was proposed as the modulator of quorum sensing and development of biofilms. UV radiation subtly damages DNA through the formation of cyclobutene pyrimidine dimmers (thymine dimers) and pyrimidine-pyrimidone (6–4) photoproducts (6–4 PPs). They account for approximately 80% of photolesions induced by UV radiation (Jones and Baxter 2017) (Table 3.1).

Radiophiles generally exploit an amalgamation of photoreactivation (phr) genes, nucleotide excision repair (uvrABCD, xpf, and rad), base excision repair (mutY and nth), and homologous recombination (recA and radA/51) to restore these DNA lesions (Jones and Baxter 2017). In addition, microbes have developed a set of photoprotective strategies to defend themselves from continuous exposure to UV radiation. They incorporate carotenoids, gene duplication via polyploidy, genome composition hydroperoxidases and superoxide dismutases (Jones and Baxter 2017), effective DNA-repair machinery, chaperone induction, and dynamic protection

against oxidative stress induced by UV radiation (e.g. accumulation of glutathione) (Pérez et al. 2017). Radiation resistance has been correlated with the ability of these microorganisms to repair DNA damage, because it was reported that radiophiles aggregate elevated levels of intracellular Mn^{2+} and reduced Fe (Pikuta et al. 2007) bestowing UV radiation resistance (Paulino-Lima et al. 2016) (Fig. 3.1).

3.10 Conclusions and Future Perspectives

Life on Earth will continue to be found in a plethora of climatic conditions previously considered to be adamant from an anthropocentric viewpoint to sustain existence of life. Studying extreme environments and extremophile biology including their position will help in predicting and hypothesising theories about situations that prevailed in the course of origin and progression of life on the ground and throughout the world. In the midst of rapid innovations made over the past few decades and latest developments in "omics" tools, extremophilic world has been thoroughly investigated, and our understanding of biosphere was evolved, extending the limits of life on Earth. Extremophiles take part in many important tasks in the environment. Their robust nature to resist, sustain, and mediate catalysts under harsh environmental conditions not only make them exceptional but also promising for environmental conservation. Extremophiles like deep-sea microorganisms contribute greatly to the atmospheric geochemical cycles. They preserve the chemical equilibrium in the environment, help to lessen the greenhouse gases (GHGs) in the atmosphere, and detoxify the hazardous chemicals in the environment. Next-generation sequencing (NGS) and next-generation proteomics (NGPs) provide important strategies for gaining insight into the molecular processes concerned with extremophilic strategies for survival (Armengaud 2016). This kind of research and methodologies may illustrate the strategies that microorganisms use to acclimatize to harsh climatic conditions and are constructive in understanding the microbial evolution with respect to extreme environments.

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Mangrove Microbiomes: Biodiversity, Ecological Significance, and Potential Role in the Amelioration of Metal Stress

4

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Abstract

The mangrove ecosystem through a rich productive ecosystem with a great diversity of flora and fauna both macro and micro is under the threat of severe pollution stress due to anthropogenic interference. Continuous input of pollutants is a major threat to this ecosystem affecting the indigenous microbial community playing a major role in the biogeochemical reactions and contributing to the richness of the biome. Being exposed to inputs from riverine sources which in turn receive huge amounts of pollutants in the form of industrial effluent discharge, agricultural runoff, domestic waste, sewage, etc., the major components in these discharges are pesticides, excessive inorganic compounds, high organic content, and metals. These pollutants especially the heavy metals tend to sink, have low solubility in water, and accumulate in the mangrove sediments, which act as the sinks for the heavy metals. Sediment contamination thus ultimately diminishes the mangrove ecosystem. Exposure to the pollutants especially heavy

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metals results in changes in the microbial communities with the prevalence of metal-tolerant species. This chapter uncovers the ecological aspects of mangrove sediments focusing on the metal-tolerant microbiome and its role in the maintenance of the biome.

Keywords

Microbiome · Metal tolerance · Mangroves · Sediments · Pollution · Ecosystem balance

4.1 Introduction

Mangroves are wetland ecosystems with fine-grained sediment. The mangrove ecosystems function as habitats and breeding sites for a variety of fishes; mangroves are a source of timber and other plant products; they serve as carbon sinks and play role in atmospheric green gas removal by sequestering the organic contents in the water and sediments. This buried detritus in the anoxic sediments make up the coastal blue carbon (Twilley et al. 1992; Chmura et al. 2003; MacFarlane and Burchett 2002; Walters et al. 2008; Nellemann et al. 2009). The mangrove ecosystem has been identified as one of the most productive ecosystems having a very high net productivity as compared to other ecosystems (Donato et al. 2011).

During the last two decades, about 35% of mangroves globally have been known to be polluted with heavy metals (Feller et al. 2010; Gopalakrishnan et al. 2020). Accumulation of trace metals in the sediments occurs because of their lower water solubility and thus gets easily absorbed and retained in sediments, making the sediments an ultimate sink (Yu et al. 2008; Alvarez et al. 2011). The presence of heavy metals affects the microbial population, and the toxic effects are ultimately seen to reflect in the increased greenhouse emissions and carbon cycling (Nath et al. 2013; Usman et al. 2013). Though mangrove ecosystems are rich in organic matter, they are however deficient in nutrients like nitrogen and phosphorous (Vazquez et al. 2000). Despite this, they are regarded as a highly productive ecosystem because the microbiome present in the sediments is very active and productive that is responsible for nutrient cycling in the ecosystem through various geochemical processes.

The microorganisms, namely bacteria, archaea, fungi, viruses, and protists, make up the mangrove microbiome. To study the role of the microorganisms in the biogeochemical changes taking play in the mangrove sediments and correlate these with the environmental processes, hypothesis-driven studies are the need of the day. Which will help to understand the mangrove interactions at various levels and the contributions of microflora thus coming out with approaches for the protection and rehabilitation of mangrove forests? This thus calls for an urgent need to study the microbiomes of the mangrove sediments, including fungi, archaea, viruses, and protists, apart from bacteria, and understand their contribution to the ecosystem

and overall environment functioning. Even though the mangrove ecosystem is greatly beneficial to man and the environment, its importance is neglected and it suffers from anthropogenic pressures.

4.2 Ecological Assessment of Sediments of Mangrove Ecosystems

Most mangrove ecosystems have human settlements in close proximities which are a source of contamination, especially the industrial discharge into the mangroves (Alongi 2002; Kong et al. 2015; Ren et al. 2015). Mangrove ecosystems are highly influenced by these humanoid activities leading to accumulation of the pollutants especially heavy metals and recalcitrant compounds (Bodin et al. 2013). Another factor leading to the contaminants reaching the sediments is the weaker winds along the coast and hence slower water movements which cause the pollutants to sink in (Cai et al. 2009). Contamination of the sediments thus deteriorates the quality of the aquatic system. Sediment Quality Guidelines are used to determine the contamination levels in sediments for monitoring and quality management (US EPA (United States Environmental Protection Agency) n.d.; Bakan and Özkoc 2007).

4.2.1 Heavy Metal Pollution

Numerous studies have been carried out worldwide on the degradation and pollution of mangroves with trace metals, especially heavy metals (Defew et al. 2005; Fernandes et al. 2012a; Bodin et al. 2013; Usman et al. 2013; Fernández-Cadena et al. 2014; Li et al. 2016). Heavy metals in low concentrations are required for growth and are toxic at higher concentrations. The metabolism of the organisms is affected leading to changes in growth patterns as well as reproduction and ultimately causing an imbalance in the food chains (Wright and Welbourn 2002). The organisms growing in heavy metal contaminated systems accumulate the metals in their body, and humans are the final link in the food chain (Stewart 1999; Mwevura et al. 2002; Wang et al. 2015; Yin et al. 2016).

Marine sediment composition and structure impact the accumulation of heavy metals in the sediments. The presence of clays, mud, and sands leads to the formation of complexes and these interactions make the heavy metals sink in the sediments (Nobi et al. 2010; Gao and Chen 2012). In a study carried out along the Saudi Arabian sea, the presence of heavy metals was assessed and showed a high concentration of Cr followed by Cu and Ni while other metals like Pb, Cd were at a lower concentration but enough to cause damaging effects on the biota of the sediments as well as waters of the mangrove econiches (Bouillon et al. 2003). Another study on the Saudi coast of the Arabian Gulf also gave similar results when the heavy metal concentration was analyzed, with chromium being the dominant metal in the surface sediments. The authors point out that dredging, landfilling, oil pollution, reclamation, sewage disposal, etc. are the sources of the pollution

(Youssef et al. 2015; Almasoud et al. 2015; El-Sorogy et al. 2016; Almahasheer 2018). Reports by Al-Kahtany et al. (2018) and Almahasheer (2019) assessed the heavy metal concentrations in Tarut island mangroves. Similar studies carried out in the Zhangjiangkou Mangrove National Nature Reserve of China assessed sediments for the presence of heavy metal concentrations of Cu, Cd, Pb, Cr, Zn, As, and Hg along with studies of its effects on the biotic components for studying ecological risk assessment and environmental management (Wang et al. 2016).

4.2.2 Assessment of Other Parameters

The mangrove sediment is rich in organic matter content which is majorly contributed by the plant litter from the canopy above (Alongi 2002; Asaeda and Kalibbala 2009; Wang and Sousa 2009). According to reports, the highest organic carbon accumulation occurs in the mangrove forests, i.e., up to 26 Tg/year (Breithaupt et al. 2012).

The organic content of mangrove sediment was found to vary from organic matter in surface layers which receive fresh plant litter. The elemental ratio of C:N was observed to be 20–30 as against the ratio of 10 required for the growth of microorganisms (Kristensen et al. 1995; Twilley et al. 1997; Wafar et al. 1997). Thus, a rapid initial drop in carbon and increase in nitrogen is required for the degradation of litter in the initial stages. Thus, it is observed that mangrove soils are deficient in dissolved inorganic nitrogen or DIN acting as its sinks from the surroundings (Alongi 1996; Rivera-Monroy and Twilley 1996). However, mangrove sediments remain nutrient-deficient, particularly in nitrogen and phosphorus (Holguin 1992; Vazquez et al. 2000; Skov and Hartnoll 2002).

The microbiota present in the sediment degrades and utilizes the organic matter present which contains mostly tannins, polyphenols, cellulose, lignin, and lignocellulose (Lee 1998). Numerous analyses carried out in India show high organic contents of up to 37% dry weight and C: N ratios reaching 27.3 (Bouillon et al. 2003). The organic carbon stocks, which are of mangrove origin, are thus very high. This organic matter found deposited in the sediments of the mangroves is indeed a source of carbon and nitrogen to the microbiota. An increase in seawater levels has resulted in the saltwater intrusion of estuarine regions. Changes in the ionic composition are also observed due to the inflow of land wash-offs adding loads of nitrates, from agricultural sources. These nitrates may be lost to the atmosphere upon conversion to nitrous oxide (Maier et al. 2000).

4.3 Mangrove Sediment Microbiome and Its Ecological Role

The 91% biomass of the mangrove microbiome consists of bacteria and fungi, while the remaining 9% comprises algae and protozoa. Most of the bacteria and fungi are attached to particles in sediment and process the energy flow and nutrients in the ecosystem. The microbiome of the mangrove ecosystem is responsible for carrying

out all facets of biogeochemical cycling, including the transformation and degradation of pollutants. However, heavy metals being toxic even at very low concentrations, the microbiome gets affected and dominance of resistant microbiota is observed. The heavy metal sensitivity is more pronounced in the microbiome as compared to macro flora and fauna in the same environment (Zhou et al. 2013). Sediment microbiome studies carried out by Zhang et al. (2019) indicated the prevalence of prokaryotic alpha diversity in mangrove sediments. This biome harbors other prokaryotic groups also mostly belonging *Gammaproteobacteria*, *Deltaproteobacteria*, *Chloroflexi*, and *Euryarchaeota*.

In intertidal zones, the sediment microbiome is responsible for detritus decomposition and nutrient cycling (Campbell 2008). The microbiome is composed of nonrandom networks of bacteria, archaea, and fungi connected by positive, negative, and neutral relationships (Sul et al. 2013). While the active role of bacteria driving carbon fluxes is defined in mangrove sediment (Holguin et al. 2001), the role of fungi, in particular, is poorly understood, and the interactions of the different components are undescribed with many potential relationships in mangrove sediment. For example, fungi can promote habitat sharing with bacteria and vice versa (De Boer et al. 2005), and the relationship between methanogenic archaeal species and sulfate-reducing bacteria is well known in methane-rich sediments (Plugge et al. 2011).

Based on the functionality of the microorganisms present in the mangrove sediment, the microbiome composition is as below.

4.3.1 Nitrogen Fixers

In mangrove soils, diazotrophs play a major role as nitrogen fixers. The concentration of soluble nitrogen is seen to influence the scale at which the diazotrophic bacteria will carry out nitrogen fixation in the mangrove ecosystem. Due to the presence of higher soluble nitrogen concentrations as well as a lack of adequate carbon sources, nitrogen fixation rates were low (van der Valk and Attiwill 1984; Mann and Steinke 1989). Bacterial strains identified as the following genera *Azospirillum*, *Azotobacter*, *Rhizobium*, *Clostridium*, and *Klebsiella* have been characterized and shown to carry out nitrogen fixation in the mangrove ecosystems. These microorganisms along with nitrogen fixation carry out nitrogen reduction forming ammonia and thus it contributes to the overall balancing of nitrogen content in the mangrove ecosystems (Fernandes et al. 2012b).

4.3.2 Phosphate Solubilizers

Phosphate-solubilizing bacteria have the potential to convert insoluble phosphates to organic available forms. They play a vital role as suppliers of phosphorus to the mangrove plants. Conversion of inorganic phosphates to organic phosphates is favored in the mangrove sediments as the conditions are anoxic. In sediments

close to the plant roots, the conditions are oxic and allow bacteria to grow and bring about phosphate solubilization. Some of the phosphate-solubilizing bacteria isolated from mangrove sediments were identified as *Bacillus atrophaeus*, *B. amyloliquefaciens*, *B. licheniformis*, *Chryseomonas luteola*, *Enterobacter aerogenes*, *E. asburiae*, *E. taylorae, Kluyvera cryocrescens*, *Paenibacillus macerans*, *Pseudomonas stutzeri, Vibrio proteolyticus,* and *Xanthobacter agilis* (Vazquez et al. 2000). Bacteria identified as genus *Chryseomonas, Kluyvera,* and *Xanthobacter*, with phosphate-solubilizing potential have been reported by Vazquez et al. (2000), from the mangrove sediments of Mexico.

4.3.3 Sulfate Reducers

The mangrove sediments harbor sulfate-reducing bacteria that carry out the degradation of organic matter under anaerobic environments prevailing in the sediments (Nedwell et al. 1994; Sherman et al. 1998). Sulfate reduction by these anaerobic bacteria accounts for the availability of soluble iron and phosphorus as well as for the emission of carbon dioxide from the sediments (Kristensen et al. 1991). Thus, the mineralization of sulfur, the production of soluble iron in the form of FeS_2 and soluble phosphorus, is making these minerals available to the microbiome for its growth and metabolism. Apart from these roles, sulfate-reducing bacteria are also found to play a role as nitrogen fixers as seen in plant-associated as well as plant-unassociated sediments in mangroves of Florida (Zuberer and Silver 1978). Bacterial groups were more abundant in these mangrove sediments in the rhizospheres of *R. mangle* and *A. germinans* mangroves (Zuberer and Silver 1978).

In the studies carried out in Goa (India), spore-forming sulfate-reducing bacteria were found associated with mangroves (Saxena et al. 1988). Eight species of sulfate-reducing bacteria belonging to four different genera were identified from mangroves of Goa, namely *Desulfovibrio desulfuricans, Desulfovibrio desulfuricans aestuarii, Desulfovibrio salexigens, Desulfovibrio sapovorans, Desulfotomaculum orientis, Desulfotomaculum acetoxidans, Desulfosarcina variabilis,* and *Desulfococcus multivorans* (Loka Bharathi et al. 1991).

4.3.4 Methanogens

Studies on the Indian mangrove sediments showed a large variation in the methanogenic populations which were due to abiotic factors of the water and sediments (Mohanraju and Natarajan 1992) as well as biotic factors mainly the presence of sulfate-reducing bacteria (Ramamurthy et al. 1990). Methanogenic bacterial strains produce methane in the anoxic conditions of the sediment such as the methanogenic bacterium, *Methanoccoides methylutens* (Marty 1985; Mohanraju et al. 1997). This methane diffuses to the aerobic sediment layers and gets oxidized by aerobic methanotrophs. There are reports of anoxic oxidation of methane in hypersaline microbial mats (Conrad et al. 1995). Metagenomic studies showed the

presence of CH4-oxidizing genes from uncultured methanotrophs *Methylosarcina, Methylomonas,* and *Methylobacter* in mangrove soils (Lüke and Frenzel 2011). Mangrove ecosystems receiving high organic inputs due to anthropogenic activities show higher methane emissions indicative of higher methanogenic activities in the mangrove sediments (Giani et al. 1996; Strangmann et al. 1999). The presence of type I or type II methanotrophs in the mangrove sediments depends on the NaCl concentration and the alkalinity of the sediments (Bowman 2015a; Shiau et al. 2018; Ho et al. 2018). The type I methanotrophs *Methylomonas* and *Methylobacter* are mostly influenced by the pH of the saline ecosystems (Bowman 2015a, b; Shiau et al. 2017) and are responsible for the reduced methane emissions from these ecosystems.

4.3.5 Photosynthetic Anoxygenic Bacteria

Some research workers on mangrove sediments in India have reported purple sulfur and purple nonsulfur bacteria (families Chromatiaceae and Rhodospirillaceae) (Vethanayagam and Krishnamurthy 1995; Vethanayagam 1991). Dhevendaran (1984) and Chandrika et al. (1990) have reported the predominance of bacteria belonging to genera *Beggiatoa, Chloronema, Chromatium, Leucothiobacteria*, and *Thiopedia,* including some brown *Chlorobiaceae* species during studies carried out in mangrove regions of Cochin. A large proportion of the anaerobic microbiota was found to consist of phototrophic sulfur bacteria. While studies in Florida reported *Chromatium* species to be most abundant in the sediment samples (Zuberer and Silver 1978).

4.3.6 Viruses

This group of mangrove sediment microbiome is not studied much and remains highly uncharacterized. Most of the information available on mangrove soil viruses based on phylogenetic analyses does show the presence of diverse groups of viruses. These viral groups are postulated to bring about complex polysaccharide recycling and thus participate in global carbon cycling. Thus activities of the viral community in the sediment affect biogeochemical cycles through the organic carbon discharge as well as the release of nutrients from hosts. The viral auxiliary metabolic genes (AMGs) are responsible for driving biogeochemical cycles in the sediments microorganisms (Zhang et al. 2014; Anantharaman et al. 2014; Roux et al. 2016; York 2017). The prime role of the viruses in controlling the bacterial populations also affects the microbiome composition.

4.4 Characterizing Metal-Tolerant Microbiomes in Mangrove Ecosystem

The impact of climate change on the mangrove microbiome is a challenge. During the last two decades, around 35% of the world's mangrove ecosystem has been reportedly polluted with heavy metals (Feller et al. 2010; Gopalakrishnan et al. 2020). Mangrove ecosystems are vulnerable to various anthropogenic stresses that contain high levels of heavy metals (Zhang et al. 2014). Continuous exposure to heavy metals has drastic effects on mangrove ecosystems as the carbon cycling is disturbed. This is because of the toxicity of the inflowing heavy metals to the mangrove microbiota (Usman et al. 2013; Nath et al. 2013). Though mangrove ecosystems are organic matter rich, nitrogen and phosphorous deficiency are however relevant (Vazquez et al. 2000). Despite this, they are regarded as highly productive ecosystems because the microbiome present in the sediments is very active and productive that is responsible for nutrient cycling in the ecosystem through various geochemical processes. Most of the sediment bacteria and fungi process the energy flow and nutrients in the ecosystem. Though microorganisms drive vital biogeochemical processes, in the mangrove ecosystem, they are more sensitive to heavy metals than the higher level of organisms in the same environment (Zhou et al. 2013).

Natural benthic communities of the mangrove ecosystem are stable as long as there are external anthropogenic disturbances such as sewage disposal, oil spills, and exposure to high heavy metals loads from discharges from mining rejects and industrial wastes. Any disruption in sediments causes a shift of the microbial community leading to disturbed nutrient cycles with reduced nutrient availability and leaching of toxic microbial by-products, thereby affecting the ecosystem health.

An environment that is enriched with heavy metals places a great selective pressure on the microflora exposed, leading to the development of a specific microbiome that is resistant to different heavy metals. To defend or protect them from metal toxicity, the development of adaption and resistance is observed in microbes by sophisticated mechanisms. Heavy metal resistant microbiome comprises of metal resistome, the resistant microbiota. Metals are also of importance for respiration as well as other biological functions involved in carbon and nitrogen cycling (Andreote et al. 2012; Ragavan et al. 2016; Kandasamy 2000).

At present, most of the research work carried out on mangrove microbial communities and their characterization were focused on a temporal and spatial range that is important for the foundational understanding of the microbiome in this ecosystem. However, there is also a need to have more advanced technological research that would help to establish a link between mangrove microbe and the health of its ecosystems. The complexity of microbial communities and the technical constraints to identify and measure diversity has hampered our understanding of the functioning and microbial diversity. Since most of the microbes are unculturable, their abundance and diversity studies are not assessable by a conventional culture-based method. With the recent advances in next-generation sequencing (NGS) technology, few studies are being reported to understand the diversity and functional

genomics of microbiomes. A broad picture of the microbial life in the mangrove ecosystem is still not satisfactorily presented. Comparative microbiome studies of the distinct mangroves using metagenomics will considerably help to a better understanding of the mangrove microbial community structure and its dynamics.

Using NGS, studies have revealed the effect of heavy metal contamination on the microbiome in some ecosystems (Feller et al. 2010; Andreote et al. 2012), However, the knowledge regarding microbiome changes with the influence of heavy metal presence is very specific, and little is known about the core resistant microbiome and its role in the contaminated ecosystem. The taxonomical studies on mangrove soil sediments from Sundarban revealed the dominance of *Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Fusobacteria, Nitrospirae,* and *Planctomycetes* based on NGS metagenomic analysis (Das et al. 2018). While in Mai Po Ramsar Wetland in Hong Kong, SAR and China studies revealed *Actinobacteria, Acidobacteria, Nitrospirae*, and *Verrucomicrobia* in inner mangrove sediments, whereas Proteobacteria and Deferribacteria were detected in outer mangrove sediments (Gopalakrishnan et al. 2020). In comparing the mangrove sediment microbiomes of India and Brazil, the richness of bacteria was observed, while the Red Sea mangrove samples showed an abundance of archaea (Usman et al. 2013; Alzubaidy et al. 2016). These differences in microbial diversity could be attributed to mangrove species, geographical location, physicochemical parameters influencing the mangrove sediments, and anthropogenic activities which influence the community compositions.

Feng et al. (2017) reported *Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Desulfobacterota, Gemmatimonadetes, Myxococcota, Nitrospirae,* and *Proteobacteria* were the dominant phyla found persistently in environments polluted with heavy metals. Firmicute and Proteobacteria were ubiquitously present bacterial groups in these environments. Proteobacteria contains taxa having extensive metabolic properties, thus enabling it to colonize a range of habitats, and thus have been reported to be a predominant heavy metal-resistant phylum in many polluted environments (Zhao et al. 2019; Li et al. 2020). As they have strong adaption and tolerance, this is also confirmed by culturing methods (Gopalakrishnan et al. 2020). A literature survey carried out by Hao et al. (2021) on heavy metal-resistant bacteria from different ecosystems revealed that more than 66% of isolates are Proteobacteria, dominated by Gammaproteobacteria (52.84%) followed by *Acidobacteria*. Metagenomic studies revealed archaea to be the second most frequently found phyla in metal-contaminated mangrove ecosystems. Other major phyla found in heavy metal polluted sites are *Chloroflexi* and *Nitrospirae*, mostly involved in nitrogen cycling (Ganguli et al. 2017; Liu et al. 2018). Figure 4.1 shows the distribution of various microbial phyla in the mangrove rhizosphere.

In general, laboratory-based experiments reveal that short-term or long-term exposure to heavy metals reduces the diversity of microorganisms. The presence of a low concentration of essential metals such as copper and zinc is required for the growth of a wide range of bacteria, which eventually results in increased microbial diversity. The constant diversity indicates the strong resilience and the capacity of

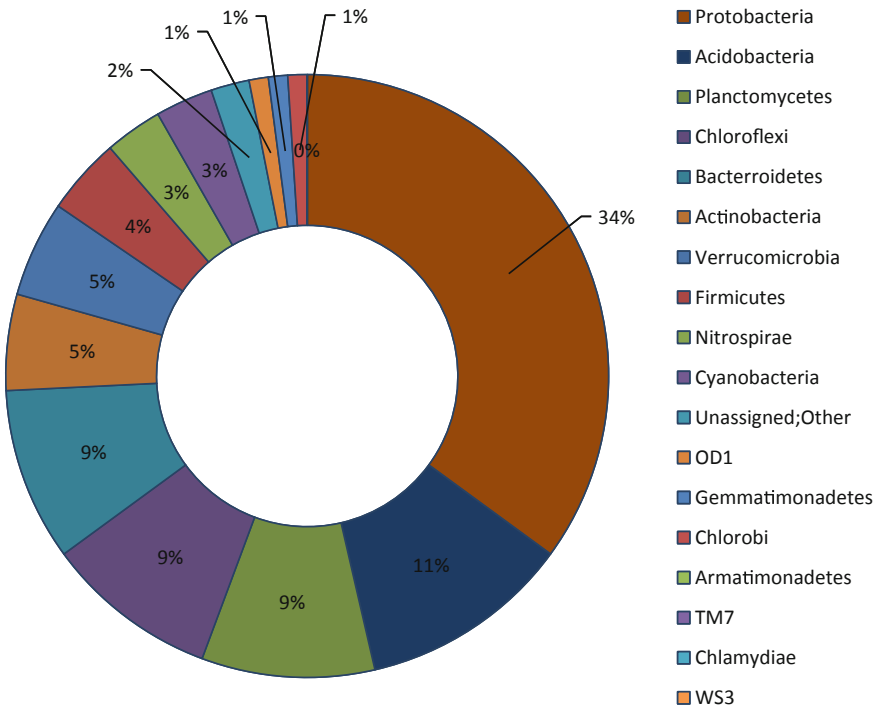

Fig. 4.1 Distribution of various microbial phylum in the mangrove rhizosphere

the microbial community to acquire resistance through horizontal gene transfer, without influencing microbial diversity (Lopez et al. 2017; Song et al. 2018).

4.5 Comparison of Microbiomes Across Diverse and Distinct Mangrove Ecosystems

Mangrove wetlands are influenced by both marine and terrestrial environments which cause gradients in salinity and organic matter in the sediments and fluctuating environmental conditions determine the microbiome in ecosystems differing significantly from others. Also, microbial diversity is influenced by mangrove species. Alzubaidy et al. (2016) reported Bacteroidetes dominance in the rhizosphere of *Avicennia* and in sediments without vegetation, *Actinobacteria* was predominant.

The heavy metal gradients can influence the microbial diversity by decreasing, increasing, or remaining constant. Mostly a decrease in the diversity is observed in studies following a severe heavy metal contamination exposure. Strong selection of microbiome occurs due to heavy metals purging sensitive taxa, leading to the enrichment of the resistant taxa but subsequently resulting in a decrease in diversity. Exposure to a low concentration of essential metals will result in a proliferation of a

wide range of bacterial groups and thus an increase in the microbial diversity of that habitat.

Several studies on the phylogenetic diversity in mangroves are located across regions such as Brazil, China, and the Red Sea mangroves of Saudi Arabia (Alzubaidy et al. 2016). Ghosh et al. (2010) reported a predominance of Proteobacteria in the mangrove areas of Sundarbans. However, in some other regions of tropical mangrove swamps, metagenomic results revealed, Deltaproteobacteria (43.88%) as the major class, followed by Alphaproteobacteria and Gammaproteobacteria dominance of *Desulfococus* spp., which is attributed to their involvement in the sulfur cycle.

Reports show that methane fluxes are largely contributed by activities of methanogens and methanotrophic communities especially in the wetlands (Cai et al. 2016; Das et al. 2018; Sierocinski et al. 2018; Yu et al. 2020). Methane production is carried out by methanogens which are placed in Euryarchaeota phylum in the archaea domain, and methane oxidation is carried out by methanotrophic communities (Deng et al. 2016), including two main groups of Gammaproteobacteria, e.g., *Methylococcaceae* and Alphaproteobacteria, e.g., *Methylocystaceae* (Yu et al. 2020). Many studies have reported the relative abundance of methanogenic communities is promoted by the presence of heavy metals (Feng et al. 2017; Giannopoulos et al. 2019). Some studies show the inhibition of carbon dioxide emissions due to heavy metal pollution thus impeding organic matter decomposition (Jaiswal and Pandey 2019; Enya et al. 2020). Overall, there seems to be a lack of understanding of the effects and long-term consequences of heavy metal pollution on the methane emission as well as carbon dioxide emissions from contaminated mangrove ecosystems.

The microbiome structure of the affected mangrove areas showed a different structure as compared to the microbiome structure of pristine areas, especially metal-contaminated mangrove ecosystems. These structural alterations were less evident in the high taxonomic groups. The observed prevalence of Proteobacteria, Bacteroidetes, Chloroflexi, and Firmicutes suggest these groups be members of the core microbiome of mangrove sediments in subtropical areas (Andreote et al. 2012; Dias et al. 2012). The Proteobacteria group is highly influenced by anthropogenically affected mangroves (Mendes and Tsai 2014).

4.6 Implications or Applications of Sediment Microbiome

Various methods used for the quantitative evaluation of heavy metal concentration help to understand the potential risk to an ecology of an ecosystem (Yuan et al. 2011). However, the application of sediment microbiota as indicators is preferable as these are sensitive environmental variation indicators. Various cultural characteristics of the microbial cells are responsible for this application which includes a large surface area to volume ratio due to their small sizes, the permeability of the cell membranes, and the ability to utilize the various nutrients available in the ecosystems (Billings and Ziegler 2008; Ikenaga et al. 2010; Troxler et al. 2012). The

ability of the microbiome to overcome numerous stress conditions also makes them preferable indicators. Some studies related to this aspect have been reported for substrate quality (Bossio and Scow 1998; Morrissey et al. 2014a), flooding (Mentzer et al. 2006; Unger et al. 2009), temperature (Zogg et al. 1997), salinity (Morrissey et al. 2014b), pollution (Córdova-kreylos et al. 2006), etc.

In particular, prokaryotic populations are highly susceptible to heavy metal pollution than eukaryotes (Frossard et al. 2017; Rajapaksha et al. 2004) in terms of biomass, activity, and diversity. Thus, the microbiota is frequently considered as potential indicators of ecological changes like monitoring them for heavy metal pollution (Li et al. 2020). The mangrove ecosystem and in particular the microbiome of the sediment contribute toward remediation of this ecosystem from metal pollutants. The heavy metals are redistributed between the sediment and the water columns above, by the metal-tolerant microbiome. The microbial community is shifted toward the dominance of the resistant genera as a result of the heavy metal pollutants in the sediments. These could be used as indicator species of environmental stress and toxicity changes in the sediment and mangrove ecosystems as a whole. Studies indicative of sea-level rise stressors, for example, showed changes in microbial diversity within weeks' duration which shows the early warning signals from the microbiomes which are indeed very useful for immediate action plan implementation (Wright and Welbourn 2002). The quality of aquatic ecosystems can be monitored by using benthic organisms because of their features like their geographical distribution, fixed lifestyle, capturing ease, and the bioturbation promotion (Cantillo et al. 1997; Nordhaus et al. 2006; Wang et al. 2015; Yin et al. 2016).

4.7 Conclusions and Fture Perspectives

The process of carbon sequestration, as well as carbon storage, is much faster in the mangrove wetlands as compared to forests or any other ecosystems. Hence, the mangrove ecosystems are the most productive and are also referred to as the blue carbon sinks. Anthropogenic activities cause disturbances in the physicochemical parameters of the econiches thus influencing the microbiota. Trace element pollution in general and heavy metal pollution, in particular, have been the focus of several studies. It is the need of the day to understand the effects of heavy metal pollution on methane, the carbon dioxide released in the atmosphere, and ultimately the variations in the associated microbial communities. This knowledge is very important to evaluate their ecological consequences and global warming implications in mangrove wetlands. Microbial biodiversity depending upon its resistivity and the concentration of the polluting heavy metal, can decrease, increase, or remain the same. There is a reduction in microbial biodiversity observed in ecosystems receiving heavy metal contamination. Strong selection of microbiome occurs due to heavy metals purging sensitive taxa, or short-term, leading to the proliferation of a few specific resistant groups and resulting in a subsequent decrease in diversity.

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Dynamics of the Coral Microbiome and Its Link to Climate Change

5

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Abstract

Coral reefs are one of the most diverse ecosystems in the world. Due to climate change and several anthropogenic activities such as overfishing, coral mining, waste disposal, marine pollution, etc., corals have been greatly affected. Corals are sessile animals that are in multipartite symbiosis with various microbes, forming the basic framework for the reef ecosystem. Microbes are considered a crucial part of the marine ecosystem as they play a key role in ecological functions, primary productivity, nutrient cycling, and producing chemical defense to protect hosts from invading microbes, etc. Coral microbiome

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investigations are gaining focus in recent years by incorporating various conventional and high-throughput sequencing technologies for determining the diversity of bacteria present in corals concerning their genera, location, health status, etc., as their diversity tends to be both static as well as dynamic in nature.

Keywords

Coral ecosystem · Holobiont · Climate change · Response · Coral reef management

5.1 Introduction

Oceans are one of the most under studied environments. They have the central importance in detecting changes in structural habitat, food web, and biodiversity. There is a limited scope for sampling in the ocean environment (Smith et al. 2015). The influence of the pollutants, eutrophication, and rapid change in temperature, along with the hypoxic conditions, affect the ocean health and the microbiota living in it (Gruber 2011). Due to the increase in climatic variations, the ecosystem in all parts of the world is affected (IPCC 2014). The most biodiverse ecosystem on the planet Earth is the coral reefs (Muller-Parker et al. 2015). Due to human activities in the late eighteenth century, there has been a tremendous change in the Earth's atmosphere which leads to rise in the Earth's temperature rapidly (Trenberth et al. 2007). Most of the coral reef ecosystems are located in the vulnerable part of the Earth's atmosphere where climate change takes place very frequently (Hughes et al. 2003; Hoegh-Guldberg et al. 2007).

The coral reef ecosystem has a greater role in the persistence of Anthropocene, the species that are present in the corals are sensitive to various changes (anthropogenic disturbances) like extreme fishing, ocean pollution, coastal development, and ocean acidification. The acidification of the ocean has a direct impact on the ocean calcifiers (corals); acidification inhibits growth and limits calcification (Doney et al. 2009). It also reduces the reef's structural integrity and in turn increases the bioerosion process which leads to the destruction during severe weather like storms and cyclones (Andersson and Gledhill 2013). In 1998, >45% of the coral cover has been lost across the Indian ocean due to climate change (Hoegh-Guldberg et al. 2007). The coral community has associated microorganisms and also comprises metaorganism which is together known as coral holobiont (Rohwer et al. 2002). These bacterial communities help in the nitrogen fixation, sulfur cycling, and protection against pathogenic attacks (Glasl et al. 2016; Lema et al. 2012; Lesser et al. 2004; Raina et al. 2009; Ritchie 2006). These microbial associations with corals change with the variation in the depth allowing the corals to obtain a wide range of nutrients from the surroundings (Hernandez-Agreda et al. 2016). Heatwaves in the oceans disrupt the symbiotic relationship between the coral and its associated microbes leading to the loss in physiological function and its nature (Glynn 1984; Eakin et al. 2016; Berkelmans et al. 2004). The increase in climatic change disrupts

the natural microbiome of the coral reef leading to the destabilization and emergence of pathogenic taxa ultimately leading to host mortality (Littman et al. 2011; van Oppen and Blackall 2019). It is clear that the change in climatic conditions directly affects the coral microbiome, and it can be considered an early warning signal (Bourne et al. 2008; Glasl et al. 2017; Lee et al. 2016).

In this review, we discuss the diversity of the coral microbiome and its beneficial effects on the reef system and survival mechanism, how these beneficial microbes act and change during various stress conditions like bleaching, high temperature, carbon dioxide, lower pH, etc., and the possible ways to mitigate these conditions.

5.2 Significant Terminologies

5.2.1 Holobiont

A eukaryotic host with all its associated microbial partners. This multispecies assemblage includes viruses, phages, eubacteria, archaea, fungi, and protozoa.

5.2.2 Hologenome

Genetic information is encoded in the eukaryotic host and all of its associated partners. This collective genome forms the theoretical genetic repertoire of a holobiont (definition by Deines et al. 2017).

5.2.3 Metaorganism

In order for a holobiont to function properly, it must have a stable hologenome, which is dependent on the hologenome's associated partners, their activity, abundance, and the transcriptionally active regions of their genomes all in balance. This results in host-microbe and microbe-microbe interactions that must be maintained in homeostasis to keep the holobiont stable. To underline this extremely dynamic functional condition (capacity) of a holobiont, we will in the following use the term "metaorganism" (Deines et al. 2017).

Microbiome refers to an "ecological community of commensal, symbiotic, and pathogenic microorganisms within a given host" (Lederberg and McCray 2001).

5.3 Coral Ecosystem

5.3.1 Coral Habitats

Corals are the richest marine ecosystem on the planet Earth. These are threatened due to pollution and rapid climatic changes (Hoegh-Guldberg et al. 2007; Graham et al. 2014; Mora et al. 2016; Casey et al. 2014). They have a diverse range of bacterial associates (Brown and Bythell 2005), and some of them are species-specific (Ritchie and Smith 1997; Rohwer et al. 2002). Fossil records show that the existence of reef-building corals dates back >400 million years in which the reef-building corals are thought to emerge at the end of 250 million years (Stolarski et al. 2011). Coral-associated microbes have some important physiological and ecological roles in the coral reef ecosystem. The nitrogen fixation process of microbes present in the coral reef was first proposed in 1987 (Williams et al. 1987). When the oxygen concentration is low intracellularly, the endosymbiotic eukaryotic dinoflagellate photosynthesis takes place (Lesser et al. 2004; Kvennefors and Roff 2009). Some of the coral-associated microbes can protect the host from predation by producing antibiotics (Ritchie 2006). It is found that 30% of the coral-associated bacteria have antibiotic capabilities (Castillo et al. 2001). Coral metaorganism is a collection of bacteria, fungi, and viruses (Knowlton and Rohwer 2003; Bang et al. 2018), and they also have a symbiotic association with the algal family Symbiodiniaceae (LaJeunesse et al. 2018). For the development of the three-dimensional structure of the massive calcium carbonate skeleton system of the host coral, more than 95% of the energy is obtained by exporting photosynthates from micro-algal endosymbionts (Jones et al. 1994). The bacterial community of the coral is highly sensitive to environmental changes (Reshef et al. 2006; Santos et al. 2014, 2015, 2016; Thompson et al. 2015).

5.3.2 Coral Complexity

Generally, the coral microbiome comprises living organisms like prokaryotes, microeukaryotes, viruses, and coral polyps (Hernandez-Agreda et al. 2017; Marcelino and Verbruggen 2016). The coral prokaryotic distributions are found to be too high in the coral skeletal system by the use of metabarcoding studies. The prokaryotic and eukaryotic organisms of the coral skeleton structure altogether are called endoliths (Marcelino and Verbruggen 2016; Marcelino et al. 2018). Some of the eukaryotes can create boreholes on the limestone of the coral skeleton (Marcelino and Verbruggen 2016; Yang et al. 2016; Wegley et al. 2007; Le Campion-Alsumard et al. 1995a). Among the microbiome, the endolithic algae play an important ecological role as a microbial agent for reef erosion (Tribollet 2008). Among them is the genus *Ostreobium* (*Ulvophyceae, Chlorophyta*) which is considered to be the most abundant of all (Vroom and Smith 2001). Mostly the balancing role of these algae is unknown. The microbial community changes with the change within the coral colony and also within the coral reef environment. Studies show that the community of the coral colony and reef exhibits distinct nature (Rohwer et al.

2002). The endosymbiotic dinoflagellates present on the coral polyp secretes mucus which contains the polymer of sulphated glycoprotein (mucocytes) (Brown and Bythell 2005). These mucous were made up of amino acids like serine, threonine, aspartate, glutamate, and glycine (Meikle et al. 1987, 1988). They also contain a small number of monosaccharides like arabinose and xylose which were believed to be produced during photosynthesis (Molchanova et al. 1985).

5.3.3 Coral Fungi

Several fungal species have been found in every marine habitat (Gao et al. 2010). The depth gradients and climatic conditions show variations in these types of microbes (Giovannoni and Stingl 2005; DeLong et al. 2006; Zinger et al. 2011; Barberán and Casamayor 2010). Mostly the geographical scales are limited to 100 s of km within the Pacific Gyre of North (Gao et al. 2010). Further studies showed that wood-inhabiting fungal composition is favored by temperature and salinity (Booth and Kenkel 1986). The first isolation and culturing of ascomycetes and basidiomycetes fungus were from the skeletons of hermatypic corals belonging to the Atlantic and Pacific oceans (Kendrick et al. 1982). By RNA analysis, it is found that there is a link between physiochemical properties and the active fungal composition of that particular area (Orsi et al. 2013). Endolithic coral fungi can develop resistance to environmental stress; some of them are symbiotic which helps in the nitrogen fixation to benefit the associated coral species (Wegley et al. 2007). Some of the coral-associated fungal species produce mycosporine-like amino acids which in turn prevent the corals from UV damage (Dunlap and Shick 1998). Like other marine microbes, marine fungi also has some similar qualitative biogeographic patterns even if there is a change in environment and dispersal mechanism (Duarte et al. 2016). It has been reported that the presence of various factors like temperature, and pH, helps in the strong structural development of hyphomycete morphospecies at the regional scale. Fungi can live symbiotically without harming the host coral along with associated coral microbiome.

5.3.4 Coral Algae

The skeleton of live coral contains high amount of eukaryotic green algae which is 16 times higher than that of the *Symbiodiniaceae* that is present in the coral tissue (Odum and Odum 1955). Among them are the green algae *Ostreobium* found to be present in most of the stony coral reefs (Marcelino and Verbruggen 2016). Most of the algae are found at >100 m below sea level and are also found in deep caves underwater (Odum and Odum 1955; Gonzalez-Zapata et al. 2018; Hoeksema 2012). Algae Dinoflagellate survives within the coral cells and provides the host with the energy they needed to perform most of their metabolic tasks (Muscatine 1990). Dinoflagellates belonging to the genus *Symbiodinum* commonly called zooxanthellae are referred to as reef-building corals (Freudenthal 1962).

Symbiodinums are well developed in balancing the sunlight absorbed in turn converting it into useable energy by the corals through photochemistry; thus, the fixed energy source (carbon) is utilized in the development of coral and calcification process (Goreau 1959; Muscatine 1990). More amount of oxygen is produced during the process is proportional to the calcification rate in the coral (Colombo-Pallotta et al. 2010). Fluctuations in the temperature and light condition lead to the destruction of the coral-algal symbiosis; this process is termed coral bleaching (Lesser 2011). Thus, the bleached corals are very difficult to regenerate even if they are prepopulated with the desired host *Symbiodinum* spp. (Jokiel and Coles 1977; Goreau and Macfarlane 1990; Meesters and Bak 1993; Ward et al. 2002). Records show that reef-building coral was found in the photic zone at about 165 m (Maragos and Jokiel 1986).

5.3.5 Coral Virus

The amount of virus present per milliliter of seawater is 10^6–10^8 which is higher than that of the other microbial cells present per milliliter (Wigington et al. 2016; Wommack and Colwell 2000; Fuhrman 2009). By the use of transmission electron microscopy, the virus that is present inside the cnidarian tissue was found to be 60 nm in diameter which is icosahedral in shape (Wilson and Chapman 2001). The sea anemones (stony corals) which are close relatives to the coelenterate are the first to show virus-like particles (VLP) (Wilson and Chapman 2001). Later, these viruses particles were characterized by comparing stressed and non-stressed coral animals where the normal non-stressed coral showed virus particles ranging from 30–40 to 50–60 nm in diameter, whereas stressed coral animal shows 40–50 and 60–80 nm in diameter along with more abundant viral particles (Wilson et al. 2005). Among 60 virus families, 58 of them live in corals around the world with 7 orders, 104 families, and 410 genera of viruses and were found to be recognized by the International Committee on the Taxonomy of the Virus (ICTV) (King et al. 2011). With recent advancements in data collection methods like metagenomics and transcriptomics, it is evident that all coral samples have the order *Caudovirales* with double-stranded DNA with particular three families *Siphoviridae, Podoviridae,* and *Myoviridae* (Lawrence et al. 2014). A recent investigation shows that the 24 coral reefs which were infected by the virus are mostly by temperate virus where the microbial densities are found in higher concentrations (Knowles et al. 2016). By using RNA-dependent RNA polymerase and shotgun sequencing methods, it is found that the vast majority of the ssRNA virus belongs to the family Picornaviridae, a virus that is known to affect marine protists (Culley and Steward 2007). The metagenomic analysis helps in the identification of the double-stranded DNA virus as a potential lysogen in the tropical coastal waters (McDaniel et al. 2014; Knowles et al. 2016). This virus also plays a very important role in the microbial evolution process called transduction (horizontal gene transfer method) (Rohwer and Thurber 2009; Paul and Sullivan 2005).

5.4 Coral Endoliths

5.4.1 Endolithic Algae

In the coral reef environment, endolithic microbes form the major component of the food chain (Hutchings 1986; Radtke et al. 1996). The first visible band appearance on the coral reef by an endolithic microbe was characterized in 1902 (Duerden 1902). More than 50–60% of the nitrogen available to the host coral was provided by the endolithic microbes says Ferrer and Szmant (1988). During the process of thermal bleaching, the process of translocating the photosynthetic carbon to the nearby host coral takes place (Fine and Loya 2002). Endolithic microbes are highly capable of nitrogen fixation and regeneration of nutrients to the coral reefs (Shashar et al. 1994; Cardini et al. 2014). Endolithic microbes that are associated with the coral skeleton systems include algae, fungi, bacteria, archaea, and viruses (Rosenberg et al. 2007; Schönberg and Wisshak 2012). Underneath the corals, there is a noticeable green band appearance formed by the green algae *Ostreobium* spp. (*Siphonales, Chlorophyta*) which were considered a coral symbiont (Kornmann and Sahling 1980; Del Campo et al. 2017). These are also found in the aragonite skeletons belonging to the coral reefs of the Caribbean, South Pacific, and Atlantic oceans which mainly include certain species like *Pocillopora* spp., *Stylophora* spp., *Acropora* spp., *Favia* spp., *Montastrea* spp., *Porites* spp., and *Goniastrea* spp., these organisms can penetrate dead as well as live carbonate coral substrates (Le Campion-Alsumard et al. 1995b; Zubia and Peyrot-Clausade 2001; Godinot et al. 2012; Halldal 1968). The green sulfur bacteria like *Prosthecochloris* are dominantly seen in *Isopora polifra* (coral spp.); some of the green sulfur bacteria were found in the tissue and mucus of the corals (Yang et al. 2016; Koren and Rosenberg 2006; Reis et al. 2009; Kimes et al. 2013; Li et al. 2015; Cai et al. 2017). By using the latest molecular techniques, it is found that these endolithic microalgae have varied diversity with 80 taxonomic units (Marcelino and Verbruggen 2016; Marcelino et al. 2017, 2018).

5.4.2 Endolithic Fungi

The endolithic fungi have the advantage of coral penetration and ultimately interacting with the *Ostreobium* cells; among these, the primarily isolated endolithic fungi that belong to *Ascomycota* and *Basidiomycota* are from the Caribbean and South Pacific (Le Campion-Alsumard et al. 1995b; Kendrick et al. 1982). The certain fungus will penetrate the polyp zone of the coral *Porites lobata* which in turn activates the defense mechanism resulting in the heavy deposition of the carbonate material giving a pearl-like appearance (Le Campion-Alsumard et al. 1995b). There are more than 10,000 species in the phylum Cnidaria which was studied for its abundance in fungal association (Zhang 2011). There is a threefold increase in the fungal sequence in the bleaching sample of the *A. millepora* by the metagenomic analysis method, yet the proper role of this fungi is unclear (Littman

et al. 2010). When the spatial distribution is disturbed in *A. formosa*, the fungi present in the healthy tissue develop into a new skeletal cavity (Yarden et al. 2007). Endolithic fungi exhibit a parasitic association rather than that of the saprophytic way of association with the corresponding coral by activating the defensive mechanism (Le Campion-Alsumard et al. 1995b; Golubic et al. 2005). The *Aspergillosis* of sea fans was believed to be caused by the dust-borne propagules that were introduced from the Sahara (Garrison et al. 2003). Along with the aspergillosis, it is believed that other opportunistic fungal infections of the sea fan are caused by the combination of more than one fungal species (Barrero-Canosa et al. 2013).

5.4.3 Endolithic Prokaryotes

The endolithic microbiome (prokaryotic and eukaryotic) performs various functions like providing nutrition to the coral, bioerosion, cycling of the nitrogen, etc. (Schlichter et al. 1995; Tribollet 2008; Miller et al. 2011). Based on the green band appearance on the coral skeleton and mussel shells, some of the first described marine *Cyanobacteria* are *Plectonema terebrans, Mastigocoleus testarum*, and *Halomicronema excentricum* (Le Campion-Alsumard et al. 1995b; Yamazaki et al. 2008). *Cyanobacteria* and other non-oxygenic phototrophic bacteria were present in higher abundance which can be seen by the appearance of the deeper darker green bands that were caused by the bacteriochlorophyll 2 pigments (Ralph et al. 2007; Magnusson et al. 2007). Targeted amplicon sequencing on 16SrRNA has identified more than 90 non-classified cyanobacterial operational taxonomic unit (OTUs) in 132 coral fragments (Marcelino and Verbruggen 2016). In coral *Isopora palifera*, the green sulfur bacteria *Prosthecochloris* was found to be in higher abundance (Yang et al. 2016). The distance decay relationship (DDR) measures beta diversity and heterogenicity habitat and was used to understand the decrease in the community similarity inside the skeletons of the corals (Nekola and White 1999; Anderson et al. 2011; Martiny et al. 2011).

5.5 Climatic Impact on Coral Reef

5.5.1 Effects of Climatic Change on Coral Ecosystem

There is an enormous change in the ocean temperature over the past 15 years also resulting in the disappearance of the arctic ice covers; it is also recorded that the precipitation is more variable with heavy rainfall, intense hurricanes, and early occurrence of the spring season (Johnson et al. 2011; Wernberg et al. 2011; Solomon et al. 2007). Most of these changes occur due to rapid climatic changes (Hoegh-Guldberg and Bruno 2010).

5.5.2 Bleaching-Associated Changes

The dinoflagellate symbionts zooxanthellae help the host by providing them with photosynthates along with coral calcification, due to extreme environmental conditions like radiation and high-temperature damage; this machinery leads to the overproduction of oxygen radicals leaving the symbionts to cellular damage and pigment degradation; this process is referred to as "bleaching" (Muscatine and Porter 1977; Lesser 2006). Heat stress damages the biological property of a coral holobiont (Littman et al. 2011). Once the symbionts were lost, more amount of solar radiation and the CO_2 enters the coral skeleton and helps the photosynthesis of endolithic algae which results in the harmful algal blooming during these bleaching periods (Fine and Loya 2002; Shashar and Stambler 1992; Diaz-Pulido and McCook 2002; Fine et al. 2006). The primary bleaching of the coral results in secondary bleaching of the nearby *Symbiodiniaceae* of the adjacent coral such a mechanism is called an optical feedback loop (Swain et al. 2018; Wangpraseurt et al. 2017). During these high temperatures caused by the irradiation, the lipid composition of the thylakoid membrane of the symbiont gets deteriorated; this compromises the photosystem II, paying the way for the increased production of nitric acid synthase which also tends to increase the bleaching of the coral reef (Tchernov et al. 2004; Trapido-Rosenthal et al. 2005). Metagenomic of the bleached coral microbiome shows increase in the carbohydrate utilization and processing along with the rapid increase in the virulence-associated gene (Littman et al. 2011; Thurber et al. 2009). If there is an increase in the wavelength of visible light (400–700 nm) and ultraviolet light (290–400 nm) it has also been linked to coral bleaching (Hoegh-Guldberg and Smith 1989; Brown et al. 1994; Fitt and Warner 1995).

5.5.3 Response to Climatic Disturbances

When the coral microbiome is exposed to ocean acidification or higher temperature, there is a shift from beneficial bacteria to more pathogenic ones like *Endozoicomonas* to *Alteromonadaceae* and *Vibrionaceae* (Bourne et al. 2016; Littman et al. 2011). Thus the variation in the species dominance and evenness disturbances shows the coral has undergone a stress response (van der Voort et al. 2016). It is found that not only stressor like climate or temperature changes the microbiome of the coral, but coming in close contact with macroalgal spp. tend to shift the microbiome to a macroalgal microbiome. This was well studied in *A. millepora* and *Seriatopora hystrix* microbiomes that they maintained stability even under lower pH and higher temperature, whereas the *S. hystrix*'s microbiome showed a considerable change when compared with similar taxa like *Foraminifera* and crustose coralline algae; this sudden shift leads to diseases like black band disease, yellow band disease, dark spot syndrome, etc., especially when the corals were exposed to multiple stressors (Zaneveld et al. 2016). During high temperatures, the nitrogen fixation process continues to increase, dramatically changing the nutrient balance in the coral holobiont, leading to an induced phosphate starvation state in

the *Symbiodiniaceae*; this ultimately leads to the poor translocation of photosynthates to the host coral also termed as selfish symbiont (Baker et al. 2018; Morris et al. 2019). When there is a dimension of corals at a specific reef due to bleaching, it signals a positive feedback loop which increases algal dominance (Rädecker et al. 2015). There will be increase in dissolved organic carbon (**DOC**) which interacts with temperature and accelerates the bleaching by inducing the proliferation of diazotrophs, thus increasing DOC leads to a further decline in the coral mortality by forming **hypoxic zones** (Pogoreutz et al. 2017; Rädecker et al. 2015; Silveira et al. 2017).

Changing climate results in a destabilized microbiome, dysbiosis, etc. which ultimately leads to the development of a more stable state characterized by increased mortality, bleaching events, and disease (Egan and Gardiner 2016; van Oppen and Blackall 2019). Certain corals show little resistance to stressors like lower pH and higher temperature (Meron et al. 2012; Epstein et al. 2019). Certain corals can revert to their original state once the stressor is gone, whereas some change irreversibly which can be either beneficial or detrimental to the holobiont (Bourne et al. 2008; Tracy et al. 2015). The effects of coral bleaching have been increased due to climatic change and various stress factors which tend to decrease reef framework and depress reef accumulation rates.

5.6 Coral Reef Management

5.6.1 Reef Microbiome Recovery and Restoration

Most of the coral degradation signs will show up once the coral reef has reached its advanced stage of decline; hence, it releases certain stress markers (Glasl et al. 2017). In the early decades, microbes act as an indicator system for various responses to environmental disturbances (total coliform count in drinking water (Faust et al. 2015; Glasl et al. 2019; Tallon et al. 2005). Coral reef conservation and restoration were primarily formed to prevent water quality and to handle massive coral depletion due to climate change in efforts to restore it (Hughes et al. 2017; Silveira et al. 2017). Most of the restoration practices were done by coral aquaculture gardening or by fragment transplantation onto the specific reef (Rinkevich 2008). Microorganism plays an important role in the maintenance of the coral animal and the proper functioning of the ecosystem; thus, it is important in preserving the threatened species (West et al. 2019). The recovery and loss of coral reefs from eastern pacific sites have been recorded for 10–28 years showing both 100% elimination to 100% total recovery (Wellington and Glynn 2007), whereas in costa Rica, live corals have been increased by 4–23% from 1987 to 2002 with other similar species in which is found in the pre-disturbance community utilizing sexual regeneration. In Panama, asexual regeneration has been observed in *Pocillopora elegans* and *Pavona clavus*, whereas *Acropora* which is found in the Arabian gulf regenerates sexually (Riegl 2002). Transplanting asexually regenerating colony tissue or replacing a sexually developing larva in the disturbed place tends to

increase live coral cover (Harrison and Wallace 1990; Richmond 1997). To support proper regeneration of the coral reef regrowth, it needs stable and firm lighting with relatively sediment-free substrates. Most importantly the substrates should be free from any other taxa (Bellwood et al. 2003).

Developing coral stocks with higher stress tolerance by inducing evolutionary changes to the coral microbiome community is referred to as assisted evolution (Van Oppen et al. 2017). The highly successful recovery rate was seen in *Acropora, Pocillopora,* and branching *Porites*, whereas the *Porites* spp. can survive for longer periods. When these taxa are found in the corals, they can regenerate their coral cover back to normal structure (Rogers et al. 2008). The ecosystem-based management (EBM) approach suggests removing local stressors from the affected area will increase coral health and improves coral health (Marshall and Schuttenberg 2006; Marshall et al. 2006). Certain studies demonstrated that the application of specific phages can act only over the coral pathogen and does not affect the integrity of the resident microbiota (Atad et al. 2012; Cohen et al. 2013; Efrony et al. 2009).

5.6.2 Management and Conservation of Coral Reefs

The change in the climate either has direct changes (like warming, aridity) or indirect effect (elevated carbon dioxide) on the coral microbiomes' diversity, distribution, and functions; one of the major change that occurs were the change in the nutrient cycle (Singh et al. 2010). Thus, if there is a loss in the microbial diversity, it leads to dysfunctionality, low stability, and increases in the unknown consequences (Delgado-Baquerizo et al. 2016). Apart from these changes, the microbes have an indirect effect on the environment by the production of greenhouse gases, even though there is enough evidence showing the link between climate change and the microbes involved the focus-based studies on microbes for climatic change is less studied (Cavicchioli et al. 2019). Beneficial Microorganisms of Corals (BMC) can be incorporated into coral reefs through methods like microencapsulation and nanoparticles to feed adult corals heterotrophically; the use of biodegradable substrates like alginate act as a bio-friendly encapsulation of BMC to deliver it to the respective coral (Martínez Cruz et al. 2012). Human-Assisted Evolution (HAE) helps naturally enhance their stress tolerance including random mutation, acclimatization, and also drastic changes in microbial symbiont communities (van Oppen et al. 2015). Before the field application, it is advisable to test in a controlled experimental system; it is also to be noted that the selected BMC should not be a pathogen to the microbiome (Sweet and Bulling 2017). The variation in the microbial symbiont occurs through switching of the symbiont, shuffling of the symbiont, and also through horizontal gene transfer methods; this helps in the development of the overall population fitness of the coral generations (Torda et al. 2017; Quigley et al. 2019). The proper developmental phase should be made together by marine ecologists and coral reef conservationists to know in-depth about the long-term survival, preservation, and conservation of these diverse environments (Kelly et al. 2018; West et al. 2019).

5.7 The Way Forward

The benefits of the coral holobiont for the healthy survival of the coral reef is suffering due to rapid change in the climate and other anthropogenic disturbances; when stress is high, they lose their natural tendency to benefit the environment (Hughes et al. 2017a). But the microbial response of different spots should be studied separately to get a good insight for proper long-term conservation (Hutchins et al. 2019). Advanced research methodology should be preferred to gain access to microbial diversity of the coral reef, rather than a DNA-based molecular approach; most of the findings were performed and analyzed based on 16S rRNA analysis. Combining metatranscriptomics, with advanced microscopic techniques, should be used to validate and record the response of the microbial holobiont under varying stress situations finally leading to the identification of the fluxes over the ecosystem (Trevathan-Tackett et al. 2019). Recent findings show that there is a chance of increasing the ability to resist climatic stress response by modifying microbial holobiont or by the application of coral-specific probiotics (Trevathan-Tackett et al. 2019). Lastly, the interdisciplinary approach should be preferred for the in-depth understanding of climate-linked coral reef microbiome variations.

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A Paradigm Shift in the Role of the Microbiomes in Environmental Health and Agriculture Sustainability

6

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Abstract

In recent days, environmental pollution has been a major global setback, which emanates from the increasing human population and anthropological activities. The microbiomes are ubiquitous and have occupied every single part of our natural environment. They are very diverse in carrying out their activities in the environment such as their metabolism and association with other flora and fauna components, processing and monitoring the environment health besides upgrading our agriculture system. In this chapter, we detail the significant role of microbiomes in environmental health and agriculture sustainability. Here, we try to investigate the ability of the microbiome to transform contaminants into toxic-free or nonhazardous bioactive compounds in other to improve the health of the environment and emphasize the beneficial roles played by the microbiome in agriculture sustainability. Many authors have reported that biotransformation is one of the key elements that can be used to scan and treat contaminants in our environment. Studies reported the effectiveness of microbes in the degradation of crude oil in contaminated soil. The substantial aid of biofertilizers in improving the health of the environment and agriculture sustainability was also reported by

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many studies. Therefore, we suggest a holistic approach to solving and maintaining a healthy environment for all.

Keywords

Biotransformation · Biofertilizer · Bioremediation · Endophytes · Phyllosphere · Microbiome

6.1 Introduction

Any collection of microbiota is referred to as a microbiome. They are found everywhere, including in or on the body of an organism. All animals and plants have a microbiome that fosters key roles in their health and immediate surroundings. Microbiomes that live in symbiotic associations with organisms frequently provide their hosts with useful biological services. They are often referred to as useful microbiome because of their mentioned effect.

Now, more than ever, the productivity of plants and animals for agriculture sustainability has become the focus of utilizing microbiome natural adaptive potentials while causing the least amount of environmental disruption possible. This new strategy has the potential to replace the usage of hazardous agrochemicals with the deployment of symbiotic microbes in other to enhance crop and livestock nutrition with less environmental pollution. According to Noble and Ruaysoongnern (2010), because plants are genetically dependent on the favorable tasks supplied by their symbiotic cohabitants, it is possible microbes can be widely used in sustainable agriculture. The plant microbial symbioses have agronomic potential, which is derived from an examination of their environmental effects studied most extensively for nitrogen fixation (Franche et al. 2009). Currently, a broad array of microbial species preparations can be utilized to boost the production of crops. Beneficial symbiont impacts also have increased in tandem with their host specificity, making it ideal for nutritional types to colonize their plant-specific hosts (Provorov and Vorobyov 2009).

The microbiomes have a significant impact on reducing chemical contaminants in the environment by converting these contaminants into harmless bioactive materials (De Lorenzo 2008; Ufarté et al. 2015). In terms of mass balance, the microbial transformations are normally measured to be the most vital degradation processes, as compared to other types of transformation processes. However, there is rising proof that the increasing levels of environmental chemical pollution have a direct negative effect on the health of the environment, (Diamond et al. 2015) for example, the invertebrate biodiversity losses in streams polluted by pesticides (Beketov et al. 2013).

Given the growing need to address environmental and human health issues, microbes can no longer be relied on to reduce contaminant levels in a passive manner (Bernhardt et al. 2017). Rather, we should capitalize on their abilities to purify our environmental resources by developing engineered systems that are

optimized for effectively wiping out these anomalies or by modifying new chemicals to preserve their activities and make them easily transformable to harmless compounds in the environment. It is therefore essential to tackle environmental pollution practically and cost-effectively by employing microbial remediation, which is a process for reducing environmental contaminants by using microorganisms and their metabolites or other by-products.

The approach of next-generation sequencing (NGS) and whole metagenome shotgun (WMS), conversely, are used to investigate the overall genomic content of a sample and uncover novel metabolic functions of microorganisms. Furthermore, when compared to 16S rRNA gene sequencing-generated taxonomic profiles, a higher taxonomic resolution can be achieved (Liu et al. 2018; Megharaj et al. 2011). The thorough characterization of microbial diversity, metabolic roles of microorganisms, and other factors that influence their metabolism could help researchers figure out the genetic pool of enzymes essential for pollution tolerance and survival (González-Toril and Aguilera 2019; Techtmann and Hazen 2016). In some cases, polluted sites may already have microorganism species that tolerate or transform the contaminant. However, because of a deficiency in a suitable carbon source, those aren't necessarily the most numerous species (Techtmann and Hazen 2016).

6.2 Microbiome Pollutant Transformation

Surface runoff and wastewater treatment release environmental waste products into the environment regularly. People migrate to anaerobic conditions and aquatic environments that receive wastewater effluent. Anaerobic bacteria can alter the structure, destiny, and transit of these newly found pollutants once they reach their destination. Many of these transformed contaminants are new micropollutants that have not yet been thoroughly investigated. Their ability to retain waste product activity is a source of concern as in the case of pharmaceutical activities (Haddad et al. 2015). The phenylmethyl ether drug, known as venlafaxine, which is responsible for a serotonin-norepinephrine uptake inhibitor, has been reported to be demethylated by anaerobic microorganisms. However, its metabolite is a prescription serotonin-norepinephrine uptake inhibitor (Gasser et al. 2012). These effects point to some important roles played by the microbiome in environmental and human health.

Any advanced approaches aimed at reducing contaminants to environmental exposure will require an understanding of the causal links between contaminant removal, the key driving agents of biotransformation at reduced accumulation, and how their presence and activity are affected by environmental conditions.

Any recorded contaminant biotransformation outcome is the result of a complex interaction of multiple factors, including the pollutants' bioavailable concentrations, the composition, and capabilities of the microbial community, and the existence of suitable substrates and electron donors or acceptors (Meckenstock et al. 2015; Poursat et al. 2019). These factors are determined by various environmental factors

(e.g., redox conditions, temperature, humidity, nutrient status, chemical exposure, microbial residence time, etc.) and thus exert control over contaminant biotransformation. The extent to which each contaminant is biotransformed is determined in large part by those factors, as well as its intrinsic recalcitrance, or the ability to interact with specific enzymes which are determined by their chemical structure. Many waste products, for example, contain phenylmethyl ether substructures that determined demethylation substrates in anoxic environments. The phenylmethyl ether structure occurs naturally in lignin, and lignin metabolites containing phenylmethyl ether have long been recognized as carbon sources for anaerobic microbes (Frazer and Young 1985).

Over decades of years now, a substantial body of biotransformation knowledge has been accumulated for many major legacies in treating contaminants, primarily from the perspective of bioremediation (Tratnyek et al. 2020). Contaminants in this environment are metabolically destroyed, meaning they can be utilized by microorganisms as carbon or other nutrition sources, as well as electron donors or acceptors, and therefore drive the overall biodegradation setting.

6.2.1 Bioremediation

Among the existing environmental pollution control strategies, bioremediation technology is gaining traction and is quickly becoming a research hotspot because of its high efficacy, low cost, ease of operation and management, and lack of environmental impact. Besides, some environmentally friendly heavy metals passivation rejuvenation technologies and modern agronomic technologies can alter the occurrence of heavy metals elements in the soil to stabilize their effective state and potentially stop the migration and transformation of biologically effective means of ecological heavy metals. This has evolved as a key area of advancement for environmental in situ restoration technology. Bioremediation technology usually employs the knowledge of plants, animals, fungi, and microorganisms strategies in solving existing environmental problems.

6.2.1.1 Microbial Remediation

Microbial remediation technology employs the use of native or artificially domesticated microorganisms with specialized activities to lower the activity of contaminants or convert them into harmless chemicals using their metabolism under suitable environmental circumstances. Microbial remediation is primarily separated into bacterial and fungal remediation, with biosorption, bioconcentration, and biotransformation as the primary remedial principles.

According to prior research in the field of microorganisms, there are hundreds of different kinds of bacteria that may digest crude oil in contaminated soil. *Pseudomonas* bacteria, alkali-producing rod bacteria, colorless rod bacteria, *Trichoderma* fungi, *Penicillium* fungi, *Aspergillus* fungi, and other fungi, for example, have substantial effects. These fungal microorganisms exhibit excellent results in the cleanup of crude oil-contaminated soil. Conditions apply to the usage of

microorganisms. Only enzyme-active fungal microorganisms that disintegrate quickly and have a high level of environmental adaptability can effectively remediate polluted soil. And, according to biological specialists, the combined action of several fungal-bacteria greatly outweighs the breakdown effect of a single fungal microorganism (Yang et al. 2011). Microorganisms have the benefits of small individuals and large specific surface area, rapid reproduction and strong metabolism, a broad array of types and distribution, high adaptability, and ease of cultivation, among others. Microbial remediation includes limitations such as low genetic stability, which makes it easier for contaminants to mutate, the inability to eliminate toxins, and the need to compete with indigenous strains, which makes it easy to be influenced by the environment.

6.2.1.2 Bacteria Remediation

The most prevalent way in microbial remediation technology is to use microorganisms to clean up heavy metal-contaminated soil. There are abundant bacteria in the natural world, particularly in soil, and provide a rich source of research materials for this technique. In general, it has been shown that bacteria that can withstand high amounts of heavy metals from polluted soil may be identified and purified and engineered for soil repair (Tuo et al. 2021). Xiaoming et al. (2018) demonstrated the isolation of *Rhodobacter sphaeroides* in the oil field after improving the cultural conditions. The microorganisms were employed to treat simulated lead-contaminated soil at various pollution levels, and the most optimal temperature, pH value, and inoculums amount of the cultivation substrate were determined. Similarly, Valentina (2005) also identified microbial strains for bioremediation of hazardous heavy metals from metal-polluted soil, mud, and water.

According to the findings, first and secondary screening yielded 72 species of acidophilic thermophilic-altered microorganisms with metal resistance and biosorption capacity. PSB (phosphate-dissolving bacteria) were isolated from coral, seaweed, and mangrove and tested for their ability to detoxify heavy metals (Kailasam et al. 2018). A bacterium that makes extracellular polysaccharides was isolated using 16S rRNA to identify it as *Bacillus cereus* vk1. The bacteria can successfully adsorb Hg^{2+}, providing a bioremediation method for Hg^{2+}-polluted ecosystems. Kang and So (2016) again investigated the link between heavy metal resistance and antibiotic resistance in ureolytic bacteria and discovered that heavy metal resistance is closely associated with antibiotic resistance in these isolates.

In comparing both the chemical and the physical treatment procedures, bioaugmentation is more practical and cost-effective (Hussain et al. 2019; Tao et al. 2017). It is also noted that the addition of lipophilic bacteria can result in the achievement of bioaugmentation (Abena et al. 2019). Oleophilic bacteria can exist in an array of petroleum-contaminated habitats, including saltwater, coasts, sludge, and soil (Kumari et al. 2018). They may be able to survive, but only on hydrocarbons as a result assists in degrading or mineralizing dangerous and hazardous petroleum pollutants (Bacosa et al. 2012; Lee et al. 2018).

In various polluted situations, we can discover several species of bacteria that degrade. DNA isotope probing (DNASIP) technique is used to determine the types

and soil organisms' actions (Wang et al. 2021). *Actinomycetes* are a prevalent phylum in soil contaminated with polycyclic aromatic hydrocarbons. In contaminated soil in the Philippines, *Acidovorax*, *Rhodoferax*, *Hydrogenophaga*, and *Polaromonas* were discovered. In a typical contaminated soil, several hydrocarbon products and *Acidobacteria* coexist (Sui et al. 2021).

Several bacteria, such as *Rhodococcus, Pseudomonas,* and *Scedosporium species,* have been shown to break down petroleum pollutants in studies (Pi et al. 2017; Yuan et al. 2018). Bacteria break down hydrocarbons mostly through aerobic mechanisms (Wang et al. 2010). Hydrocarbon catabolism is often expedited when oxygen serves as an electron acceptor (Cao et al. 2009). Degradation is mediated in aerobic mode by utilizing redox reactions, hydroxylation, as well as dehydrogenation. Cytochrome P_{450}, monooxygenase, peroxidase, dehydrogenase, dioxygenase, and hydroxylase are some of the enzymes that aid in the biodegradation of hydrocarbons (Wang et al. 2010; Huan et al. 2019). *Rhodococcus* species, *Pseudomonas* species, and *Acinetobacter* species are the bacteria that have the greatest impact on the breakdown of petroleum pollutants. According to research, cytochrome P_{450} enzyme or non-heme iron oxygenase (AlkB) may oxidize short- and medium-chain alkanes (C5-C16). Alkane long-chain oxidation is mediated by monooxygenase putative flavin binding (AlmA) and monooxygenase long-chain alkane (LadA) (Liu et al. 2021).

6.2.1.3 Mycoremediation

In fungal remediation, the ability of some fungi in the soil ecosystem to digest contaminants is employed to clean up contaminated soil. Some fungi are super-enriched organisms that can absorb enormous amounts of heavy metal ions from the environment and store them in the fruit body to help the soil heal. Mohammadi et al. looked examined fungal populations in soil samples from lead and zinc contaminated locations in Zanian Province, Iran (2017). Microflora's cadmium, lead, and zinc effects were determined by measuring the "minimum inhibitory concentration following exposure to escalating concentrations of heavy metal chlorides," while their copper resistance was established by evaluating the total metal adsorption capacity after combustion. Heavy metal biosorption is obligate for halophilic fungus, according to Bano et al. (2018). Fungi that can clean up heavy metal-polluted soil are usually adaptable to their surroundings. They may have devised strategies to shield themselves from the dangers of heavy metal (Bazzicalupo et al. 2020).

For example, mycorrhizal fungi contain mycelia that grow into the soil, thereby increasing the surface area of plant roots (Trellu et al. 2016). Bissonnette et al. (2010) found that mycorrhizal inoculation improves the capacity to take up Cu^{2+}, Cd^{2+}, and Zn^{2+}. Endophytic mycorrhizae can aid in the development of heavy metal ion resistance in host plants. Acidification, the synthesis of chelating agents, iron transporters, organic acids, and the activation of metal phosphates are the key ways that plant-endophytic mycorrhizae synergized. When heavy metal levels in the soil approach dangerous levels, mucus generated by the fungal cell wall can mix with polyphosphate and organic acid ions in the fungal tissue to bind the heavy metal ions

and restrict mobility. It was found that arbuscular mycorrhizal fungi's adsorption capability on Mn^{2+}, Zn^{2+}, and Cd^{2+} was 1.6%, 2.8%, and 13.3% of their dry weights, respectively (Jin et al. 2018). Furthermore, after the fungus infects the plant roots, the number and composition of root exudates alter, impacting heavy metal oxidation in the rhizosphere (Niu et al. 2011).

6.2.1.4 Integrated Remediation

Some methods are developed to allow rhizosphere nitrogen-fixing bacteria and leprosy plants to thrive in varying amounts of heavy metal solutions containing Cr (VI) and Cd(II) (Sobariu et al. 2017). In the bacteria-plant system, nitrogen-fixing bacteria may increase Jatropha's seedling growth, root length, stem length, and dry biomass, while the plant's reaction to heavy metals improves, implying that the two have a good symbiotic relationship and can build a reliable joint remediation system. Alfalfa phytoremediation, pseudomonas bioremediation, and bioaugmentation facilitated phytoremediation (growing alfalfa plants in soil infected with *Pseudomonas aeruginosa* to jointly repair metal pollutants and hydrocarbon-contaminated soil) have the best benefits, according to Agnello et al. (2016).

The combined plant-microorganism remediation approach improves plant root systems while oxidatively degrading crude oil and other pollutants by using the complementary link between plants and soil microorganisms (Fig. 6.1). The effect of the conventional microbial remediation technique is insignificant when the amount of crude oil is too tiny to support the survival and growth of soil microbial. To some

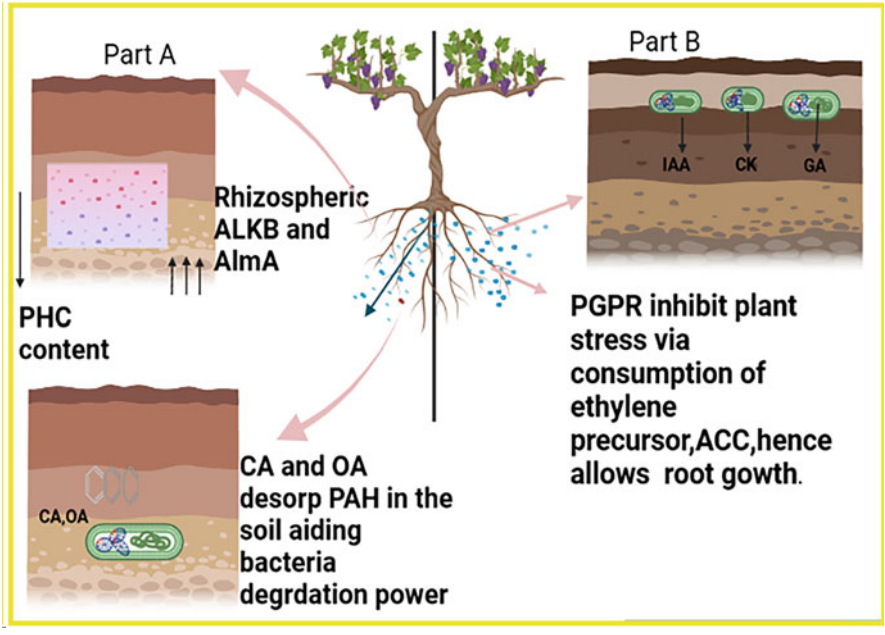

Fig. 6.1 Plant microbiome interaction

extent, the oxygen absorbed by green plants and some chemical products formed by their rhizomes can aid in the decomposition and destruction of soil microorganisms. As a result, it is possible to combine traditional biodegradation technology with integrated plant-microorganism remediation technology to boost soil remediation capabilities by combining their benefits. The effect of combined plant-microorganism remediation technology is superior to standard biodegradation technology in the same soil environment (Yue et al. 2014). The current integrated plant-microorganism remediation technology, on the other hand, has a limited application range because it only uses plants to remediate and optimize soil quality; plant growth is usually affected by climate, environment, and soil quality; soil remediation effect is poor when only one type of plant is used; recycled dead leaves and branches must be treated, and so on (Zuo et al. 2020).

During rhizoremediation, major plant-microbe interactions occur. Plant root exudates stimulate hydrocarbon-degrading bacteria and aid in the desorption of pollutants to bound to soil particles, increasing rhizobacteria's access to them in part (A). In part (B), rhizosphere microorganisms enhance plant development by producing plant hormones and degrading 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of the stress hormone ethylene, among other methods. PHC stands for petroleum hydrocarbons; alkB stands for alkane monooxygenase; AlmA is a flavin-binding monooxygenase; OA stands for oxalic acid; CA stands for citric acid; PAH stands for polycyclic aromatic hydrocarbon; PGPR stands for plant growth-promoting rhizobacteria.

Electrospun cyclodextrin fiber (CD-F) can encapsulate bacteria for bioremediation, according to Zuo et al. (2020). They encapsulated bacteria in an electrospun cyclodextrin fiber matrix to treat wastewater as a result the bacteria/CD-F had a greater pollutant removal potential than free bacteria. The reason for this is because CD-F fiber biological composites have natural and nontoxic qualities. The substance improves the survivability of bacteria, and bacteria can use CD as an additional carbon source to help them proliferate. New bio-composite materials with bioremediation potential are developed using the notion of bacterial cell encapsulation for the treatment of heavy metal contamination.

According to a study, it is vital to use effective degrading bacteria to break down crude oil pollutants while increasing the electric charge and electric field to decompose and degrade crude oil pollutants. The crude oil pollutants may be quickly dissolved using DC voltage, which will help to improve soil quality and speed up the decomposition of crude oil pollutants (Dongyi et al. 2010).

The advantages of physical, chemical, and bioremediation methods can be integrated using a combination of the three. Physical and chemical technology can lessen the inhibitory effect of toxicity on microorganisms, while microbial restoration can reduce the remediation burden both physically and chemically. Zhi-Yong et al. (2019) and colleagues coupled EDTA-assisted electrokinetic remediation techniques with biodegradation technology to treat crude oil and lead-contaminated soil. The toxicity of EK treatment with EDTA has been reduced significantly, allowing microorganisms to continue degrading pollutants. Direct microbial treatment will limit microbial development when the original hazardous metal

concentration is high, whereas direct use of physical and chemical procedures will result in high costs and time-consuming operations; therefore, the two can be combined to achieve economic and environmental harmony.

6.3 Beneficial Microbiome Diversity: Application in Environmental Health

Extreme habitats are one-of-a-kind ecosystems that are home to a varied range of bacteria that can live in diverse situations. Microbiomes have been found in many environments, such as high and low temperatures, hypersalinity, water scarcity, and high and low pH. Extremophiles have evolved adaptive traits that allow optimal growth at one or more environmental extremes, whereas polyextremophiles grow ideally under many environments. Temperature (psychrophiles: 2–20 °C; thermophiles: 60–115 °C), salinity (2–5 M NaCl; halophiles: 2–5 M NaCl), and pH (acidophiles: 4 acidophiles; alkaliphiles: >9) are all factors that these extremophiles can thrive in. Thermal springs are unique ecological niches among different harsh settings, containing both mesophilic and thermophilic archaea and bacteria (Saxena et al. 2015; Yadav et al. 2015a, b).

Microbiota phylogenetic characterization is done for geothermal springs all around the world. In India, thermal springs like Bakreshwar, Balarampur, Chumathang, Manikaran, and Vashisht provide an uncommon environment for thermophilic microorganisms (60–100 °C), which can be potential sources of novel genes, alleles, and microbiota (Suman et al. 2015; Sahay et al. 2017). The segregation of a broad range of psychrophilic/psychrotrophic microbiomes has resulted from the exploration of low-temperature habitats. The frigid habitat of the Indian Himalayas provides a niche for the psychrotrophic microorganisms in agricultural and biotechnological industrial uses (Yadav et al. 2017a, b, c, d). Novel colors (as food additives), extracellular enzymes (amylase, cellulase, chitinase, laccase, lipase, pectinase, protease, xylanase, galactosidase, and glycosidase), exopolysaccharide synthesis, and antifreeze chemicals are all potential bioresources for psychrophilic microorganism or biocontrol agents in extreme cold and high-altitude habitats (Yadav et al. 2015a, b).

In deserts, water scarcity/low moisture conditions combined with high temperatures result in the enrichment of microbial populations that can withstand temperature and drought extremes. Poor soils with little organic content and limited levels of accessible inorganic nutrients are characteristic in such locations. Desert microbiomes are responsible not only for the neogenesis and enhancement of soil structure but also for productivity, biogeochemical element cycling, and ecosystem balance (Verma et al. 2014). Under rainfed conditions, drought-tolerant microorganisms from hot deserts were isolated and characterized as plant growth promoters (Verma et al. 2017). PH extremes have an impact on microbial population growth and enhanced soil productivity. The availability of key micronutrients such as P, Ca, Mg, and molybdenum is harmed when the soil is acidic. Mangrove ecosystems, which are typically nutrient-deficient, notably in terms of N_2 and P,

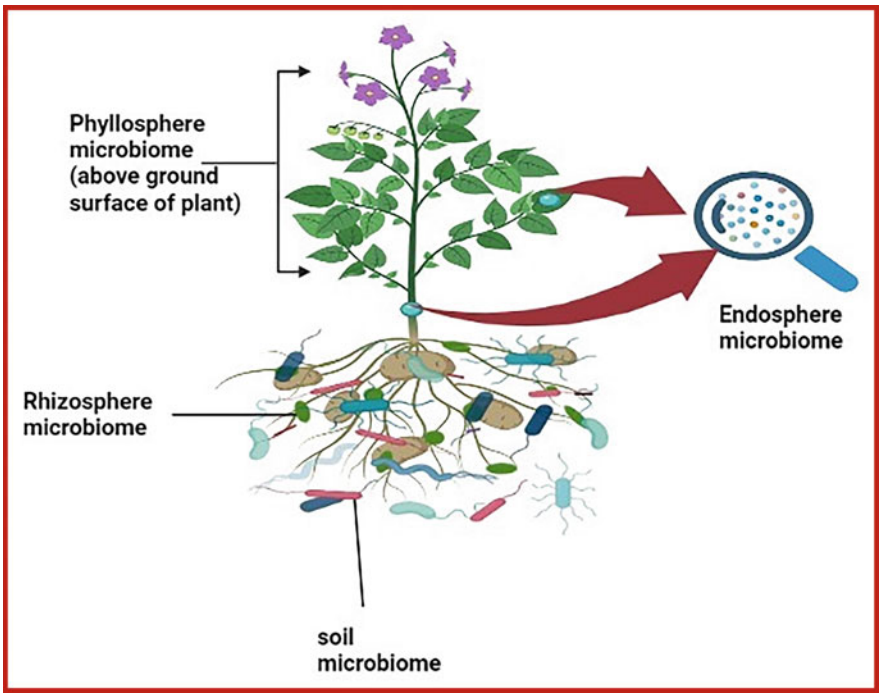

Fig. 6.2 Microbiome compositions of plants

are another useful extreme environment. However, mangroves are quite productive, because of microbial activity that results in large nutrient changes (Yadav 2017).

Rhizospheric microorganisms (dwelling near the roots in the soil), epiphytic microbes (comprising the endophytic microbes), and phyllosphere organisms are the three types of plant microbiomes. In general, three types of plant-microbe interactions are considered: epiphytic, endophytic, and rhizospheric interactions. The zone of soil is the rhizosphere where roots influence microbial activity by releasing substrates (Fig. 6.2). The ability of rhizospheric bacteria to bind to root surfaces allows them to gain the most advantage from root exudates. Several factors, for instance, soil form, soil pH, and other environmental circumstances surrounding any plants have influenced the population and abundances of rhizospheric microorganisms. From the rhizosphere of various crop plants, several microbial species from diverse genera were discovered, including *Bacillus*, *Arthrobacter*, *Aspergillus*, *Acinetobacter*, *Azospirillum*, *Enterobacter*, *Burkholderia*, *Flavobacterium*, *Haloarcula*, *Halococcus*, *Haloferax*, *Paenibacillus*, *Methylobacterium*, *Piriformospora*, and *Penicillium* (Verma et al. 2014, 2017; Yadav et al. 2017a, b, c, d; Suman et al. 2016).

Phyllosphere provides a common and unique environment for microbe-plant synergy. Plant parts, particularly leaves, are exposed to dust and air currents, resulting in the growth of characteristic flora on their surface, which is aided by

cuticles, waxes, and appendages, which aid in microbe attachment (Fig. 6.2). Phyllospheric bacteria may survive or multiply on leaves to varying degrees depending on the number of material impacts in leaf diffusates or exudates. The phyllosphere microbes may engage in an essential role in suppressing airborne infections that cause plant disease. Extremophiles are microbes that can endure extremes of temperature (5–55 °C) and UV light, such as those found on leaf surfaces. Many crop plant's phyllosphere harbors many microbes, including *Achromobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Flexibacterium*, *Methylobacterium*, *Micrococcus*, *Micromonospora*, *Nocardioides*, *Pantoea*, *Streptomyces*, *Pseudomonas*, *Planomonospora*, *Penicillium*, and *Xanthomonas* (Mukhtar et al. 2010; Meena et al. 2012; Dobrovolskaya et al. 2017).

Most plants host diverse communities of microorganisms including bacteria, fungi, protists, and archaea. A variety of microbes normally colonize the above-ground parts of plants, known as epiphytes or phyllosphere microorganisms. They normally inhabit leaf surface waxes and form colonies. Endophytes (microbes recovered from inside plant tissues) are microorganisms that colonize the interior parts of plants, including the root, stem, or seeds, without causing harming the plant host. Rhizospheric microorganisms colonize soil zones where roots release substrates that influence their activities.

Endophytes (microbes recovered from inside plant tissues) are microorganisms that inhabit the inner portions of the plant, such as the inner root, stem, or seeds, without hurting the host plant (Fig. 6.2). Endophytic microorganisms penetrate host plants mostly through wounds that arise in nature and when the plant grows, as well as through the hair root and epidermal junctions. Endophytes can be passed down either vertically (from parent to child) or horizontally (from offspring to parent) (among individuals). Chemotaxis, or the movement of microbes toward root exudates, leads to attachment in the rhizosphere. The favored site of attachments and subsequent penetration is the apical root zone with a thin root layer, including the cell elongation zone and the zone of rhizosphere with small fissures generated by the formation of lateral roots. Endophytic colonization of plant roots may be aided by microbial characteristics. Microbes must create cellulolytic enzymes, such as endoglucanases and endopolygalacturonidases, to hydrolyze the exothermal walls to penetrate (Suman et al. 2016). Most plant species have entophytic microorganisms that range from symbiotic to somewhat harmful within their living tissues. From various host plants, a great number of entophytic microbes have been identified, including *Achromobacter*, *Burkholderia*, *Curtobacterium*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Microbispora*, *Streptomyces*, *Pantoea*, *Pseudomonas*, *Serratia*, *Planomonospora*, and *Nocardioides* (Verma et al. 2015a, b; Rana et al. 2016; Yadav et al. 2016).

Salinity, droughts, acidification, fluctuations of low and high temperature, soil metals, and saline are all abiotic stressors that cause significant yield loss in crops. Many research on microbial diversity in severe conditions have been published, including low temperatures (Yadav et al. 2017a, b, c, d; Yadav 2015; Shukla et al. 2016), high temperatures (Yadav et al. 2015a, b; Sahay et al. 2017), saline soil, drought, acidic soil, and alkaline soil (Yadav 2015). Plant development-promoting

properties may exist in microorganisms isolated and sorted from harsh environments, allowing these abiotic stress-tolerant bacteria to be employed for plant growth under the right abiotic stress conditions.

6.4 Microbiome in Agriculture Sustainability

Bacteria, fungi, protozoa, microalgae, and viruses are some of the microbes commonly found in agricultural environments. These organisms are abundant in the soil, water, food, animal intestines, and a variety of other places. Various microbial environments show an immense variety of biochemical and metabolic properties that have evolved in microbial populations on account of genetic variation and natural selection. The knowledge of microbial variation is used in the manufacture of fermented foods like bread, yogurt, and cheese. Certain soil bacteria produce nitrogen that plants need for growth, as well as compounds that help to maintain the Earth's atmosphere in check. Other bacteria cause problems to the food supply by infecting food-producing plants and animals with illnesses that reduce productivity. Different bacteria in our bodies aid in the digestion of food, the defenses against foreign species, and the skirmishes and pitched fights with the human immune system in the natural illness process.

Obtaining a microbes full genome sequence provides important information about its biology, but it is only the first step toward understanding its biological capabilities and, if necessary, changing them for agricultural uses. Microbial biotechnology is a vital field that can help to improve, food security, food safety, nutrition in humans, animal protection, as well as agricultural science fundamental research. Microorganisms in the soil aid in the absorption of nutrients by plants. Plants and these helpful microorganisms work together to recycle nutrients. The microbes assist the plant in "absorbing" vital energy sources. Plants give their waste by-products to the microbes in exchange for food. Plant microbiomes are important agricultural bioresources because beneficial microbes can improve plant growth and nutrient uptake through, zinc, potassium, and phosphorus solubilization, fixation of nitrogen, and mechanisms such as the production of siderophore (Fe biofortification with microbial mediation in different crops). Crop yields may be increased, pollutants removed, diseases inhibited, and fixed nitrogen or new compounds produced by beneficial bacteria. Plant microflora can encourage growth through nitrogen fixation, biological control of phytopathogens through the yield of antibiotic, antifungal, or antibacterial agents, the generation of Fe-chelating compounds, nutrient competition, and the induction of procured host resistance, and the generation of plant growth regulators such as IAA, gibberellic acids, and cytokines, and biocontrol of phytopathogens through the manufacturing of antibiotic, antifungal, or antibacterial agents, and the production of plant growth regulator.

Sustainable agriculture necessitates the implementation of measures that enhance or uphold current levels of food production while minimizing environmental and human health risks. Plant microbiomes such as PGP (plant growth promoter) agents/biofertilizers are a more environmentally friendly alternative to traditional

agriculture technologies. In a variety of studies, various PGP microorganisms directly assist the growth of their plant hosts. The PGP bacteria can fix nitrogen from the air and provide it to plants. Plant microbiomes with diverse PGP abilities synthesize a variety of plant growth regulators that can aid plants at different phases of development; they may have processes for increasing the availability of Zn, P, and K for plant growth and development; and they may synthesize some lesser-known low-molecular-mass production or enzyme-modulating plant growth and development. When microorganisms produce ammonia, hydrogen cyanide, Fe-chelating chemicals (siderophores), β-1,3-glucanase, chitinases, cellulase, lipase, antibiotics, and other fluorescent pigments, they hinder the growth of other plant harmful microbes. Infection by pathogenic organisms costs the world's agriculture billions of dollars every year. The employment of microbes for disease management is the most promising technique to boost agricultural output. Nitrogen is the most significant limiting element for plant growth, and using nitrogen-fixing microorganisms as biofertilizers has proven to be the most effective and environmentally friendly way to boost crop plant growth and output. Chemical nitrogen fertilizers could be substituted with nitrogen-fixing bacteria, resulting in more productive and environmentally friendly agriculture.

6.4.1 Microbial Biofertilizers

Biofertilizers are helpful bacteria that aid plant growth and soil enrichment by increasing nutrient availability to crops. The production of healthy crops to meet the demands of the world's growing population is largely dependent on the type of fertilizers used, which are essentially used to supplement all of the nutrients in the plants. However, a greater reliance on chemical fertilizers is wreaking havoc on the environmental ecology and negatively impacting human health. As a result, using microorganisms as biofertilizers is being studied as an alternative to chemical fertilizers to improve soil fertility while also enhancing crop output. These bacteria are thought to have biopotential and to be a novel tool for offering significant agricultural advantages. These microorganisms colonize and accelerate the growth of the roots. PGP microbes have a variety of PGP characteristics that aid plant growth directly in the production of hormones and N2-fixation in plant growth, solubilization of phosphorus, potassium, and zinc and indirectly employing production of ACC deaminase, lytic enzymes, antibiotics, ammonia, siderophores, and hydrocyanic acid (Kour et al. 2017). Biofertilizers have been studied extensively, and it has been discovered that these microorganisms can give the needed nutrients to the crops at adequate levels to increase agricultural yield. Microbes with multifunctional PGP properties are environmentally benign biofertilizers for long-term agriculture (Yadav et al. 2017a, b, c, d).

Plants require both phosphorus and nitrogen to flourish. These substances are found in nature, but plants can only extract a little amount of them. Phosphate is required for crop oxidative stress, development, and quality, as well as direct and indirect nitrogen fixation. Penicillium bilaii is a fungi that aids in the release of

phosphate from the soil. It makes an organic acid, which dissolves phosphate in the soil and make it accessible to the roots. The biofertilizer of this organism is applied by immunizing seeds with the fungus or planting them directly in the ground. Rhizobium is a type of bacterium that is used to make biofertilizers. This bacterium lives in nodules on the plant's roots, which are clusters of cells. Nodules are biological factories that collect nitrogen from the air and convert it into an organic form that the plant can use.

Nature has devised this way of fertilization. The legume can use naturally occurring nitrogen instead of the pricey typical nitrogen fertilizer since it has a large population of friendly bacteria on its roots. Biofertilizers assist plants in utilizing all of the food available in the soil and air, allowing farmers to need fewer artificial fertilizers. This contributes to the long-term preservation of the ecosystem for future generations.

6.4.1.1 Biopesticides and Herbicides

Plants do not create amicable relationships with all of the soil microbes. These diseases have the ability to infect or harm plants. Scientists developed ecological "tools" that use disease-causing bacteria to control weeds and pests in an organic way. Weeds are a problem for farmers. They compete for water, nutrients, sunlight, and space with crops, as well as harboring insect and disease pests, clogging drainage and irrigation systems, denigrating crop quality, and stashing weed seeds into agricultural harvests. Bio-herbicides are an alternative to synthetic herbicides for weed management that do not have the same environmental dangers. Invasive genes in the bacteria can assault the weeds' protection genes, causing them to die. Bioherbicides have the advantage of lasting a long time in the environment, allowing them to infect additional weeds the next growth season. It has the potential to dramatically reduce farming costs if properly handled because it is less expensive than synthetic pesticides. It's also less harmful to the environment than standard herbicides, and it doesn't harm nontarget creatures.

6.4.1.2 Microbial Bioinsecticides

Biotechnology can also aid in the development of alternatives to synthetic insecticides to combat insect pests, as well as soil microorganisms that attack fungus, viruses, or bacteria that cause root diseases. To safeguard the plant during the vital seedling stage, formulas for seed coverings (inoculants) that convey these helpful organisms can be devised. Bioinsecticides do not last long in the environment with minimized shelf lives. They are efficient in minute amounts and are safer for humans and animals than insecticides, They are very specific, frequently affecting only a single insect species, and have a very specific mode of action; they are slow to act and the timing of their application is relatively critical; they are slow to act and the timing of their application is relatively critical.

6.4.1.3 Fungal Bioinsecticides

Fungi-infected diseases are reported in more than 200 species of insects, and their disease-causing features are employed in the production of bioinsecticides. Fungi are

mass produced using fermentation technology. Spores are gathered and packed before being spread across insect-infested areas. The spores utilize enzymes to attack the outer skin of the insects' bodies when they are applied. They begin to develop once inside and eventually die. Some experts believe that fungal agents have the best long-term insect control potential. This is because bioinsecticides attack in multiple ways at the same time, making insect resistance extremely difficult.

6.4.1.4 Virus-Based Bioinsecticides

Insect pests such as aphids, potato beetles, corn borers, and flea beetles are affected by baculoviruses. Bertha armyworms infect, flax, vegetable, canola, and crops, and one strain is utilized as a control agent. Traditional insecticides do not kill the worm until it reaches this stage, by which time it has already done a lot of damage.

6.5 Conclusions and Future Perspectives

The microbiome is now recognized as an integral part of our environment, according to research. The number of studies looking at the microbiome from the background of environmental toxicology is rapidly growing, and there are unique challenges for toxicology when it comes to understanding the role of the microbiome in environmental health and agriculture, such as issues with the initiation of their metabolites and toxins, the mechanism of pollutant transformation, and how environmental factors like drought and acidification affect their activities. We must not overlook the critical role they play in agriculture's long-term viability. Bacteria and algae, for example, make up the majority of epiphytic microorganisms that colonize the lower surface of floating plants. Bacterial biofilms are responsible for the removal of organics, inorganic, and metals from environmental systems. The nature and variety of bacteria are influenced by plant species and pollution concentrations in the environment. In addition, nutrient accessibility affects bacteria metabolism as well as the efficacy of pollutant removal. Endophytes and the rhizosphere both play an important role in pollution removal.

The rhizosphere microbes remove contaminants in the root complex, while the endophytes are responsible for removing pollutants within the shoots and roots. By relieving pollutant stress, increasing tolerance to environmental changes, and regulating plant growth through direct and indirect processes, a community of endophytic and rhizospheric bacteria improves the pollution removal process. Using bacteria to inoculate plant roots enable certain bacteria strains to help speed up the removal of the pollutant process. The importance of plant-microbe interaction in the pollution removal process in the environment is obvious from this knowledge. Environmental parameters such as temperature, pH, and nutrient availability contain a major effect on microbes' ability to remove pollutants. As a result, we recommend that agriculture and environmental protection organizations should plant their feet firmly on the ground to aid in the safeguarding of our ecosystem.

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Conflict of Interest The authors declare no conflict of interest.

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Modifications in Environmental Microbiome and the Evolution of Viruses Through Genetic Diversity

7

Pola Sudhakar and Dhanalakshmi Padi

Abstract

The microbiome present in the environment is changing due to climate, chemical composition, biodiversity, and also even human activities. Biodiversity is vital for the maintenance of a healthy ecosystem and environment, and this includes genetic diversity which helps to maintain the gene variations among species and also aids in the evolution of better species that can withstand the changing environment. Therefore, genetically diverse species are more resistant to infectious pathogens, which cause dreadful diseases.

Keywords

Microbiome · Biodiversity · Genetic diversity · Major histocompatibility (MHC) · Evolution · Biogeography

7.1 Introduction

7.1.1 Modifications in Environmental Microbiome

Microbes are ubiquitous on the Earth; they can be found on the surface and in the deeper layers of the Earth. Microbes can also be found in the air and even in higher altitudes. The environmental microbes are continuously changing due to many factors such as climatic changes, chemical composition, and biodiversity. Also,

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human activities like plant and animal extinction may lead to the loss of useful microbial strains; therefore, this affects the microbes present in the environment. The richest amounts of microbes are found in the soil because it has all kinds of nutrients that are necessary for the growth of microbes (Bardgett and Van Der Putten 2014). Microbes help modulate the biogeochemistry; therefore, the chemical composition and properties in the Earth are due to the integrated microbial action. Microbes cause many diseases to humans and their secondary metabolites such as antibiotics have medicinal properties (Zhu and Penuelas 2020).

7.1.2 Microbiome Diversity

The changes that occurred due to biodiversity are necessary for evolutionary development; however, the Earth's microbiome is highly redundant and extremely diverse. Biodiversity is useful for a healthy ecosystem; it balances all the living organisms on the earth. Biodiversity is divided into three main categories; they are genetic diversity, species diversity, and ecosystem diversity.

- **Genetic diversity**: This focuses on the variations of genetic material (DNA) among individuals.
- **Species diversity**: In this category, different types of species in a particular area have been included.
- **Ecosystem diversity**: Differences in the ecosystem, which is within a geographical location, are focused on.

At these three levels of variations, the balances in nature are maintained and thus play an important role. The diversity in genes, species, and ecosystem among individuals, communities, and areas is helpful in evolutionary development. A wild variety of species is necessary for high biodiversity. However, the advent of molecular and genomic tools and techniques plays a crucial role in studying microbial diversity.

The depletion of biodiversity may affect the environment and the ecosystem. These effects on biodiversity impact the emergence and evolution of microbes (Rodríguez-Nevado et al. 2018). Biodiversity help in the development of human well-being, a better ecosystem, and sustainable development (Tydecks et al. 2018). European Environment Agency initiated the program to manage and protect biodiversity globally (Barbault 2011).

Low biodiversity affects the climate change and weather of the environment, which, in turn, affects the living organisms and ecosystem (Mawdsley et al. 2009). The journal, *Trends in Ecology & Evolution* mentioned that the species which are introduced show resistance to the parasite's prevalence. In contrast to that, global homogenization increases the susceptibility to diseases (Young et al. 2017). Review literature by the researcher Jessica says that the pathogens are increased due to the agricultural practices, and the domestic animals play a crucial role in spreading the diseases by pathogens to humans. Also, it is said that modification in the environment affected the ecology and thus laid a path to the development and emergence of pathogens, which causes infectious diseases (Pearce-Duvet 2006).

Human-made changes are also the reason for the evolution and emergence of pathogens; the two significant changes which were mentioned in the journal *Vector-Borne and Zoonotic Diseases*, by the researcher Loh et al., depicted the land-use changes and the agricultural industry changes. Also, climate changes and medical industry changes made humans susceptible to emerging infectious diseases (Loh et al. 2013).

7.2 Genetic Diversity Drifted Through Evolution

Genetic diversity covers the areas of genomics, ecology, and evolutionary biology, which are useful for a better understanding of biodiversity. The difference in the genes among species or organisms is known as genetic diversity; thus, these differences make two individuals look different from each other. Variations in a gene are caused when mutations occur; a mutation can show a positive or negative effect on a species. However, the selection of the mutated genes is dependent on the flow of those genes. The positive mutation or the addition of the valuable genes in the genome of the reproductive genes can be carried along with the generations (Wright 2005).

Microbial biogeography occurred when microorganisms developed an ability to acquire foreign DNA; therefore, the movement of genes occurred through the ecosystems. The microbes which can withstand the genetic changes can pass these genes to other organisms (Gillings 2017; Reed et al. 2014). Newly discovered viruses are developed and implemented with new standards and transformed for our understanding of microbial ecology, evolution, and biogeochemical cycles. These are also useful in leading innovative paths in many diverse fields such as environmental, agricultural, and biomedical sciences (Call et al. 2021).

Genetic polymorphism spreads among species; the two or different forms of traits in an entire genome are drifted through evolution (Ellegren and Galtier 2016). Genetic variations can be seen in the wild species, whereas the domesticated species have a low level of genetic diversity because mankind selects few traits. The organisms which are naturally selected are highly resistant to the artificially chosen organisms.

Genetic diversity is beneficial for the evolution of a better species which can adapt to the changing environment (Frankham 2005). The species with the potential to adapt to the environment can fight off bacteria and viruses. These can pass on the favorable characteristics to the generations, whereas the susceptible organisms cannot carry on their genes further (Doehring 2020).

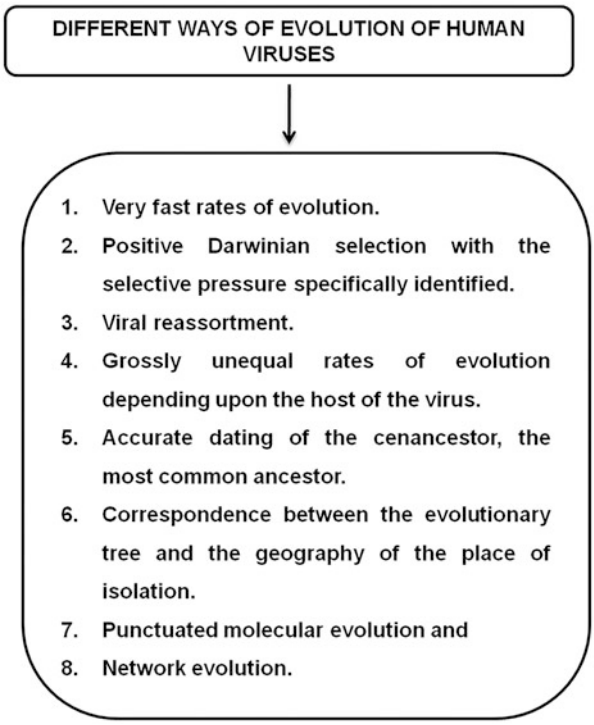
The viruses or bacteria can easily infect the species which are artificially selected as they have similar genes; therefore, the wild variety species possess genes that are diverse and can show resistance to pathogenic viruses and bacteria. Every organism has the blueprint of its genome, and thus, they vary from every individual. The genetically diverse species are more resistant to the changing environment and can quickly adapt to the changes. Therefore, these species can withstand adverse conditions.

7.3 Evolution of Viruses Through Genetic Diversity

Pathogens such as viruses, particularly RNA viruses, have the potential to reemerge and can infect multiple hosts. These pathogens cause infectious diseases, which can be a dangerous threat to human lives and economies (Cleaveland et al. 2001). There are mainly three different types of evolutionary paths for the RNA viruses (Reanney 1982); they are positive-sense single-stranded viruses, negative-sense single-stranded viruses, and double-stranded RNA viruses. Among these RNA viruses, single-stranded RNA viruses share the genes during their evolution, whereas double-stranded RNA viruses have a different evolutionary line (Baltimore 1980).

In the article, *Cellular and Molecular Life Sciences*, the mechanism of viral mutation are explained. It was mentioned that the RNA viruses (Lauring and Andino 2010) are more prone to mutations when compared to the DNA viruses, and single-stranded viruses undergo mutations faster than double-stranded viruses. Also, some viruses can adapt to the new environment and the host quickly, and therefore, their potentiality for quick adaptation is based on the generation of de novo diversity (Sanjuán and Domingo-Calap 2016) (Fig. 7.1).

In 1996, Walter M. Fitch in the article *Molecular Phylogenetics and Evolution* mentioned the different ways of the evolution of human viruses (Fitch 1996). They included:



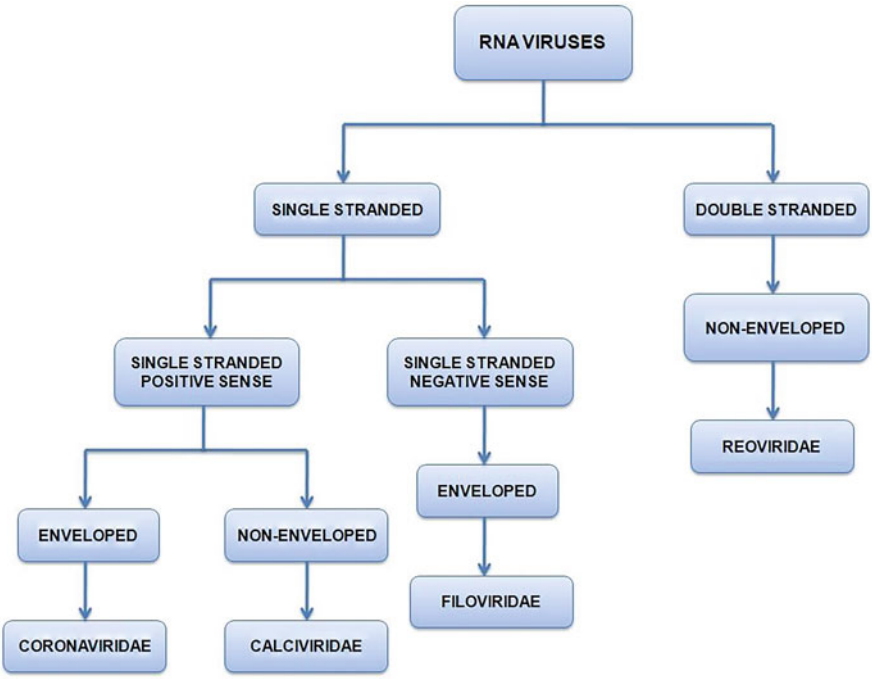


Fig. 7.1 Different types of evolutionary paths in the evolution of RNA viruses. The three major evolutionary lines are positive-sense single-stranded viruses, negative-sense single-stranded viruses, and double-stranded viruses

The rate of viral mutations and their genetic diversity depend on the multiple viruses and the host-dependent processes; also there are some selective processes involved in the development of mutations (Domingo and Holland 1997). The changes in the nucleotide bases caused the evolution and reemergence (Wang et al. 2020) of SARS-CoV-2, and many types of research are carried out to find out the mutations that occurred in the virus.

The researcher Phan. T of the University of Pittsburgh Medical Centre, USA, conducted genetic analyses on 86 genomes of SARS-CoV-2, which were collected from the source GISAID (Global Initiative on Sharing All Influenza Data) (https://www.gisaid.org/) and mentioned that they have revealed 93 mutations overall the entire genomes of SARS-CoV-2. Therefore, these analyses provided the data regarding the mutations and deletions in the coding and noncoding region of SARS-CoV-2 and also gave the evidence for the evolution of the novel coronavirus-2019 (nCoV-2019) (Phan 2020a, b).

The emergence and reemergence of infectious viruses need to understand better to overcome the global issues related to outbreaks and pandemics. The researches covering the areas such as evolutionary biology, epidemiology, and genomics need to be focused more. The advanced genome technologies and computational biology are useful to sort out the problems and, however, focus on the evolutionary

emergence of viruses to be increased. Various methods and challenges need to be fulfilled in controlling the disease. Also, the further possible reemergence of infectious viruses to be studied thoroughly to avoid future outbreaks (Pybus et al. 2015).

7.4 The Role of MHC (Major Histocompatibility) in the Evolution

Jones and Partridge in 1983 in the *Nature* journal explained that the MHC system is used primarily for sexual selection, and thus to achieve gene recombination, the inbreeding of the species has been avoided. These selections of the MHC genes can be detectable when a series of selective pressure is applied for a short time (Garrigan and Hedrick 2003). MHC gene sequence's role in the reproduction, mate selection, and fitness to survive in the changing environment is also reviewed in many research papers (Zhu et al. 2019). The gene organization of MHC complexes is different among species in terms of size, complexity, and gene order (Flajnik and Kasahara 2001).

MHC diversity plays an essential role in the genetic drift of the traits and is thus involved in evolution (Kaufman 2018). The genetic drift is useful in shaping the genetic diversity and population, whereas the limited gene flow may cause massive differentiation in the genetic variations of the MHC complex (Lan et al. 2019). MHC variant molecules are useful in making the population resistant to pathogens. The pathogens are rapidly mutating and evolving; therefore, they can flow easily through the species with similar genes. Thus, the variants of MHC molecules are helpful for not passing the pathogens further.

7.5 MHC Diversity in Humans Makes Resistance Against Evolving Pathogens

In vertebrates, MHC genes, especially antigen-presenting cells, i.e., class-I and class-II MHC molecules, are highly variable. Pathogen-mediated selection has been focused more on because they play a crucial role in the selection of MHC genes (Hughes 2002). For instance, the two MHC types and the two MHC variants can produce ten different types of genotypes, which are resistant to the pathogens. The alleles of the different genotypes of MHC genes encode the different proteins; thus, the population with these genotypes shows resistance against the rapidly mutating pathogens (Fig. 7.2).

Also, during the inheritance of the haplotypes of MHC gene sequences to the offspring, the gene conversion and the gene recombination (Schaschl et al. 2006) make the MHC gene sequences polymorphic, and thus the new variants of MHC molecules are produced during the inheritance of haplotypes (Yamaguchi and Dijkstra 2019). The MHC genotypes, which are produced in response to the pathogens, are having the genes which show the phenotypic character fitness to the pathogens (Wegner et al. 2003).

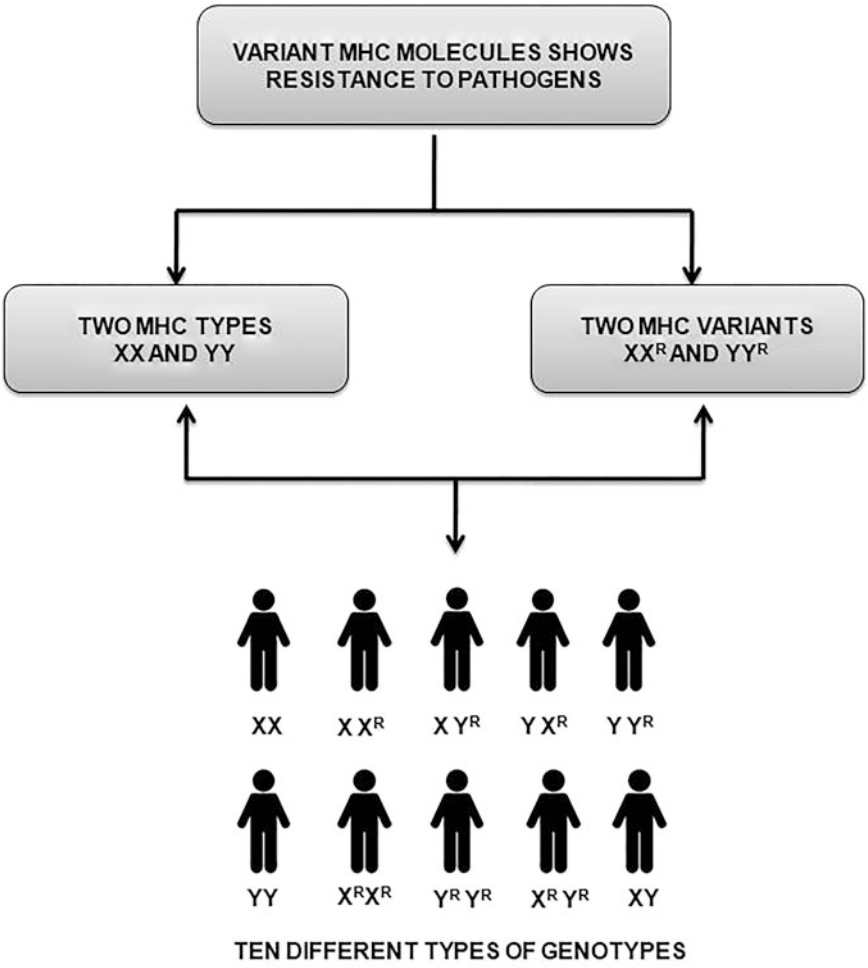


Fig. 7.2 Variant MHC molecules that are produced during the evolution show resistance to the pathogens. Ten different types of genotypes which are produced from two MHC types and two MHC variants help to avoid the infection caused by the viruses among species of similar genes

MHC complexes are mainly involved in the recognition of the foreign particles that invade our immune system; these genes at the MHC loci produce antibodies by activating the immune responses to fight against antigens (Dawkins and Lloyd 2019). MHC is represented by the immune cells and acts as a stalk that anchors the pathogens to the cells and, therefore, produces antibodies against them (Altuvia and Margalit 2004; Kelly and Trowsdale 2019).

7.6 Concluding Remarks

From this review, we conclude that the changes in the environmental microbiome need to be controlled as it affects the helpful bacteria in the environment. Also, maintenance of biodiversity is essential for sustainable development, and also genetic diversity is necessary among the species to stop the reemergence of infectious disease. Genetic diversity also makes the species to be fit and resistant to emerging pathogens. Thus, the research should be more focused on the areas of epidemiology, evolutionary biology, and genomics, which help us to be prepared for the subsequent outbreaks or pandemics.

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8 Novel Insights into Environmental Niche Adaptations and Secondary Metabolite Biosynthesis Potential of Marine Sponge Microbiome

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Abstract

The microbial diversity of the sponge differs from a very rich non-polluted site to polluted sites; it is unclear whether the intra-species variation changes with the change in the environment. Sponges are a collection of species with close association and having complex interactions in which the host-microbiome has been less characterized when compared to host species. Based on the environment the sponge-associated microbiome varies. The microbiome of sponges isolated from contaminated sites has low diversity and higher intra-species dispersion when compared to the microbiome of sponges from a rich environment. The microbiome of the sponge has abundant biotechnological applications along with the range of other inhibitory substances; the role of these substances was unclear, especially with regard to their ecological roles. Sponges which are

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less complex in nature that thrive under degrading environmental conditions have more variable microbiomes following the ecological paradigm that correlates community diversity and environmental degradation. Operational taxonomic units (OTUs) are shared between the microbiome of sponge within the same family or order. These OTUs show great variation between different sponge families and orders. These taxonomic units of a particular sponge can be taken as their signature identity. Eighty percent of these are present in uncultured microbiota in a vast amount. Studying these OTUs reveals a deeper understanding between host specificity of the sponge microbiome and the hidden sponge-associated microbial resources.

Keywords

Sponge · Microbiome · Diversity · Environmental · Marine

8.1 Introduction to Sponges

The phylum Porifera contains filter feeding and sessile animals which help the benthic environment worldwide (Van Soest et al. 2012). It mainly includes four classes: Calcarea, Demospongiae, Hexactinellida and Homoscleromorpha (Gazave et al. 2012). Hexactinellida are characterized by hexactin-structured siliceous spikes and syncytial tissue organization. Demospongiae is a diverse community of the non-monophytic group (Boury-Esnault 2006). They usually have a mineral skeleton made of siliceous spikes, but like the usual bath sponge, many species do not have a mineral skeleton, but rather a network of fibres. Modern sponge phylogeny also incorporates molecular approaches and sponge morphological characteristics, but sponge spikes remain the most important features for the discovery of sponges and the discovery of sponges in fossil records.

Most of the sponge species dwell in marine environments (9162 species), the world Porifera database also contains 248 species in freshwater (van Soest et al. 2019). Despite their numerical abundance, domination of biomass and durability in many ecosystems, their practical importance often continues to be underrated. However, sponge ecologists have long recognized the functional importance of sponge organisms in the benthic climate (Rützler 1975, 1978; Ruetzler 2004; Rützler and Macintyre 1978; Diaz and Rützler 2001; Wulff 2006).

Over 6000 described species were found in both freshwater and marine environments in the tropical, temperate and polar regions (somewhat more limited) (Hooper and Van Soest 2002). Sponges have received a lot of attention recently due to the following two fundamental (and sometimes interrelated) factors: (1) their association with varied microorganisms and (2) the presence of richer metabolites. The design of the sponge is distinct from any other taxon, and the anatomy of the sponge greatly influences other areas of sponge biology, including encounters with microorganisms. The general structure of the body consists of many separate layers of cells (Simpson 1984). The exterior portion (pinacoderm) comprises pinacocytes

(epithelial cells). Via pores (ostia) on the surface of the sponge, these cells often spread along the internal channels of the sponge, through the ostia (pores) located on the surface of the sponge, which also spreads along the internal channels of the sponge. In these galleries, collectively called the choanoderm, the flagellated choanocytes beat to pump water through the ostia and along the complex aquifer structures of the sponge (Taylor et al. 2007a, b). The choanocytes filter food particles (including bacteria and microalgae) and transfer it to the mesohyl, a large layer of connective tissue. The food particles are digested in the mesohyl by another group of sponge cells, the archaeocytes, via phagocytosis. These totipotent cells can differentiate into any other type of sponge cells. The sponge mesohyl layer contains a huge microbial community (Friedrich et al. 1999; Wilkinson 1978).

Sponges and other marine invertebrates (corals and sea squirts) rely heavily on chemical production as a natural defence mechanism against enemies, such as predators and competitors (Taylor et al. 2007a, b). In this respect, marine sponges have attracted particularly intensive scrutiny, with a wide range of natural sponge products to date. Each year various bioactive metabolites are obtained from marine sponges (Munro et al. 1999; Blunt et al. 2006). These compounds have also been proposed for various environmental roles, including defence against predators (Becerro et al. 2003; Chanas et al. 1997; Pawlik et al. 1995), competitors (Thacker et al. 1998; Turon et al. 1996; Engel and Pawlik 2000), fouling organisms (Sears et al. 1990; Willemsen 1994) and microbes (Thakur et al. 2005; Newbold et al. 1999; Becerro et al. 1994).

Sponges are known to be a prolific source of biologically active metabolites with a unique structure. They are known to produce a wide variety of secondary metabolites. They are therefore more relevant in recent studies.

8.2 Contrasting Environment

Since Pre-Cambrian times, microbes have been intimate partners of sponges (Willemsen 1994). In some sponge species, they can account for up to 50% of the volume of the microbial sponge holobiont (Uriz et al. 2012) which are taxonomically and metabolically diverse (Weisz et al. 2007; Thomas et al. 2016a, b). Therefore, regardless of the bacterial microbiome that accompanies them, it is difficult to imagine the causes of sponge success or failure. Their microbial imbalances cause widespread mortality in the Mediterranean (Webster et al. 2008; Cebrian et al. 2011) and Red Sea (Gao et al. 2014).

Contrary to the spatial and temporal variations reported for microbial communities in seawater (Zeglin 2015; Glasl et al. 2017), there is no significant change in the sponge microbiome by geographical and bathymetrical ranges or over temperature, eutrophication, or irradiance shifts (Hentschel et al. 2002; Erwin et al. 2012; Pita et al. 2013a, b; Luter et al. 2014; Strand et al. 2017). While numerous experimental studies have reported microbial change in some host species subject to severe environmental changes (Mohamed et al. 2008; Fan et al. 2013; Lesser et al. 2016; Webster and Reusch 2017; Pineda et al. 2017; Weigel and Erwin 2017;

Ramsby et al. 2018; Glasl et al. 2018), there will be short-term changes in the sponge microbiome in response to environmental stress.

8.2.1 Responses of Sponge Microbiomes with Environmental Stresses

The sponge microbiome is complex and host-specific in nature, affected by a variety of environmental conditions (Cleary et al. 2013). Variations in extent linked to seasonal changes were reported when the same individuals were examined repeatedly over a period of time (Wichels et al. 2006; Anderson et al. 2010). Schmitt et al. (2012) studied 32 sponge species located in different regions with varied geographical locations. The results showed that their microbial communities shared a small percentage of the core bacterial taxa and a large percentage of the species-specific taxa (Schmitt et al. 2012). It was also proved by another study by Schmitt et al. (2011) on other sponge species. These studies showed that the response of the sponge microbiome to different environmental conditions comes from the following reasons: sponges are highly stable and have specific microbial communities; in terms of their diversity and structure, sponges have highly variable microbial communities supported by environmental factors or stresses (Yang et al. 2019).

The stability of sponge-microbe associations and their response to different environmental conditions contribute to the significant ecological and economic role of symbionts in nature and society. Further research has revealed that the sponge-associated bacterial population is stable not just across biogeographical regions, but also across time, as seen by seasonal shifts (Yang et al. 2019).

Other recent studies report that driving shifts in the microbial sponge community can be caused by certain environmental factors, including places and living conditions. For example, an ecologically important sponge species, *Carteriospongia foliascens*, has been studied to understand how its microbial community responds to variations between coastal and offshore sites (Luter et al. 2015). The biomass of Cyanobacteria increased steadily over Bacteroides in the locations examined, suggesting that the *C. foliascens* were induced by specific environmental factors. The sponges *Hymeniacidon heliophila, Paraleucilla magna* and *Petromica citrina* were examined by Turque et al. (2010) in two settings with varied amounts of pollution. The diversity and composition of seawater and sponge-related archaea were compared. The complexity of the archaeal community for sponges in the inland seawater bay was higher than that of the *Cagarras Archipelago*, according to the findings. In sponges living in polluted locations, *Crenarchaeota* displayed more diversity, which might be explained by the altered structure of their associated microbial communities that reflect their approach to adapting to the damaged surroundings (Yang et al. 2019).

Some sponge microbiomes have habitat- and host-related differences, implying that host phylogeny and living circumstances are both important in influencing microbial community composition and structure. Two sponge species, for example, have been chosen to assess their microbial makeup in both marine lakes and nearby

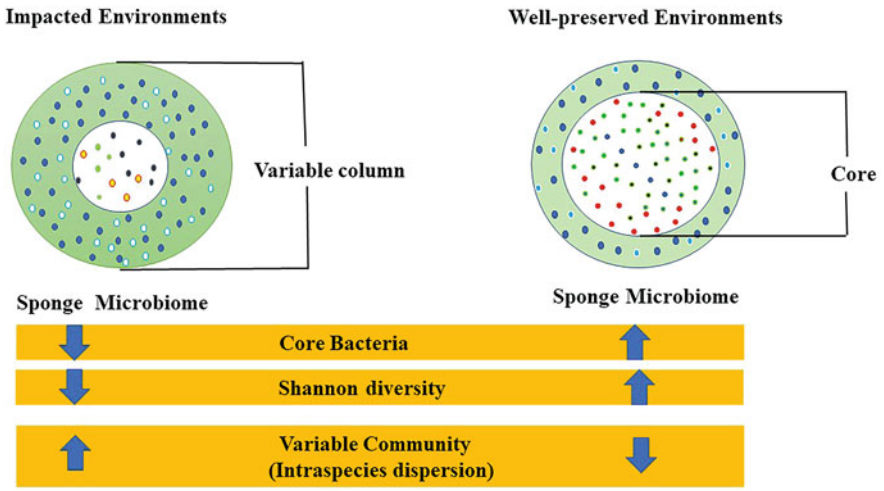

Fig. 8.1 The figure shows the nature of the sponge microbiome in well-preserved and impacted environments. The green area represents the variable community and the white part represents the core community. The coloured dots represent single bacterial species. (Adapted and modified from Turon et al. 2019)

open coastal systems (*Suberites diversicolor* and *Cinachyrella australiensis*) (Cleary et al. 2013). They found a significant difference in microbial diversity between the species. Within *S. diversicolor*, their bacterial populations somewhat differed between samples from inside and outside the lakes. In contrast, habitat variation in *C. australiensis* has resulted in a significant shift in the associated microbial communities. As a result, sponges residing in the same environment had a microbial community that was distinct to the host species rather than the shared living conditions; different sponge species in different environments responded to environmental influences to varying degrees (Yang et al. 2019) (Fig. 8.1).

8.2.2 Temperature Fluctuation

In order to better understand specific symbiotic connections, stability correlations with temperature fluctuations were investigated in addition to the generalized biogeographic stability of tropical and temperate sponge-microbe partnerships. Shifts in symbiotic microbial communities will influence sponge health, growth rates and their ability to defend themselves against predation, fouling and illness as a result of climate change or environmental stress. Meanwhile, sponges' ability to adapt to changing environmental conditions may be aided by the adjustment response of microbial populations (Rohwer et al. 2002; Reshef et al. 2006), although little is known about the sponge-microbe association's ability to adapt.

In this century, sea surface temperature (SST) was predicted to rise to 4 °C (Solomon et al. 2007), which is a significant environmental threat to coral reef populations and marine sponge populations (Hoegh-Guldberg et al. 2007). Because elevated SST could induce changes in the microbial community associated with sponges with regard to its diversity, function and structure (Webster and Blackall 2009), it could directly/indirectly damage the body of the sponge (López-Legentil et al. 2008, 2010; Lemoine et al. 2007). Sponge health is typically linked to the characteristics and stability of sponge symbionts over time and geographic and environmental gradients, as well as during vertical transfer from generation to generation (Webster et al. 2010; Steger et al. 2008). The Crenarchaeota population associated with the gigantic barrel sponge *Xestospongia muta* appears to be stable during sponge mortality, with a composition comparable to seawater and ambient sediments. This shift revealed a link between the drop in sponge health and a decrease in the richness of microbial populations (Lopez-Legentil et al. 2010).

Webster et al.'s (2008) study revealed that *Rhopaloeides odorabile* was exposed to temperature in the range of 27–33 °C. They investigated that sponge was considered highly vulnerable to the impact of global climate change, as a 1 °C increase in temperature could lead to a rapid decline in host health due to loss of symbionts. Sponge larvae therefore allow for a highly stable symbiosis in the face of changes in seawater temperatures up to those predicted under current climatic conditions (Yang et al. 2019). Sponge microbiomes are very susceptible to environmental stress (Webster et al. 2008). It was discovered that environmental change (e.g. increased temperature) can irreversibly destroy the symbiosis in the sponge-microbe community with similar highly dependent microbial members, with major implications for host health (Yang et al. 2019).

8.2.3 Irradiance and Depth Variation

Erwin et al. (2012) studied the sponge *Ircinia* sp. to determine their microbial community response to changing irradiance. This showed that the microbial community maintained species-specific stability over the monitoring period with significant fluctuation of irradiance. A recent study analysed the microbial communities of three species of sponges collected from regions of the Caribbean over a depth range of 10–100 m (Olson and Gao 2013). The results showed that the stability and specificity of associated microbial communities varied with host phylogeny, but that each species supported a separate population, implying that various sponge species have different degrees of stability in their associated microbial communities with depth. Over a wide range of depths, each sponge species shared "core" microbial taxa, with the composition of the remaining community potentially modified by biotic and abiotic variables (Yang et al. 2019).

During sponge culture, the microbial communities' stability varies depending on the host species. Because of the variable quantities of nutrients and the chemistry of the seawater, the microbial community of sponges kept in big aquarium systems differed (Yang et al. 2019).

8.2.4 Chemical Variance: Heavy Metals and Nutrients

Based on their adaptation ability to changing environmental conditions, sponges are called "dynamic multicellular systems" (Gaino and Magnino 1999). In addition, there is evidence that sponge-symbiotic microbes play an important role in sponge-microbe-associated nutrition and secondary metabolite biosynthesis (Taylor et al. 2007a, b). Marine sponges were proposed as heavy metal pollutant sentinels (Perez et al. 2005; Patel et al. 1985; De Mestre et al. 2012). Some studies have shown that they can accumulate high quantities of metals depending on the contamination in their environment (Cebrian et al. 2007; Hansen et al. 1995). The sponge *Rhopaloeides odorabile* has shown a decrease in the density and diversity of its total microbial community under cupric ion (Cu^{2+}) treatment, particularly for bacteria with high copper tolerance (Webster et al. 2001a, b).

The sponge *Haliclona cymaeformis* has shown its associated microbial community a selective response to copper treatment (Tian et al. 2014). Two dominant associated microbial taxa such as sulphur-oxidizing Ectothiorhodospiraceae and photosynthetic Cyanobacteria were significantly reduced after treatment with a high concentration of copper. The change in the microbial community associated with copper-entrained sponges has been revealed in terms of restructuring their composition and functional diversity. Some microbial taxa have been enriched to reorganize the community for copper stress survival. It has also been reported that the cultured bacterial community associated with sponges tolerates heavy metals (Bauvais et al. 2015; Mangano et al. 2014; Wanick et al. 2013). Metal tolerance is often considered relevant for antibiotic resistance and can also affect microbial biochemical activities. Furthermore, the comparison of the heavy metal and antibiotic resistance patterns at the phylogenetic level of the associated microbes revealed various characteristics. Interestingly, the different strains vary between growth patterns to affect a distinct tolerance of heavy metals. Hence the patterns of heavy metal sponge tolerance are more likely to be specific to the symbiotic microbial community.

In addition to the high tolerance to environmental heavy metals, sponge-microbe associations also have a high nutrient concentration threshold, such as the microbial communities of the sponges *Aplysina cauliformis* (Gochfeld et al. 2012), *Rhopaloeides odorabile* (Simister et al. 2012a, b), *Ircinia fasciculata* (Pita et al. 2013a, b) and *I. oros* (Pita et al. 2013a, b), as well as *Cymbastela stipitata* (Luter et al. 2014).

8.3 Microbial Community Changes in Disease Affected Sponges

Sponges not only protect themselves but also protect other beneficial microbes through the production of bioactive compounds (Cebrian et al. 2007). Interestingly, the symbiotic microbes can also help the host defence (Lee and Qian 2003). In addition, the sponges' innate immune systems are also thought to play a role in

preventing microbial invasion (Taylor et al. 2007a, b). Numerous studies focused on the shifts in microbial and chemical patterns during an outbreak of sponge disease (Yang et al. 2019).

By analysing affected and unaffected portions of the diseased sponge *Aplysina aerophoba*, the microbial community composition was evaluated to determine the role of microbes in the disease process (Webster et al. 2008). Microbial diversity was found to be higher in diseased sponges than in healthy sponges. Only diseased sponge tissues were found to have Deltaproteobacteria, Epsilonproteobacteria and Firmicutes. In disease-affected sponges, the bacteroidetes and prokaryote communities showed significant differences and increased abundance. The first disease affecting the *Geodia barretti* deepwater sponge was described by Luter et al. (2017). Between different health states, very different community profiles were discovered, with distinct community changes involving higher relative abundances of Bacteroidetes, Firmicutes and Deltaproteobacteria in sick people. Furthermore, three OTUs (operational taxonomic units) were missing in sick people but were found in disease lesions and apparently healthy tissue from sick people, suggesting a non-localized infection.

Besides the complexity of defence mechanisms, sponges host a wide range of microbial communities, which could form stable symbiotic associations and help host sponge maintain healthy growth (Taylor et al. 2007a, b; Thacker and Freeman 2012). Importantly, the majority of the bioactive secondary metabolites that protect sponges from predation were in fact produced by their symbiotic microbes (Esteves et al. 2013). The sponge carries out multiple defence mechanisms to protect itself from a wide range of potential predators, such as *Paracentrotus lividus* and some fish, including *Chromis chromis*, *Oblada melanura* and *Diplodus vulgaris* (Yang et al. 2019).

8.4 Sponge Microbiome

Marine sponges often contain microbial communities that are highly diverse and include bacteria, archaea fungi and microalgae. Sometimes these microbial associates account for as much as 40% of the volume of sponge and can significantly contribute to host metabolism (e.g. through photosynthesis or nitrogen fixation) (Taylor et al. 2007a, b). The first in-depth study, looking at 81 species of sponges, found that the sponge microbiomes cover at least 39 microbial phyla and some candidate phyla (Thomas et al. 2016a, b).

Phyla Proteobacteria (Gamma- and Alphaproteobacteria), Actinobacteria, Chloroflexi, Nitrospirae, Cyanobacteria and Candidatus Poribacteria belong to the most dominant bacterial symbiont groups, while Thaumarchaea is the dominant archaeal group (Moitinho-Silva et al. 2017; Thomas et al. 2016a, b). The microbial communities are mostly species-specific, but are composed of both generalist microbes as have been reported in most sponge species from different geographical regions and specialist microbes as have been found to be enriched in particular

species as these are rare or completely absent in other species (Gili and Coma 1998; Thomas et al. 2016a, b).

Generally, in 16S rRNA gene sequencing studies of sponges, the most frequently reported sequences include those from acid bacteria, Actinobacteria and Chloroflexi (Hentschel et al. 2006). Members of several bacterial phyla, namely, "Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Planctomycetes, Proteobacteria and Verrucomicrobia", have also been isolated from marine sponges (Enticknap et al. 2006; Hentschel et al. 2001; Kim et al. 2005; Lafi et al. 2005; Lee et al. 2006; Lopez et al. 1999; Montalvo et al. 2005; Olson et al. 2000; Pimentel-Elardo et al. 2003; Santavy et al. 1990; Webster et al. 2001a, b). Unlike marine sponges, available (limited) information suggests that bacterial diversity and abundance are substantially lower in freshwater species.

Eukaryotic microbes have also been reported to be associated with sponges. There have been reports of sponge-inhabiting dinoflagellates (Garson et al. 1998; Hill 1996; Hill and Wilcox 1998; Sara and Liaci 1964; Scalera-Liaci et al. 1999; Schönberg and Loh 2005; Steindler et al. 2001; Webster et al. 2004; Wilkinson 1992) and diatoms (Totti et al. 2005; Webster et al. 2004; Taylor et al. 2004; Gaino et al. 1994; Cerrano et al. 2000), with the latter apparently most prevalent in polar regions (Bavestrello et al. 2000; Cerrano et al. 2000; Totti et al. 2005; Webster et al. 2004; Gaino et al. 1994). Sponges in freshwater often contain endosymbiotic microalgae, mainly zoochlorellae (Bil et al. 1999; Frost et al. 1997; Jensen and Pedersen 1994). Wilkinson (1992) has reported cryptomonads in sponges, whereas marine sponge-associated fungi receive more attention because of their biotechnological applications (Wilkinson 1992). Of the 681 fungal strains isolated from 16 sponge species worldwide, the majority of these belonged to genera that are omnipresent in terrestrial habitats (e.g. *Aspergillus* and *Penicillium*) (König et al. 2006; Höller et al. 2000; Bugni and Ireland 2004). In most cases, therefore, it remains unclear whether such fungi are consistently associated with the source sponge, or dwell as an independent marine species (Höller et al. 2000).

Complex host-associated microbial communities are divided into a core microbiome with "members of the microbial community that are highly prevalent in all host individuals of the same species" and a variable microbiome with "members of the microbial community that are only recovered from some individuals or vary in relative abundance" in terms of community structure (Hester et al. 2016). Surveys on different environmental conditions (e.g. geographic distance (Pita et al. 2013a, b), season (Erwin et al. 2012, 2015), depth (Steinert et al. 2016) and habitat (Cárdenas et al. 2014)) have consistently observed that sponges harbour species-specific and stable microbiomes at different taxonomic prokaryotic levels (Steinert et al. 2017) and prevalence thresholds (Astudillo-García et al. 2017) (Fig. 8.2).

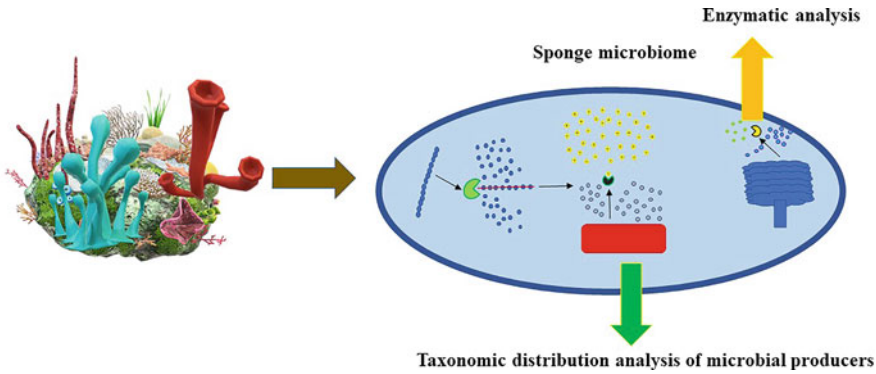

Fig. 8.2 The figure shows the diverse capability of the sponge microbiome which harbours various enzymes with different distribution of microbial populations. (Adapted and modified from De Oliveira et al. 2020)

8.4.1 Sponge Microbiome Diversity

The sponge microbiome is well known to protect the host from external agents like predators and epibionts by synthesizing various secondary bioactive compounds; this forms the major basis of success for sponge survival (Hentschel et al. 2012). The sponge-based microbiomes are not yet fully characterized; however, some of them have been analysed with the help of 16S rRNA sequencing technology (Reveillaud et al. 2014). The 16S rRNA gene reveals the distinctive nature of the OUT (operational taxonomic unit) richness (Ghyselinck et al. 2013). Most of the sponge microbiomes are host-specific in nature and can thrive well under varied climatic conditions, whereas some showed community shifts under varied locations and seasons (Luter et al. 2015).

Microbes have been found to have an association with sponges from the Pre-Cambrian time. Any sudden change in the microbial symbiosis can lead to the death of the specific sponge (Cebrian et al. 2011). In *Demospongiae*, most of the orders have large numbers of bacteria. In *Aplysina cavernicola* and *Ceratoporella nicholsoni*, more than 56% of the volume is taken up by the bacteria (Willenz and Hartman 1989). It has been reported that sponges *A. aerophoba* constituting $6.4 \pm 4.6 \times 10^8$ g^{-1} and *Rhopaloeides odorabile* 8.3×10^9 mL^{-1} bacterial counts from sponge tissue sample have microbial counts that are much higher than that which is present in seawater (Webster and Hill 2001). The microbial diversity of the sponges has been categorized into 14 bacterial phyla (Hentschel et al. 2001). Denaturing gradient gel electrophoresis reveals the bacterial phyla using 16S rRNA, which include Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Gemmatimonadetes, Nitrospira, Planctomycetes, Proteobacteria (Alpha, Beta, Delta and Gammaproteobacteria), Spirochaetota and Verrucomicrobia (Althoff et al. 1998). Certain sponges bear sponge-specific phyla, e.g. "*Poribacteria*".

The reported archaea in marine sponges belongs to the phylum Crenarchaeota, which is also reported in artic sponges (Holmes and Blanch 2007). The most abundant sponge derived archaeal sequences belongs to *Crenarchaeota*, which is more prevalent in the marine environment (Karner et al. 2001). The sponge *Axinella mexicana* has 65% volume of the "Candidatus *Cenarchaeum symbiosum*" (Hallam et al. 2006).

8.4.2 Sponge-Specific Microbes

Based on electron microscopy and laboratory cultivation methods, the sponge-associated microbes are categorized into three: (a) larger population of microbe in the sponge mesohyl, (b) smaller population occurring intracellularly, and (c) bacteria from the surrounding seawater (Wilkinson 1978). Both molecular-based study and cultivation-based approach revealed unique bacterial communities between sponges and surrounding seawater (Hill et al. 2006). According to Hentschel et al. (2002), some sponge-specific microbes have been identified and they belong to Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Nitrospira and Proteobacteria. The 16S rRNA sequence results from the Indonesian sponge 01IND 35 have 50% gene sequence similarities which were closer to genes obtained from other sponges, which include species belonging to Acidobacteria, Nitrospira, Bacteroidetes and Proteobacteria (Hill 2003).

There are 16 bacterial phyla and two main archaeal lineages (*Crenarchaeota* and *Euryarchaeota*) that are recovered from marine sponges, the microbes which are sponge-specific in nature are present in larger quantity due to the presence of the very rich microbial communities (high microbial abundance sponges) (Hentschel et al. 2006). The superphylum Planctomycetes-Verrucomicrobia-Chlamydiae (PVC) consists of some variable associations during phylogenetic analysis, and such sequences containing the sponge species are *Agelas dilatata, Aplysina aerophoba, Discodermia dissoluta* and *Theonella swinhoei* (Taylor et al. 2007a, b). Caribbean, sponges such as *Agelas dilatata, Discodermia dissoluta, Plakortis* sp. and *Xestospongia muta*, *Xestospongia testudinaria* from Indonesia, *Theonella swinhoei* from the Red Sea and *Dysidea avara* from the South China Sea contain 54 sequences of sponge-specific clusters Acidimicrobiaceae (Taylor et al. 2007a, b).

8.4.3 A Generalized Pattern of Sponge-Associated Microbes

There is a generalized pattern of how these sponge-associated microbes localize themselves to the sponge. The light-exposed outer surface generally consists of Cyanobacteria (*Aphanocapsa* sp., *Synechocystis* sp., *Prochloron* sp.) and eukaryotic algae. *Aphanocapsa feldmanni* is the most abundant and is present in at least 19 different sponge species along with *A. aerophoba*. Then the inner part of the sponge is populated by heterotrophic bacteria occupying the mesohyl layer of the sponge, which are located in the extracellular layer within the mesohyl. Some of the

known bacteria are *Astrosclera willeyana* and *Petrosia ficiformis*. Lysis takes place inside the sponge vacuole, in healthy sponges in the canal system, and the outer surface area is free from bacterial growth. The sponge *A. aerophoba* has a large number of filamentous bacteria that are present within the sponge nuclei; the number of bacteria present is correlated to its pathogenicity of the host. Similarly, the bacteria belonging to the genus *Holospora* infect eukaryotic ciliate *Paramecium* (Görtz and Brigge 1998).

When compared to planktonic variations, most of the sponges have very little difference within their communities. When compared to the human microbiome (Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria), in sponges the phylum Proteobacteria (class Alpha and Gammaproteobacteria) was found to be in higher amount, with Chloroflexi, Cyanobacteria and Crenarchaeota rarely showing relative abundances (Thomas et al. 2016a, b). There is an estimation that between 9000 and 20,000 operational taxonomic units (OUTs) have been identified from the surface of the seawater using 16S rRNA sequencing of the V6 region by pyrosequencing (Zinger et al. 2011). A single-sponge species can harbour a large number of OUTs from its surrounding seawater, sponge species like Carteriospongiafoliascens and *Ircinia variabilis* harbour more than 12,000 OUTs, and the same results were seen in other sponges like *Cliona delitrix, Ircinia strobilina, Ircinia oros, Mycale laxissima, Plakortis halichondrioides, Sarcotragus fasciculatus, Xestospongia* sp. (Zinger et al. 2011).

8.4.4 Sponge Microbiome-Associated Natural Products

Since 1960 the number of natural products that were discovered mostly come from marine sources, with sponges being on the top of the list (Blunt et al. 2011). Each year, more than 5000 different natural products from sponges have been reported along with 180 metabolites (Laport et al. 2009). Some of the important sponge metabolites with profound pharmacological activities include halichondrins, discodermolide, hemiasterlins and arenastatin A, which are used in the treatment of tumours, inflammation and several other diseases. Some of the derived substances have not been derived from sponges but from the sponge-associated microbes that can be either present in them or transported in through the filter-feeding mechanism (Proksch et al. 2002).

8.4.4.1 Manzamine Alkaloids

They form about one-quarter of the 25,000 products that were reported from the ocean. The manazamine alkaloids were first reported in sponge *Haliclona* (Okinawan sponge) with cytotoxic activity against the leukaemia cell line P388 showing activity of IC50 of 0.07 mg/mL (0.13 mM) (Sakai et al. 1986). The alkaloids ircinols A and B which were isolated from sponge *Amphimedon* comparatively have opposite configurations against ircinals A (2) and B (3) from the Okinawan sponge *Ircinia* sp. (Tsuda et al. 1994). They have a wide range of activities like cytotoxicity, insecticidal and antibacterial as well as curative activity

against malaria in vivo (Ang et al. 2000). The compound zamamidines A-C(13-15) isolated from the sponge *Amphimedon* showed inhibitory activity against parasite *Trypanosoma brucei brucei* (IC50 values: 1.4, 1.4, 0.4 and 0.07 mM) as well as against the malarial parasite *Plasmodium falciparum* (IC50: 9.6, 16.3, 0.8 and 1.8 mM) (Yamada et al. 2009).

8.4.4.2 Bromopyrrole Alkaloids

These are compounds (Oroidin 17) that are found especially in marine sponges, first isolated in 1971 from sponge *Agelas oroides* (Forenza et al. 1971). Due to the presence of the pyrrole-imidazole part in bromopyrrole alkaloids, it can be considered as the metabolic derivative of oroidin. They exhibit good activity against predators in the Caribbean reef sponges *Agelas* (Wilson et al. 1999). Compounds such as Hymenidin (2-debromooroidin), clathrodin (2,3-debromooroidin), sventrin (pyrrole N-methyloroidin) were obtained from marine sponges like Hymeniacidon, and the Caribbean sponges *Agelas clathrodes* and *A. sventres* (Rosa et al. 1992). The compounds oroidin, hymenidin and clathrodin were found to have anticholinergic and antiserotonergic activities. The compound tauroacidin A (28) and B (29) from sponge *Hymeniacidon* sp. and taurodispacamide (30) from *Agelas oroides* showed an inhibitory activity over EGF receptor kinase and c-erbB-2 kinase activities (IC50 ¼ 20 mg/mL), whereas the compound taurodispacamide showed antihistaminic activity, and the debromo derivative from the sponge *Axinella verrucosa* has been shown to act as a glutamate and serotonin antagonist (Aiello et al. 2006). The compound hymenin (46), which acts as an alpha-adrenoceptor antagonist, was isolated from the sponge *Hymeniacidon* sp. has antibacterial activity against *Bacillus subtilis* and *Escherichia coli* (Kobayashi et al. 1986).

The compound hymenialdisine has inhibitory activity against CDKs, GSK-3b, CK1 and Chk1, which helps in the treatment of Alzheimer's disease, type II diabetes and cancer (Martinez et al. 2002). The compound agelastatin A (41) was obtained from *Agelas dendromorpha* and from *Cymbastela* sp.; they exhibit higher antiproliferative activity against cancer cell lines (D'Ambrosio et al. 1996). The bioactive compounds belonging to nagelamides have a broad range of activity against bacterial and fungal pathogens like *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Trichophyton mentagrophytes, Cryptococcus neoformans, Candida albicans and Aspergillus niger*, having an MIC range of 7.7 and 38.4 mM (Endo et al. 2004).

8.4.4.3 Bromotyrosine Derivatives

The first reported compound of the bromotyrosine derivative is (+)-aeroplysinin-1 (100) obtained from the sponge coming under the order Verongida (*Verongia aerophoba*) in 1970; later on, it was isolated from different sponges like *Psammoposilla purpurea, Aplysina laevis, Aplysina caissara*, whereas the isomer of this compound was isolated from *Ianthella ardis* (Fulmor et al. 1970). This aeroplysinin-1 (100) is highly antiproliferative against cancer cell lines such as Hela, L5178Y, human mammary cell lines and colon carcinoma cell lines (Koulman et al. 1996). It also inhibits the growth of BAECs (bovine aortic endothelial cells)

with apoptotic cell death. The cytotoxic compound aplysinones A–D (124–127) was isolated from the sponge *Aplysinagerardogreeni* has been reported to show cytotoxic activity against MDA-MB-231 (breast adenocarcinoma), IC50 values between 3.0 and 7.6 mM, A-549 (lung carcinoma) and HT-29 (colon adenocarcinoma) (Hernández-Guerrero et al. 2007). Aplysinones A (124), B (125) and D (127) have proven cytotoxic activity against HT-29 cells (colon adenocarcinoma) with corresponding IC50 values of 9.1, 3.0 and 11.3 mM (Hernández-Guerrero et al. 2007). The compound bastadins isolated from the sponge *Ianthella basta* has shown higher antibacterial activity against gram-positive bacteria, anti-inflammatory activity, topoisomerase II, dihydrofolate reductase, inosine 5′-phosphate dehydrogenase, and 12- and 15-human lipoxygenases (Jaspars et al. 1994).

8.4.4.4 Sponge-Microbiome-Derived Peptides

The sponge-microbial-derived peptides have a unique structure when compared to other source peptides. They also contain unusual linkage between amino acids *kapakahines* from sponge *Cribrochalinaolemda* (Nakao et al. 2003). The first bioactive peptide discodermin A (142) was isolated from the sponge *Discodermia kiiensis*; discodermins A–D (142–145) possess antibacterial activity, and phospholipase A2 (PLA2) inhibitor activity (Ryu et al. 1994). The discodermins F–H (146–148) shows cytotoxic activity against murine leukaemia cells resulting in IC50 values of 0.6, 0.23 and 0.6 mM, discobahamins A and B show mild antifungal activity against *Candida albicans* (Gunasekera et al. 1994). The peptide polydiscamide A helps in the inhibitory activity of the A549 (cancer cell line of the lungs) ($IC_{50} = 0.4$ μM) and also inhibits the growth of the bacterium *Bacillus subtilis* (MIC of 1.8 μM) (Gulavita et al. 1992). The peptide jaspamide (150) has a broad range of activity that includes antifungal, antihelminthic, insecticidal and cytotoxic, and there are 16 derivatives of jaspamide (B–H and J–R) that have been isolated from the marine sponge *Jaspis splendens* (Zampella et al. 1999). Almost all these derivatives show antiproliferative activity with IC_{50} values 0.01–10 μM when tested against MCF-7 human breast adenocarcinoma, HT-29 colon carcinoma, or L5178Y muse lymphoma cell lines (Gala et al. 2009).

The bioactive peptide hemiasterlin (157) is an antimitotic tripeptide that was primarily isolated along with geodiamolide TA in 1994 from the sponge *Hemiasterella minor*; it has cytotoxic activity against the P388 leukaemia cell line with an IC_{50} value of 0.02 μM (Talpir et al. 1994). The bioactive peptide halipeptin A (176) has an anti-inflammatory activity which can inhibit 60% of oedema in a mouse model when it was given a dosage of about 0.3 mg/kg (i.p.) when compared with the known indomethacin and naproxen (ED_{50} of 12 and 40 mg/kg) (Randazzo et al. 2001). The bioactive compound theonellamide F (177) has a wide range of antifungal activity against *Candida* sp., *Trichophyton* sp. and *Aspergillus* sp. With MIC values 1.8 and 7.3 μM, it also has cytotoxic activity on L1210 and P388 leukaemia cells with IC50 values of 1.9 and 1.6 μM (Matsunaga et al. 1989).

8.4.4.5 Industrial Enzymes from Sponge Microbiome

The metaproteome of the Mediterranean sponge *Aplysinia aerophoba* comprises 3.6% of the CAZymes. They play a major role in metabolising chitin, and N-acetylglucosamine also helps in the bioconversion of the complex polysaccharides (glycoproteins and glycolipids) (De Mares et al. 2018). The enzyme carboxylesterases that was derived from the marine source has been utilized for various purposes like the food industry, leather industry, textile industry, pharmaceutical production and biofuel production. After CAZymes (polysaccharide-degrading enzymes), the enzyme carboxylesterases are the most characterised enzyme that was obtained from the sponge microbiome (Navvabi et al. 2018). The bacterial strain *Pseudomonas* sp. MSI057 obtained from *Dendrilla nigra* shows elevated relative activity even in the presence of low detergent concentrations like Triton-X and SDS, and the sponge *Halichondria rugose* yields a mild lipase active enzyme from the *Bacillus pumilus* B106 strain (Zhang et al. 2009). The novel esterase 7N9 isolated from *Stelletta normani* (deep-sea sponge), which has slight alkaliphily, salt tolerance and reactivity to metal ions, and mainly, psychrophily highlighted its potentiality for low-temperature processes (Borchert et al. 2017).

8.4.4.5.1 Proteolytic Enzymes

A collagenolytic enzyme isolated from the bacterial strain *Pseudoalteromonas agarivorans* NW4327 that belongs to the sponge *Rhopaloeides odorabile* has a pathogenic activity which was proved by the Azocoll-degrading assay and electron microscopic study, which was later purified and tested against gelatine, casein, bird feather and collagenous spongin obtained from different demosponge skeletons. Interestingly the enzyme production reached the max when a natural source of seawater along with host-specific spongin was used later with the help of structural prediction, and functional sequence analysis was used to identify them as the U32 peptidase family (Bhattacharya et al. 2018, 2019).

The collagenase enzyme-producing *Pseudoalteromonas* strain was successfully used in the process of meat tenderization and also helps in the production of antioxidant hydrolysates from seafood waste (Yang et al. 2017).

Many of the isolated protease enzymes from the marine source (sponge microbiome) shows similar activity when compared with previously isolated protease enzymes. A heat-stable alkaline protease was isolated from the *Fasciospongia cavernosa* sponge which was produced by a gelatinolytic strain *Roseobacter* strain which shows 92% activity at pH 9.0 and 50 °C (Shanmughapriya et al. 2008). A fibrinolytic protease was isolated from the bacteria *Streptomyces radiopugnans* strain, which can degrade casein plasminogen and also has the potential to release the red blood cells at greater efficiency (91%, 100% and 100% at 10, 20 and 30 in, respectively) when compared to the known drug streptokinase (87%, 94% and 100% at 10, 20 and 30 min, respectively) by performing clot lysis assay which can be used as a thrombolytic agent for cardiovascular disorders, such as acute myocardial infarction and stroke (Dhamodharan 2019).

8.5 Environmental Factors and Sponge Microbiome

The sponge microbiome is resilient to most of the environmental factors like starvation and exposure to various physical and chemical agents. Sometimes the process of translocation to *Aplysina aerophoba* and *Aplysina cavernicola* shows only a few changes in their microbiome composition (Thoms et al. 2003). Bacterial profiling of the three sponge species *Lubomirskia baicalensis, Baikalospongia intermedia* and *Swartschewskia papyracea* by pyrosequencing shows the richness of Cyanobacteria. The microbiome diversity of *S. papyracea* is richer when compared to other sponges such as *L. baicalensis* and *B. intermedia*, especially when the Actinobacteria is in enormous amount (Seo et al. 2016).

The increase in the temperature can cause the sponge microbiome to shift or even decline in its overall health. A sudden decline in the microbiome population in *Rhopaloeides odorabile* was noticed when the temperature shifts from 31–32 to 33 °C, leading to sponge tissue necrosis and decline in the microbial symbiont (Simister et al. 2012a, b). The process of eutrophication also leads to changes in the sponge microbiome, due to a sudden increase in the nutrients such as nitrogen and phosphorus which indeed increases the phytoplankton population thus creating a biological oxygen demand situation (BOD) and higher sedimentation rate (Nogales et al. 2011). At the starting stages of nutrient influx, in Chesapeake Bay the dominant groups of the microbiome are the SAR11, SAR86 and picocyanobacteria, due to continued depletion in the oxygen causing a drastic shift in the community to anaerobes Firmicutes, Bacteroidetes and sulphur-oxidizing Gammaproteobacteria (West et al. 2016)

8.6 Conclusion

The marine sponge microbiome has a richer source of microbial community that is useful in many applications. The role of microbiome dynamics has important functions in the synthesis of bioactive compounds and protection against predation. Most of the high-potency enzymes were isolated from the sponge or sponge-associated microbiome, whereas in the sponge *Spongia officinalis* growing in polluted areas, it harbours a very rich source of microbial diversity when compared to its surrounding seawater. By applying advanced techniques like metagenomic analysis, transcriptomic analysis brings out the unexplored part of the sponge-microbiome interaction and its potential.

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Microbiome-Based Sustainable Agriculture Targeting Plant Protection

9

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Abstract

The plant rhizosphere hosts a vast array of microbes including bacteria, fungi, and others that provide nutrient absorption and plant protection among other crucial functions. Recent research shows that the plant defense system through the influence of secondary metabolites in root exudates and defense hormones shapes the rhizosphere and endosphere microbiome, promoting certain taxa while removing others. The root-associated microbiota deploys their repertoire of secondary metabolites to antagonize pathogens even before they get to the plant, acting as the true first line of defense while also priming systemic plant defense. Attempts to promote plant protection through the use of one or more such beneficial microbes have not yielded consistent results in field settings. Disease-protective soils that confer strong plant protection have spurred interest in the use of the microbiome to bolster plant protection. The consistent theme arising in recent research has been that healthy resilient microbiomes corresponding to better plant protection are characterized by a higher diversity of microbes, likely nurtured by richer host root exudates. Relatively higher

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microbial diversity is detected in wild relatives of crops, organic farms, and disease-suppressive soils as opposed to domesticated crops with inorganic fertilizer farming, which also display reduced symbiotic interactions. These observations suggest that a good investment in sustainable farming would be to harness diverse beneficial microbial communities for agriculture and to engineer crop plants to recruit and retain the same, akin to their wild relatives. Microbiome-based agriculture, free from toxic and polluting pesticide and fertilizer use, is, therefore, an exciting advance towards sustainability.

Keywords

Plant rhizosphere · Resilient microbiomes · Microbiome-based agriculture · Sustainability

9.1 Introduction

All eukaryotes display complex associations with microbial communities (Lareen et al. 2016). The rhizosphere microbiome refers to the teeming diversity of microbes including bacteria, fungi, oomycetes, archaea, viruses, and protists inside, on, or around plant roots in the soil forming a complex ecosystem (Compant et al. 2019); specifically, the term microbiome indicates the genetic information that identifies these microbes. Rhizosphere microbes compete with each other and the plant for soil nutrients and organic compounds and often assist the plant in accessing the trove of nutrients from the soil. Some of these microbes are free-living, and some colonize the root surface (rhizoplane), while others can live inside the roots and are referred to as endophytes. The best-characterized endophytes include the nitrogen-fixing *Rhizobium* and arbuscular mycorrhizal fungi. Fungi are also vital members of the rhizosphere microbiome and an estimated 80% of angiosperm species are supposed to associate with mycorrhizal fungi (Wang and Qiu 2006).

The microbiome of an organism serves as an extension of its genome (Turner et al. 2013), conferring new genomic and biochemical functional capabilities. The rhizosphere microbiome bestows on the plants a vastly extended capability of nutrient absorption, disease resistance, immune regulation, and stress tolerance and is an important determinant of growth and productivity (Berendsen et al. 2012; Perez-Jaramillo et al. 2016). The rhizosphere microbiome of each plant is influenced by many factors—primarily the soil microbial diversity which is used to seed the microbiome; the nature of the soil, including water, nutrient, mineral content, and pH; plant genotype; and other environmental conditions. Microbes are attracted to root exudates and other organic material secreted by the roots which contain nutrients and signals to attract microbes for colonization through a process referred to as rhizodeposition, which alters the chemical nature of the rhizosphere environment. Up to 40% of photosynthetically fixed carbon and 20% of plant nitrogen may

be released into the soil environment (Odelade and Babalola 2019; Whipps 1990). This highlights the significant investment made by the plant to nurture its microbiome. The quality of the root exudates is dependent on the host genotype and its products of primary and secondary metabolism and is also influenced by the environment; hence, the rhizosphere microbiome composition is a function of the genotype-environment interactions with soil being the major seeding factor.

From a plant defense standpoint, the microbiome functions as an additional layer of protection against pathogens. The existence of certain microbes in the rhizosphere can reduce or avert plant disease (Newitt and Prudence 2019). Rhizosphere microbes add to the repertoire of defense proteins that plants produce such as chitinases and proteases to suppress pathogens (Pinski and Betekhtin 2019). The microbes can synthesize novel antimicrobials that the plant cannot make (Rout 2014), restricting the growth of certain microbes, including potential pathogens. Rhizosphere colonization of bacteria can also induce systemic defense in a process referred to as induced systemic resistance (ISR), wherein plants are primed for a faster and stronger response for defense against infections.

The plant immune system also plays a significant role in selecting microbes from the soil environment (Leach et al. 2017). Plants respond to microbes in the rhizosphere through a process is known as MAMP-triggered immunity (MTI), which senses microbial structures and secretions and limits microbial access to the root environment. In addition, plants utilize a large diversity of secondary metabolites to selectively retain certain microbes while targeting others. For example, the plant stress hormone salicylic acid gates the plant endosphere and limits access to certain microbes, thus shaping the microbiome composition (Lebeis et al. 2015). The plant commensals and symbionts have evolved to tolerate or dampen plant immunity to survive in the rhizosphere and endosphere. The beneficial survivors in the rhizosphere not only stimulate plant growth but also protect them from stress in return for organic carbon and other nutrients. Thus, the plant immune system and the selected rhizosphere microbiota mutually benefit each other.

Crop disease accounts for major losses in agriculture and disease resistance can be bred into crops, but evolving pathogens can overcome the resistance in field settings (Wille et al. 2019). Modern agriculture has been based heavily on chemical application and the effect of pesticides has adverse effects on the environment (Gomez Exposito et al. 2017). For generations, humans have unwittingly as well as knowingly manipulated the rhizosphere microbiome to optimize plant growth. Soil amendments ranging from manure to compost involving microbe-driven fermentation processes constitute an important part of organic farming and enrich the root microbiome. In recent decades, farmers have used one or more beneficial plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* and *Bacillus* species to enhance plant growth and protection through biological control of pests and pathogens (Rosier et al. 2016; Kloepper et al. 1980). Appreciating that microbes are crucial drivers of agricultural productivity (Qiu et al. 2019), recently the focus has shifted to utilizing the soil microbiome to sustainably improve crop production without the use of polluting fertilizers and harmful pesticides (Philippot et al. 2013). To realize this, it is important to approach plants that need protection as holobionts

that are intimately and inseparably tied to their microbiome and maximize the positive effects of the microbiome (Wille et al. 2019). The one plant-one pathogen model is now giving way to the pathobiome concept, which considers that the effects of the pathogen are moderated by the action of the commensals and symbionts such as those in the rhizosphere microbiome as well as the environment (Bass et al. 2019).

Only a fraction of the microbiome can be cultivated in artificial media and early estimates ranged from only 1% to 10% (Conn et al. 1918) as it is a challenge to reproduce the conditions required to sustain many species; recent research shows that these predictions are an underestimate. Culture-independent identification of bacteria through DNA sequencing has enabled the identification of bacteria recalcitrant to culture. The development of high-throughput next-generation sequencing has facilitated shotgun metagenome sequencing and made possible the identification of millions of sequences per sample and dramatically improved the resolution of identification to include even rare species (Turner et al. 2013). Most importantly, this has led to the identification of microbes that are recalcitrant to culture and broadening of our understanding of three-way plant-rhizosphere microbiome-pathogen interactions in an unprecedented fashion (Wille et al. 2019). The study of the metaphenome, which encompasses not only the metagenome and metatranscriptome but also the metaproteome and metametabolome can help appreciate the full functional potential of the rhizosphere microbiome on a global scale (Jansson and Hofmockel 2018).

The improved ability to culture bacteria has also enabled the development of synthetic communities (SynComs) of bacteria that have enabled a deeper understanding of microbial community functions, their interactions with the plant, and plant responses to them. This information can facilitate the development of new strategies including improving plants to adopt better microbiomes, applying optimal microbial communities, plant probiotics, and microbe-derived products for better plant growth and biological control of pests and pathogens (Levy et al. 2018; Rosier et al. 2016). The discovery that plant genotype influences microbiome composition has also important connotations to improve agriculture (Leach et al. 2017).

Sustainable agriculture is a priority in serving the burgeoning human population, which has increased sevenfold since the beginning of the nineteenth century. It will be an important strategy to combat the rising challenge to grow food and fodder in less than ideal conditions including dwindling arable land and more hostile climate conditions triggered by climate change (Tilman et al. 2002; McNear 2013). Harnessing the rhizosphere microbiome could improve crop productivity, decrease losses from plant disease, and reduce the use of pesticides (Turner et al. 2013). In this chapter, we discuss the rhizosphere microbiome in the context of agriculture and how the understanding of plant immunity-microbiome interactions can be utilized for sustainable agriculture.

9.2 Understanding Rhizosphere Microbiome Interactions with Plant Defense

9.2.1 Rhizosphere Colonization

Plant-microbe association in the rhizosphere is largely driven by mutual metabolic needs. Competing or cooperating microbes influence each other's survival and abundance, while the plant recruits and selects microbes from the pool in the soil environment. The plant genotype, as well as the environment, can affect the morphology of the root as well as the chemical composition of the root exudates and other plant material. The amount of organic compounds like sugars and amino acids and inorganic nutrients can dictate the composition and abundance of microbial species in the rhizosphere (Fierer 2017; Rout 2014). The colonization of the rhizosphere by microbes proceeds through several steps: recruitment and motility, root surface colonization, and in some cases biofilm formation (Pinski and Betekhtin 2019). Additionally, endophytic microbes also invade the host tissue for colonization.

9.2.1.1 Recruitment

The recruitment of specific microbes by plant roots to form the rhizosphere microbiome is an active process involving rhizodeposition (Quiza et al. 2015). Rhizodeposition involves the secretion or release of root exudates, gases, macromolecules, sloughed-off cells, and intact root border cells enriched in organic compounds into the rhizosphere environment (Jones et al. 2009). Root exudates are predominated by sugars, organic acids (as in tomato) (de Weert et al. 2002), and amino acids (as in rice) (Bacilio-Jiménez et al. 2004) and also include metabolites such as fatty acids, sterols, vitamins, secondary metabolites like phenolic compounds and putrescine, volatile compounds as well as macromolecules such as proteins, and complex carbohydrates such as cellulose and mucilage (Badri and Vivanco 2009; Bertin et al. 2003; Quiza et al. 2015; Mendes et al. 2013). The molecules in root exudates, released mainly from root cap cells, can attract microbes in the surrounding soil, which can utilize them as carbon and nitrogen sources or as signals that trigger chemotaxis (Reinhold-Hurek et al. 2015). Only microbes that survive host defenses and competition among each other and sense these molecules as preferred substrates venture into the rhizosphere for successful colonization (Zhalnina et al. 2018). Many beneficial bacteria like rhizobia and *Bacillus* and *Pseudomonas* spp. migrate to the plant through chemotaxis and can colonize on or inside the plant. Thus, root exudates are critical determinants of the root and rhizosphere microbiome composition (Rout 2014; Turner et al. 2013).

Certain metabolites in root exudates help recruit beneficial bacteria. For instance, the release of the organic acid malic acid in exudates triggered by foliar infection with *Pseudomonas syringae* enlists the beneficial bacterium *Bacillus subtilis* (Rudrappa et al. 2008). Likewise, citric acid and malic acid released by tomato, watermelon, and cucumber roots promoted positive chemotaxis of beneficial *Pseudomonas fluorescens* WCS365, *Paenibacillus polymyxa*, and *Bacillus*

amyloliquefaciens SQR9, respectively (de Weert et al. 2002; Ling et al. 2011; Zhang et al. 2014). Thus, the attraction of beneficial bacteria by exuding organic acids is a common phenomenon in the rhizosphere.

9.2.1.2 Surface Colonization and Biofilm Formation

Root exudates attract a variety of bacteria, but only those that can make contact with the root can colonize the root surface and access the interior (Pinski and Betekhtin 2019). Protein-based fimbriae, pili, adhesins, and curli fibers can facilitate the physical attachment of bacteria to surfaces (Mohan et al. 2018). Bacteria can then autoaggregate and form microcolonies. Bacteria communicate through a process known as quorum sensing (QS), which is fundamental to the colonization of plants by bacteria. Through this process, they sense or estimate the density of their population or that of other bacteria by monitoring levels of certain secreted signaling molecules called autoinducers and regulate gene expression accordingly. Autoinducers include *N*-acyl homoserine lactones (AHLs) (e.g., *Pseudomonas*), lipid-based diffusible signal factors (DSF) (e.g., *Xanthomonas, Stenotrophomonas*), and oligopeptides (e.g., *Bacillus*) (Eberl 1999) (reviewed in Mohan et al. 2018). Different bacterial species may share the same signal and display interspecies cooperativity, or interfere with quorum sensing in other bacteria in a process known as quorum quenching. QS communication is critical for the coordination of various population density-driven processes such as motility, adhesion, biofilm formation (Lareen et al. 2016), and virulence functions in pathogens. Once bacteria adhere to root surfaces, they can form microcolonies and in some cases proceed to develop a biofilm (Rout 2014).

Microcolonies can grow into biofilms where bacteria aggregate in several layers ensheathed in a matrix. Biofilm-forming bacteria may shed their flagella and secrete a glutinous substance called exopolysaccharide (EPS) among others to aid the formation of a biofilm (Meneses et al. 2011; Żur et al. 2016). The secretion of these substances requires cooperation between bacteria of the same or different species, coordinated through QS (Hassani et al. 2018). Root exudates, particularly amino acids, have an important role in the dynamics of biofilm formation and disassembly (Kolodkin-Gal et al. 2010). Bacteria within a biofilm can also communicate to coordinate the density-dependent discharge of plant growth-promoting compounds (Rudrappa et al. 2008). Biofilms not only serve to shield the component bacteria from other bacteria and host immunity (Van Acker et al. 2014) but also occupy niches to deny phytopathogens access to space, thus physically protecting the root surface.

9.2.1.3 Invasion

Bacteria, particularly endophytes, may enter into roots passively through cracks or may actively produce cell wall- and middle lamella-degrading enzymes (Turner et al. 2013; Viaene et al. 2016) to disrupt the barriers and gain entry into the root. The production of these enzymes (frequently hydrolases) may be triggered by root exudate components and amplified by QS (Levy et al. 2018). In sum, the bacteria that establish in the rhizosphere survive a competitive environment and go through

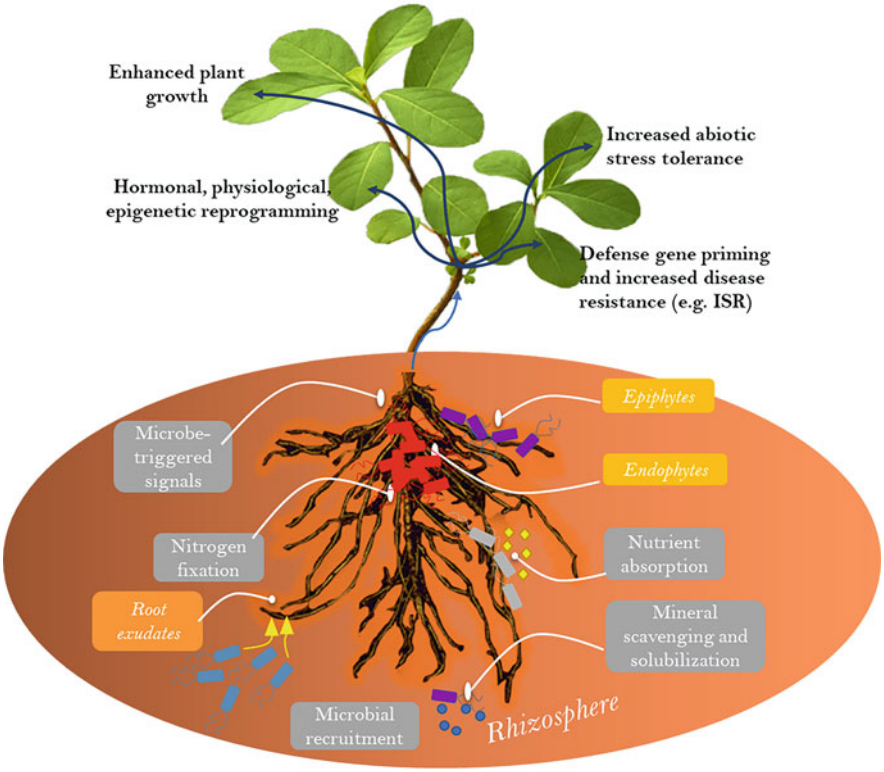

Fig. 9.1 Various benefits of the rhizosphere microbiota—belowground and aboveground. Epiphytes colonize root surfaces (purple), while endophytes colonize root interiors (red). Roots release exudates containing primary and, more selective, secondary metabolites and microbes (bacteria shown as blue) respond to the exudates; rhizosphere microbes facilitate nutrient absorption, mineral scavenging, and nitrogen fixation; they also recruit other microbes to the root; microbe-derived signals stimulate various systemic responses in the aerial parts of the plant as shown

several steps to establish contact with roots in the rhizosphere. The colonized microbes confer numerous benefits to the plant as illustrated in Fig. 9.1.

9.2.2 Selection of the Rhizosphere Microbiome by Plant Immunity

Microbial diversity decreases from the surrounding soil to the rhizosphere and is least in the endosphere, indicating that the rhizosphere and root interiors are strong selective environments (Rodriguez et al. 2019). At the same time, the abundance of microbes of each type is enriched within the rhizosphere implying that the selected microbes experience a supportive environment. Recent evidence strongly suggests that plant immunity plays a major role in selecting the microbes in the rhizosphere.

Plants appear to have a strong capacity to influence the composition of the rhizosphere through the secretion of secondary metabolites and phytohormones (Bulgarelli et al. 2015). Exudation of nutrients and antimicrobial metabolites and proteins encourages certain microbes while deterring others (Quiza et al. 2015). It appears that the competitive shield of rhizosphere microbes operates as the very first layer in plant protection, while additional layers of plant immunity exist. The first line of plant defense is the basal resistance conferred by preexisting physical and chemical defenses. Then comes the molecular machinery of induced defense that is activated when the plant perceives potential intruders by detecting microbial structures or contents. Finally, induced defense involves signaling that culminates in transcriptional and posttranslational activation of protein-based defenses in addition to refortification of physical structures and recharging of chemical defenses.

9.2.2.1 Basal Immunity

9.2.2.1.1 Physical Defenses

The waxy cuticle of the root serves as the primary physical barrier to microbial ingress (Martin 1964). The root cap and the border cells that constitute the distal part of the cap are also important defensive structures in the root. While the root cap protects the growing root tip, the root border cells are sloughed off periodically and participate in the physical and chemical defense against potentially pathogenic microbes (Gunawardena and Hawes 2002). The sloughed-off cells and root border cells serve a protective function for the plant by acting as bait to distract phytopathogens while attracting beneficial bacteria (Hawes et al. 2000).

9.2.2.1.2 Basal Chemical Defenses

Root exudates, in addition to primary metabolites like sugars, amino acids, and organic acids, are also enriched in secondary metabolites relevant to plant immunity and thus begin to target specific microbes even before they have come into contact with the plant. Several defense-related metabolites differentially influence (attract, deter, or kill) different sets of microbes, and the resultant microbial community is a consequence of the collective selective pressure exerted by the plant metabolites and proteins in combination with those released by microbes. Some defense metabolites are produced before the onset of stress and are coined phytoanticipins (VanEtten et al. 1994). Phytoanticipins include benzoxazinoids, cyanogenic glycosides, glucosinolates, and saponins (Pedras and Yaya 2015).

9.2.2.1.2.1 Phenolic Compounds

Application of a mixture of root exudate-based phytochemicals followed by 16S rRNA profiling in *Arabidopsis* revealed that phenolic compounds in root exudates had a stronger impact than other metabolites on the root microbiome composition through suppression of certain members while promoting the growth of others (Badri et al. 2013). Moreover, plant phenolic compounds induced the expression of the antifungal compounds 2,4-diacetylphloroglucinol (DAPG) and pyoluteorin (PLT) in the beneficial *P. fluorescens* CHA0 (de Werra et al. 2011). Phenolics may serve as

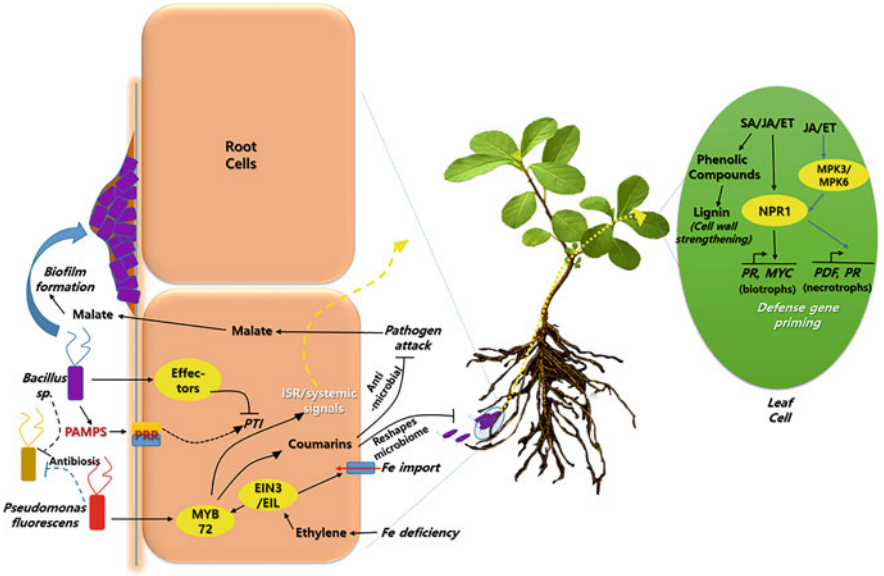

Fig. 9.2 Local and systemic protection conferred by rhizosphere microbes. Beneficial bacteria such as *Bacillus* sp. and *Pseudomonas* sp. can antagonize other microbes, including potential pathogens, through antibiosis, for instance, by producing antimicrobial compounds. *Bacillus* sp. can suppress plant defense (MTI/PTI, MAMP/PAMP-triggered immunity) using effector proteins; this allows them to colonize. Iron deficiency can signal through the ethylene pathway (EIN3/EIL), which promotes iron import through transporters. Both ethylene signaling and *Pseudomonas simiae (fluorescens)* can activate the transcription factor MYB72 which can trigger the production of the secondary metabolite coumarin and also induce ISR (induced systemic resistance). Coumarin secretion helps with the iron acquisition as well as serves as an antimicrobial to reshape the microbiome. Pathogen attack can stimulate malate release which triggers biofilm formation in *Bacillus*. The physical occupation by a biofilm protects the plants from pathogens. ISR stimulates the priming of defense in systemic tissues. While the defense hormones salicylic acid, jasmonic acid, and ethylene can also stimulate structural defenses, they can also activate primed defense gene expression (PR, MYC, PDF) against both biotrophic and necrotrophic pathogens

substrates or as signals to certain bacteria and are positively correlated with the enrichment of certain beneficial bacteria such as *Streptomyces* (Newitt and Prudence 2019). Alteration in phenolic compound profile in poplar cinnamyl-Co reductase (CCR) mutant resulted in shifts in the root microbiota composition (Beckers et al. 2016), illustrating the importance of phenolics in microbiome homeostasis.

Phenolics may be simple phenols like coumarins or complex phenols like flavonoids. Coumarins are secondary metabolites that protect plants from pathogenic fungi. The release of coumarins by roots is triggered by beneficial bacteria during iron starvation and is dependent on a root-specific transcription factor, MYB72 (Fig. 9.2) (Stringlis et al. 2018). One such coumarin, scopoletin, not only mobilizes iron but also exhibits antimicrobial activity against pathogenic fungi, while not affecting some beneficial bacteria. Recent evidence suggests coumarins also inhibit

biofilm formation in bacteria (Reen et al. 2018), thus potentially affecting the ability to compete for bacteria to establish themselves in rhizosphere niches. Thus, beneficial bacteria can restructure the microbiome by triggering the release of selective metabolites like coumarin from plants. Flavonoids are plant-specific polyphenols that are critical determinants of the root microbiome (Weston and Mathesius 2013), particularly enriched in the maize and *Arabidopsis* rhizospheres (Pétriacq et al. 2017). The role of flavonoids in plant-microbe interactions was underscored when they were identified as plant signals exuded by legume hosts to recruit modulating *Rhizobium* species (Cooper 2007).

9.2.2.1.2.2 Other Secondary Metabolites

Benzoxazinoids are an important group of secondary metabolites functioning in defense against pathogens and pests and are derived from indole (Zhou et al. 2018). They are abundant in maize and other Poaceae members. Benzoxazinoids have been shown to affect rhizosphere microbiome composition in maize by specifically affecting certain groups of bacteria (Hu et al. 2018). Additionally, a type of benzoxazinoid termed DIMBOA also helps recruit and colonize beneficial microbes like *Pseudomonas putida* (Neal et al. 2012). Saponins are constitutive phytoanticipin antimicrobial metabolites with defense functions, derived from the fusion of triterpenoid or steroid groups with sugar groups (Pedras and Yaya 2015). Well-known examples are saponins avenacin A-1 and avenacoside B that influence microbiota in oats and confer resistance to fungal pathogens (Papadopoulou et al. 1999). Strigolactones are often released by roots during nitrogen or phosphate starvation and help recruit beneficial microbes (Yoneyama et al. 2012). Like flavonoids, strigolactones also serve as signals for a symbiosis of plants with mycorrhizal fungi and parasitic plants (Perez-Jaramillo et al. 2016). Some secondary metabolites mimic bacterial AHLs and manipulate bacterial quorum sensing. For example, plants like sweet basil release rosmarinic acid (RA) in root exudates in response to infection with *Pseudomonas aeruginosa*. RA directly binds to a QS response regulator and triggers premature QS signaling to suppress microbial growth (Corral-Lugo et al. 2016). Thus, plants have a versatile array of secondary metabolites that exert a strong effect on the rhizosphere and endosphere to sculpt the root microbiome.

9.2.2.2 Induced Immunity

9.2.2.2.1 MAMP-Triggered Immunity (MTI)

An important challenge that plants face when encountering a myriad of microbes in the rhizosphere is distinguishing between pathogenic and nonpathogenic species. In some cases, plant pathogens and nonpathogens are physically not very different, and the functional differences may arise simply by the gain or loss of a few pathogenicity islands in some cases (Melnyk et al. 2019b); this complicates the distinction between pathogens and nonpathogens for the plant. Induced plant defense responses may be triggered by recognition of conserved bacterial structures (microbe-associated molecular patterns or MAMPs) on bacteria in a process known as MAMP-triggered

immunity (MTI). Plant cell surface pattern recognition receptors (PRRs) recognize MAMPs such as lipopolysaccharide (LPS), EF-Tu, and flagellin through cognate PRRs (e.g., FLS2, a leucine-rich repeat receptor-like kinase or LRR-RLK) to trigger an immune response. A typical MTI defense response includes the generation of reactive oxygen species (ROS), proton influx, calcium level spike, MAP kinase signaling, and transcription of antimicrobial pathogenesis-related (PR) genes, and collectively, these processes serve to limit pathogens (Trdá et al. 2014). MTI is important to limit microbial growth (Dangl et al. 2013) and is expected to be an important factor in gating the root microbiome.

For symbiotic bacteria and fungi, microbially produced signals are recognized by the plant to enable colonization (Pinski and Betekhtin 2019). For example, *Rhizobium*, an endosymbiont establishes symbiosis with legume hosts through a lipochitooligosaccharide NOD factor signal, while mycorrhizal fungi use chitooligosaccharides that are recognized by host roots (Leach et al. 2017). These signals are structurally similar to the bacterial MAMP peptidoglycan and the fungal MAMP chitin, respectively (Liang et al. 2014) and recognized by receptor-like kinases (RLKs) in plants to initiate symbiosis (Zipfel and Oldroyd 2017). Although MTI is an important defense response in the roots, profiling the PRR FLS2 expression in roots suggests that MTI may be more actively induced in the inner layers of the root (e.g., pericycle in stele) and in areas most susceptible to infection—the entry sites (Beck et al. 2014; Chuberre et al. 2018; Wyrsch et al. 2015). The abundance of MAMPs in the soil may prompt desensitization of the MTI response in the outer layers. Recently, mounting evidence indicates that beneficial microbes actively suppress or evade host immunity to engage in symbiosis (Yu et al. 2019).

9.2.2.2.2 Induced Chemical Defenses

In contrast to phytoanticipins that are constitutively produced, phytoalexins are secondary metabolites that are produced in response to pathogen infection. Phytoalexins are produced in both root and shoot infections (Duan et al. 2014) and can impact rhizospheric and endophytic bacteria composition (Pinski and Betekhtin 2019). A variety of phytoalexins are produced by plants, many in a genotype-specific manner; for example, camalexin in Brassicaceae members, capsidiol in capsicum, gossypol in cotton, and pisatin in pea (Preisig et al. 1990). Such defense metabolites play significant roles in defining the characteristic microbiomes of various plant species.

9.2.2.3 Plant Defense Hormones

That phytohormones are important in the regulation of microbial community composition is evident with the observation that treatment with hormones as well as defense hormone signaling mutants altered root exudate and microbial profiles (Leach et al. 2017). Three major plant defense hormones are salicylic acid (SA), jasmonic acid (JA), and ethylene. Salicylic acid mediates defense against biotrophic pathogens and is important for systemic acquired resistance (SAR), a resistance mechanism that is triggered in the shoot (Glazebrook 2005). On the other hand, JA and ethylene function in resistance to necrotrophic pathogens in the shoot, but are

also required for induced systemic resistance, a resistance pathway initiated in roots upon interaction with beneficial microbes. These three hormone pathways can function in defense signaling with additive and synergistic effects, and the loss of all three hormonal pathways results in aberrant rhizosphere microbiome composition or dysbiosis that may be linked with reduced field survival (Lebeis et al. 2015). Each of these hormones play an active role in shaping the rhizosphere and/or endosphere microbiome.

9.2.2.3.1 Salicylic Acid

Salicylic acid (SA) has been detected in root exudates of plants (Khorassani et al. 2011; Ling et al. 2013) and is among the preferred nutritional substrates for certain rhizosphere bacteria, alongside other organic acids, as observed in the oat, *Avena barbata* (Zhalnina et al. 2018). Besides serving as a nutrient, SA could also serve as a signaling molecule for some bacteria (Lebeis et al. 2015). The biosynthesis of SA in plants is suppressed by beneficial microbes; for example, an effector protein produced by the beneficial fungus *Piriformospora indica* suppressed the expression of the plant SA biosynthetic transcription factor *CBP60g* presumably to suppress SA-mediated defense and to facilitate its own colonization (Akum et al. 2015). SA also has a marked influence on the rhizosphere microbiome composition and can inhibit mycorrhizal and root nodule symbioses (Rodriguez et al. 2019). A defect in SA-mediated defense leads to increased colonization of certain bacterial species including *Salmonella enterica* and the nitrogen-fixing *Gluconacetobacter diazotrophicus* (consistent with the inhibition of nitrogen-fixing bacteria by SA), but not other bacteria such as *Klebsiella pneumoniae* (Pinski and Betekhtin 2019). *Arabidopsis* mutants exhibiting altered SA synthesis and signaling, but not JA and ethylene mutants, showed distinct core root microbiomes at the family level (Lebeis et al. 2015), while previous studies showed little effect of SA on the microbiome (Bodenhausen et al. 2014; Carvalhais et al. 2014; Doornbos et al. 2011). SA appeared to limit the growth of several families of bacteria as they were enriched in SA defense-deficient mutants in root interiors, suggesting that SA plays an important role in restricting the growth of certain taxa in wild-type plants while allowing the growth of others. The disruption of SA-mediated defense also reduced leaf endophytic diversity (Kniskern et al. 2007). Thus, it is clear that SA is a strong component of plant defense in gating rhizosphere microbes and regulating the microbiota composition. Consistently, beneficial bacteria such as *Pseudomonas putida* appear to modify the microbial community by activating SA signaling in *Arabidopsis* (Sheoran et al. 2016).

9.2.2.3.2 Jasmonic Acid

The effect of JA on symbiosis varies with plant genotype and conditions (Reverchon et al. 2019). Certain microbes not only suppress SA defenses, but some like the mycorrhizal fungus *Laccaria bicolor* also inhibit JA signaling to enable colonization; an *L. bicolor* effector prevents the degradation of the JA repressor JAZ to keep early JA-mediated defense inhibited to allow colonization (Plett et al. 2014). Other beneficial fungi, *P. indica*, and the beneficial bacteria *Bacillus subtilis* suppress early

PTI in *Arabidopsis* using the JA pathway as the defense suppression is lost in JA signaling mutants, *jar1* and *jin1* (Jacobs et al. 2011; Lakshmanan et al. 2012). While the loss of SA defense reduced endophytic diversity in *Arabidopsis* roots, on the contrary, activation of JA signaling through exogenous JA application reduced root endophytic diversity in wheat (Liu et al. 2017). The shift in microbiome composition following JA application is attributed to changes in root exudate composition (Yu et al. 2019).

9.2.2.3.3 Ethylene

Colonization of plants such as *Medicago truncatula* (Iniguez et al. 2005) and sugarcane (Cavalcante et al. 2007) with beneficial microbes triggered ethylene signaling and gene expression early on and an ethylene-insensitive mutant of *M. truncatula* was observed to be over-colonized by the endophyte *K. pneumoniae* (Iniguez et al. 2005), indicating that ethylene plays a restrictive role in microbial colonization consistent with its role in plant defense. Ethylene also inhibits root nodule symbiosis as well as the association with mycorrhizal fungi (Rodriguez et al. 2019). It is, therefore, not surprising that some bacterial species including *Bacillus* and *Pseudomonas* produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces root ethylene biosynthesis by degrading the ethylene precursor, ACC (Compant et al. 2019; Glick 2014), and this was shown to enhance plant stress tolerance and root development and possibly improved general microbial colonization. Suppression of ethylene production can be beneficial to the plant as ethylene is a stress hormone that can be detrimental to plant growth at higher levels (Vaseva et al. 2018). Unexpectedly, ablation of ethylene biosynthesis and signaling in *Nicotiana attenuata* mutants reduced endophytic microbial diversity, suggesting that ethylene affects microbial homeostasis within the plant and certain bacteria may require plant ethylene signaling for invasive colonization in roots (Long et al. 2010). In contrast, in *Arabidopsis*, root microbial diversity was not affected, but rhizosphere bacterial abundance was reduced in ethylene mutant *ein2*. Thus, ethylene, like SA and JA, functions inflict both positive and negative effects on root microbiota. SA and JA/ethylene pathways generally function antagonistically as they confer resistance to different kinds of pathogens, but in the roots, they modulate microbial homeostasis as they all appear to generally prevent microbial ingress and overgrowth of certain bacteria while in some cases promoting endophytic diversity. The activation of these pathways during stress may be further instrumental in reshaping the microbiome.

9.2.3 Modulation of Plant Immunity by the Rhizosphere Microbiome

While the root microbiome is, in large part, selected by the plant immune system, they also have a reciprocal effect on plant immunity. It is now well established that the root microbiome expands plant immunity and functions as an additional layer of defense against pathogenic microorganisms, providing unique opportunities to

develop novel tools in crop protection and enhance crop productivity sustainably. Two of the ways the root microbiota participates in plant disease resistance are direct disease suppression (DDS) and induced systemic resistance (ISR) (Fig. 9.2).

9.2.3.1 Local Disease Suppression

DDS takes place either in the rhizosphere or the root interior and is commonly based on competition for nutrients and niches, parasitism, antibiosis, or combinations of the abovementioned mechanisms. DDS has ideally exemplified in disease-suppressive soils, soils in which a soilborne pathogen cannot cause disease because of the presence and/or increased abundance of potent antagonistic microbes. The mechanisms involved in direct pathogen suppression include mainly competition for carbon and siderophore-mediated competition for iron, the production of cell-wall-degrading enzymes such as chitinases, and the production of various antibiotics including the well-studied antibiotic compounds 2,4-diacetylphloroglucinol (DAPG) and phenazines (PHZ) (Rout 2014). More recently, volatile molecules have been proposed to contribute to DDS in suppressive soils. These functions are further elaborated below in the context of disease-suppressive soils.

9.2.3.2 Induced Systemic Resistance

ISR is initiated in the roots upon microbial colonization and confers broad-spectrum systemic resistance to aboveground plant tissues against pathogens and even insects (Pieterse et al. 2014). ISR was first described in studies of the early 1990s focusing on the ability of *Pseudomonas* sp. rhizobacteria to trigger systemic resistance in carnation, wheat, and common beans. Since then, the phenomenon has been shown to occur in numerous dicotyledonous and monocotyledonous plant species, suggesting that ISR represents a conserved function of the root microbiome. Interestingly, novel findings in *Arabidopsis* suggest that plants experiencing pathogen attack in the aboveground tissues modify the composition of the exudates they excrete in the root vicinity to recruit a potent consortium of ISR-inducing rhizobacteria (Melnyk et al. 2019a). Such microbiota-dependent legacy that plants generate in the soil under stress conditions has been shown to enhance the defense capacity of future generations against pathogens thereby promoting offspring survival in hostile environments. The catalog of ISR-eliciting microorganisms is long and includes both individual strains and microbial consortia. Epiphytic or endophytic soilborne bacteria belonging to the genera *Pseudomonas*, *Bacillus*, *Serratia*, and *Streptomyces* represent typical examples of ISR-eliciting microbes. Symbiotic rhizofungi such as *Trichoderma* spp., mycorrhizal fungi like *Rhizophagus irregularis* (syn. *Glomus intraradices*), the mycorrhizal-like endosymbiotic fungus *Piriformospora indica*, and nonpathogenic *Fusarium* species are also capable of eliciting ISR. Interestingly, several of the same strains involved in LDS have been shown to be potent ISR inducers.

Epiphytic ISR-inducing bacteria capable of colonizing the root system of host plants form biofilms in the root epidermis, whereas endophytic ISR-inducing bacteria enter the root interior by either actively penetrating the external root layers or entering passively through wounds and discontinuing in the epidermis such as those

formed during lateral root emergence (Pieterse et al. 2014). Although ISR-inducing rhizobacteria are not enveloped in symbiotic organs, such as the root nodules in the *Rhizobium* symbiosis, they commonly induce significant alterations in the root system architecture. Such alterations contribute to plant growth promotion but also enhance the exudation of energy-rich compounds taking into consideration that most of the root exudation takes place in the elongation zone of young roots. Yet plant growth promotion and ISR are mediated by distinct signaling pathways in the host tissues. Evidence is also accumulating that rhizobacteria of the root microbiome, including ISR-inducing bacteria, suppress plant defense responses at the early stages of the interaction to efficiently colonize plant tissues. Yet plants have evolved immunity-based genetic networks to control the population of epiphytic and endophytic communities of microbes. In *Arabidopsis*, disruption of such networks has been recently shown to result in a form of dysbiosis.

Several microbial determinants have been proposed to function as ISR elicitors, among them, molecules with well-established immune-stimulatory properties such the MAMPs flagellin and LPS, but also iron-regulated siderophores, the antibiotics DAPG and pyocyanin, *N*-acyl homoserine lactones, and biosurfactants such as cyclic lipopeptides (Rout 2014). These elicitors are likely to act redundantly during the elicitation of ISR. More recently, volatiles emitted by ISR-inducing strains have been shown to trigger the expression of the essential for ISR establishment MYB72 transcription factor (Fig. 9.2). Despite the extended list of ISR-eliciting molecules, with few exceptions such as the volatiles mentioned above, little is known on the hierarchy that those molecules function during the initiation of ISR and the exact contribution of each determinant to the phenomenon.

The molecular mechanisms underpinning rhizobacteria-mediated ISR are well-studied in *Arabidopsis* (Pieterse et al. 2014). In *Arabidopsis*, ISR triggered upon root colonization by the model strains *Pseudomonas simiae* WCS417 depends on an intact jasmonic acid (JA) and ethylene (ET) signaling pathway and further requires the transcriptional regulators MYC2 and NPR1. In contrast to the costly plant defenses activated by pathogens or insects, the establishment of ISR is not correlated with substantial reprogramming of the host's transcriptome. Instead, upon pathogen attack, immunized plants display a boosted immune reaction resulting in enhanced resistance to the attacker encountered. This phenomenon is called priming and shares striking similarities with the potentiation of cellular defense responses in primed monocytes and macrophages in mammals. In roots, initiation of ISR is regulated by the root-specific transcription factor MYB72, a member of the R2R3 family of MYB transcription factors, and components of the ET signaling pathway that locally act in the generation or translocation of a thus-far unidentified systemic signal. Importantly, MYB72 is also required for ISR triggered upon root colonization by the beneficial fungus *Trichoderma asperellum* strain T34, suggesting that this transcription factor is a node of convergence in signaling pathways induced by diverse types of beneficial soilborne microbes. MYB72 regulates the secretion of plant-derived coumarins, suggesting that these molecules are essential components of the ISR signaling pathway. Thus, root microbes play a vital role in stimulating

local and systemic plant defenses for enhanced disease resistance, which, in turn, can reshape the rhizosphere microbiome through altered root exudation.

9.3 Microbiome and Modern Agriculture

9.3.1 Impact of Modern Agricultural Practices on the Rhizosphere Microbiome

9.3.1.1 Plant Domestication

Generations of modern agricultural practices have markedly altered rhizosphere microbes. Plant protection in agriculture has long involved breeding for resistance and, more recently, genetic modification for enhanced resistance, but the development of broad-spectrum resistant crops is time-consuming and subject to stringent regulation and public approval (Syed et al. 2018). Moreover, resistance in crops can break down over the years, as observed for grapevine mildew, wheat rust, and rice blast. One of the reasons behind the resistance breakdown is that pathogens can evolve rapidly (Peressotti et al. 2010) and recently there has been an alarming rise in new fungal phytopathogens (Fisher et al. 2012). To counter this, modern agriculture has witnessed a massive surge in the use of biocides, including toxic pesticides and herbicides and yield-promoting fertilizers that can have a telling nontarget effect on the rhizosphere microbial community either directly or indirectly through their impact on the plants (Turrini et al. 2015).

9.3.1.1.1 Changes in the Rhizosphere Microbiome

Plant domestication through agriculture appears to have resulted in a reduction in both plant and microbial genetic diversity through the loss of plant traits and wild microbial species that were originally adapted for the plants (Perez-Jaramillo et al. 2016; Compant et al. 2019). These changes in the microbiome may be small in some cases but significant, as observed in wild and cultivated barley, beans, and sugarbeet (Bulgarelli et al. 2015; Zachow et al. 2014; Perez-Jaramillo et al. 2017). In general the bacterial phylum Bacteroidetes was comparatively less abundant in the rhizospheres of cultivated crop plants such as beans and other plant species compared to their wild counterparts, which are colonized more abundantly by Proteobacteria and Actinobacteria (Perez-Jaramillo et al. 2017; Pérez-Jaramillo et al. 2018). Members of Bacteroidetes, also an abundant phylum in the human gut, are known for their propensity to metabolize complex carbohydrates, a component that may have become more limited in agricultural crop rhizospheres. Thus, changes in root microbiota composition could be associated with simplification of plant exudates.

Several studies have suggested that microbial community changes during domestication likely resulted from changes in root architecture, root exudate composition, plant physiological changes, and alteration of the chemical environment (Perez-Jaramillo et al. 2016). These changes appear to have hampered beneficial associations with mycorrhizae and nitrogen-fixing rhizobia. Indeed, wild ancestors

in maize, wheat, and breadfruit showed a greater disposition to mycorrhizal associations compared to modern varieties (Kapulnik and Kushnir 1991; Xing et al. 2012; Zhu et al. 2001). The comparison of wild and domesticated legumes grown in natural soil also revealed that the ability to attract and colonize a diverse microbial community was reduced in cultivated crops, suggesting the loss of microbial recruitment skills upon domestication (Mutch and Young 2004). The lower microbial diversity in agricultural soils may also be attributed to the reduced diversity of available microbes in agricultural soils compared to natural soils since the selection of microbes by the plant is limited by what is available in the soil. This is well exemplified in the study showing that the transformation of Amazon forest areas into agricultural land resulted in shrinkage of microbial diversity (Rodrigues et al. 2013).

The loss of rhizosphere microbial diversity has consequences to plant health. Generally, diversity in a microbial community ensures that competition for niches and resources keeps pathogens at bay. Additionally, more diverse communities are also more resilient to environmental stresses such as drought as the stress-induced loss of important microbial species (often temporary) is compensated for by the presence of new taxa that spring into action and help the plant withstand stress (Xu et al. 2018). Thus, the reduction in microbial diversity in modern agricultural soils could offer pathogenic species an opening to invade the rhizosphere and cause disease and could also render the plants less resilient to stress.

9.3.1.1.2 Changes in Plant Morphology

Soil surface watering and fertilization in agricultural plants appear to have led to the evolution of shallower root systems, as the nutrients are easily accessible at the surface negating the need for deep rooting (Jackson 1995). This change in root architecture can alter root surface niches as well as oxygen exposure near the surface and consequently affect the microbiome, as has been suggested (Micallef et al. 2009). The shallowing of roots or loss of deep rooting in domesticated plants compared to wild plants has been witnessed in many plant species including lettuce. Evolutionarily, a less deep root system may have contributed to the deselection of anaerobic root microbiota such as some members in the Bacteroidetes phylum (Pérez-Jaramillo et al. 2018).

9.3.1.1.3 Changes in Plant Physiology

Agricultural domestication of plants has resulted in an erosion of genetic diversity as witnessed in multiple plant species including rice, wheat, and bean (Perez-Jaramillo et al. 2016). A general reduction of plant genetic diversity through agriculture may be linked with a reduced ability to recruit and select rhizosphere microbial communities (Wissuwa et al. 2008). The genetic component of rhizosphere microbiome selection is evident from the analysis of maize recombinant inbred lines that revealed the significant genetic contribution to microbial selection and diversity (Peiffer et al. 2013). Specifically, plant domestication progressively selected out secondary metabolites and volatile compounds to render plants more palatable or less toxic to humans and livestock (Meyer et al. 2012) and this has

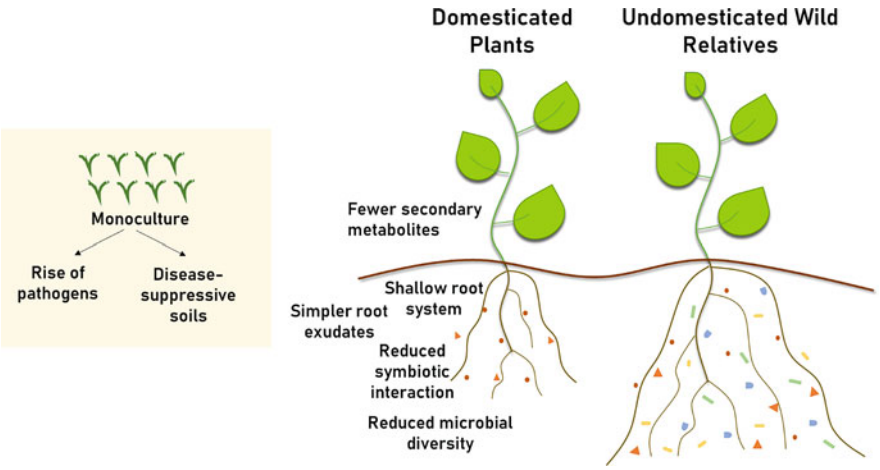

Fig. 9.3 Impact of plant domestication and agriculture on rhizosphere microbes. *Left panel*, monoculture in agriculture has resulted in the rise of pathogens, but also nurtured the development of disease-suppressive soils which have had a protective effect in limiting pathogens; *right panel*, plant domestication through agriculture had led to the loss of secondary metabolites that are key selective agents in root exudates against microbes. The regular provision of water and nutrients has led to the evolution of shallow root systems, which can alter microbial niches in the rhizosphere. The root exudate composition in domesticated plants is also simpler and correlates with reduced microbial diversity and interaction with symbiotic microbes like nitrogen-fixing rhizobacteria and mycorrhizal fungi. In comparison, the undomesticated wild counterparts have more secondary metabolites, deeper root systems, more complex components in root exudates, and higher microbial diversity

rendered modern crops more susceptible to insect pest herbivory, for instance (Chen et al. 2015). Many of these metabolites are defense compounds against pathogens and insect pests, including phenols, flavonoids, terpenes, and glucosinolates, which almost always carry a strong taste such as bitterness, acridity, or astringence (Drewnowski and Gomez-Carneros 2000). Such metabolic changes may have impacted the ability of modern crops to recruit microbiota as these secondary metabolites also play a key role in the selection and shaping of the rhizosphere microbiome as discussed above. The root exudates of crops may also be less complex than wild counterparts as modern wheat showed severalfold higher exudation of simple sugars such as glucose and fructose (Shaposhnikov et al. 2016). The impact of plant domestication on rhizosphere microbes is illustrated in Fig. 9.3.

9.3.1.2 Inorganic Fertilizers

Modern farming is largely inorganic farming and inorganic fertilizer treatment of soil undoubtedly enhances plant growth, but only about 60% of the nitrogen supplements are absorbed by the plant, and the rest leach into and contaminate groundwater and end up in water bodies causing environmental pollution such as eutrophication (Schmer et al. 2014). Furthermore, the treatment of plants with nitrogen-based fertilizers for a long time resulted in the displacement of mutualists

by less mutualistic root bacteria, negating microbe-mediated benefits to the host (Weese et al. 2015). Similar to the enrichment of certain members by eutrophication in water bodies, fertilizer treatment promoted the growth of copiotrophic bacterial taxa like Actinobacteria and Firmicutes with a reduction in oligotrophic species in Acidobacteria and Verrucomicrobia (Ramirez et al. 2012). Phosphorus is another major macronutrient for plants, but only about 5% of soil phosphorus is accessible for uptake by the plant. To sidestep this problem, farm soil is amended with phosphate fertilizers. Fertilizers do augment the biological activity in the soil (Quiza et al. 2015), but appear to restructure the microbiome with the apparent cost of microbial diversity loss.

9.3.1.3 Pesticides

Without question, pesticides can boost crop yield through protection from pests and plant growth promotion (Syed et al. 2018). Products like fungicides carry both financial and environmental costs, in addition to the development of fungicide resistance by pathogens and the need to keep developing new products (Ma and Michailides 2005). Fungicides and other agrochemicals can also inadvertently target the microbiomes and weaken beneficial interactions of the plant with rhizobacteria and mycorrhizae (Berg 2009). For instance, products like Oryzalin and glyphosate have been shown to suppress plant-associating mycorrhizae and nitrogen-fixing bacteria, respectively (Kelley and South 2017; Santos and Flores 1995).

Taken together, many modern agricultural practices appear to have collectively caused a shift in rhizosphere microbiomes with reduced interactions with beneficial microbes and diminished microbial diversity compared to their undomesticated counterparts. Soil organic matter is the driving force for rhizosphere microbiome colonization as a source of colonization signals and sustaining nutrients. Modern farming practices reduce soil organic matter content, compromising soil microbial diversity (Lareen et al. 2016). Indeed, low-input farming is correlated with higher microbial diversity characteristics of a healthy rhizosphere microbiome (Postma-Blaauw et al. 2010).

9.3.2 Contemporary, Alternative Farming Practices

9.3.2.1 Organic Farming

Organic farming is a more sustainable alternative to modern agriculture, as it aims to replace hazardous and polluting pesticides, fungicides, herbicides, and fertilizers with the more eco-friendly options—organic matter (Quiza et al. 2015). Organic farming enriches soil organic matter content and biological activity and plants cultivated in organic soil showed greater microbial diversity and species richness than those grown in conventional mineral soil in winter wheat, clover, and other species (Hartmann et al. 2015; Long et al. 2010; Lupatini et al. 2016). The increased microbial species richness may be owed to the fact that organic matter contains complex organic substrates that may nurture a distinct and more diverse set of bacteria. Microbial 16S rRNA profiling revealed that Proteobacteria members

were elevated in the organic soils compared to conventional soils which mainly contained Actinobacteria (Li et al. 2012). The enrichment of Proteobacteria is not surprising because they are among the most abundant phyla in animal feces (Shanks et al. 2011) that are often used as soil amendments and may also indicate an enrichment by the plant.

Organic farming practices emphasize soil amendments including compost, animal manure, and treated sewage sludge, rich in organic matter. Compost includes chitinous material such as crab shells, fish emulsion, and fruit pulp (Gómez Expósito et al. 2017). Often the compost possesses biocontrol activity and affords disease protection; for example, compost including chitosan, crab shell (chitin), and citrus pulp protected bell pepper from *Phytophthora* root and crown rot (Kim et al. 1997). In some cases, organic mulches have been supplemented with beneficial fungi to improve disease resistance, as observed for root rot resistance to the oomycete pathogen *Phytophthora cinnamomi* in avocado (Costa et al. 2000). Green manure, consisting of cover crop plant material left to decompose on the field, not only enriches organic matter but also acts as a mulch to retain soil moisture and suppress weed growth (Muimba-Kankolongo 2018). The application of green manure increased bacterial richness and soil microbial heterogeneity while also increasing the levels of microbes that promote nutrient cycling (Ingels et al. 2005). Thus, organic farming practices generally supported a higher microbial diversity than inorganic farming with protective effects.

9.3.2.2 Crop Rotation

Crop rotation has been utilized as an important tool to restructure the rhizosphere microbiota to benefit crop plants and is a mainstay in organic farming (Mazzola 2007), although it could also be practiced with modern inorganic farming. The alternating growth of complementary plants in crop rotation—particularly with legumes—not only increased nutrient cycling and improved soil properties but also increased disease resistance (Ingels et al. 2005). For instance, the nitrogen-fixing legume chickpea was found to recruit microbiome—including the plant-protective *Penicillium* sp. that benefited the subsequent wheat crops (Ellouze et al. 2013). Similarly, another legume red clover developed rhizobacterial communities that were beneficial to potato growth (Sturz et al. 2003). Thus, legumes make good partner crops for rotation with other crops. Oats produce terpenoid avenacin that confers resistance to the highly destructive fungal disease take-all (Begley et al. 1986). The growth of oat as a break crop before growing wheat transferred the resistance benefits to wheat as the protective effects persisted in the soil (Huang and Osbourn 2019). Thus, rotation or alternation of crops can result in complementary microbiomes that are tolerated by both crops, with additive or synergistic benefits from the mixed microbiome (Quiza et al. 2015). The mixed community has greater microbial diversity and resilience to pathogen invasion, contributing to a disease-suppressive effect. Furthermore, the alternation with incompatible hosts also discourages plant pathogen survival.

Although organic farming is ecologically friendly, drawbacks include the undefined nature of the amendments that limit the reproducibility of benefits (Quiza et al.

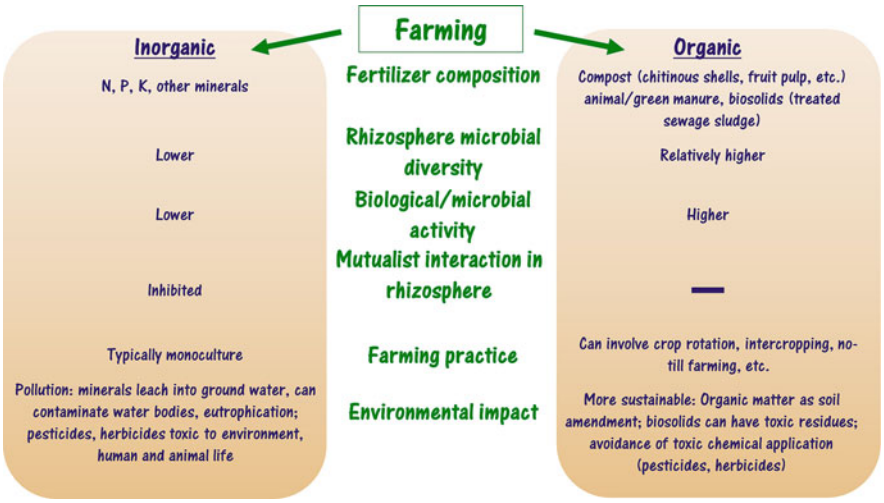

Fig. 9.4 Microbiota in organic and inorganic farming. N, P, K, nitrogen, phosphorus, potassium; "-" indicates unknown

2015). Moreover, the salinity in some of the treatments and heavy metals and therapeutic agents in biosolids and other soil amendments may be toxic to the native soil microbiota. Nevertheless, organic farming is a more sustainable alternative to modern inorganic farming. The effect of organic and inorganic farming on rhizosphere microbes is compared in Fig. 9.4.

9.3.2.3 Tillage

Tilling and turning over of soil can aerate the soil, but disrupt the soil structure and microbial community organization and expose the soil to potential erosion and runoff from precipitation. No-till farming preserves the microbial communities for the next crop season and the residual plant material can sustain microbial growth. In one study comparing the microbiomes of tilled and non-tilled farms, the bacterial communities were not observed to be significantly different (Yin et al. 2017). It was suggested that the tillage may affect fungal populations more as fungal enzymes may play a more significant role in the digestion of lignocellulosic material (Baker et al. 2019).

9.3.3 Monoinoculant Biocontrol

As an alternative to inorganic and organic fertilizers, microbes such as *Azospirillum* can be introduced in the field as biofertilizers that can promote plant growth, generally by solubilizing nutrients and promoting absorption (Maeder et al. 2002; Namvar and Khandan 2015; Qiu et al. 2019). Plant growth-promoting rhizobacteria (PGPR) go a step further by not only improving plant growth but also enhancing

protection from diseases (Compant et al. 2019). Some PGPR produce plant growth-promoting phytohormones including auxins, gibberellins, and cytokinins or modulate endogenous levels of them within the host (Compant et al. 2019; Hardoim et al. 2008). Several PGPR species including *Pseudomonas*, *Bacillus*, and *Streptomyces* have been employed in agricultural soils to enhance crop growth, yield, and survival (Sanchis and Bourguet 2008). Several *Bacillus* spp. have shown promising results in conferring plant growth promotion and disease resistance under field conditions (Syed et al. 2018). Beneficial fungal species such as *Trichoderma* have been employed for a similar purpose and function (Harman et al. 2004).

Plant protection by PGPR species involves pathogen antagonism as many of them grow aggressively and compete fiercely and these bacteria are also referred to as biological control or biocontrol bacteria. For example, *Pseudomonas* and *Streptomyces* can protect host plants through the function of antimicrobial/antibiotic/antifungal compounds such as phenazine derivatives and DAPG and antimicrobial lytic enzymes such as proteases (Newitt and Prudence 2019). Similarly, *Bacillus* spp. produce antibiotics such as iturin A and surfactants well as lipoproteins that have an antimicrobial function (Lareen et al. 2016; Turner et al. 2013). PGPR also sequester critical nutrients such as iron using iron-scavenging siderophore proteins, thus depriving their competitors and potential pathogens (Hassani et al. 2018). For instance, *Pseudomonas* spp. suppress fungal pathogens and disease through the use of siderophores (Mercado-Blanco and Bakker 2007). PGPR also prime the plant immune system to trigger a rapid defense to a wide range of pathogens through various mechanisms. One such process is induced systemic resistance (ISR), where rhizosphere colonization triggered systemic resistance in plants. For example, field trials showed that root colonization of *Bacillus* spp. enhanced resistance to the cucumber mosaic virus (CMV) in tomatoes and cucurbit wilt disease (Zehnder et al. 2000). Similar benefits of ISR have been observed in several crop species (Choudhary et al. 2007).

PGPR microbial inoculants help slash the usage of polluting biocides and fertilizers (Qiu et al. 2019), but the overall promise of biocontrol bacteria is curtailed by their limited success and unpredictability in field settings even though they were promising in laboratory and greenhouse experiments (Schlaeppi and Bulgarelli 2015). For instance, although *Pseudomonas* spp. exhibit promising biocontrol activity against take-all disease in wheat, these strains are sensitive to desiccation and only survive the early stages of growth on wheat in field settings and are subsequently outcompeted (Coombs et al. 2004; Schlatter et al. 2017). Moreover, plant protection is even more imperative in the context of climate change, which is expected to be hostile to monoinoculant PGPRs—where all eggs lie in one basket. These observations suggest that overreliance on single PGPR inoculants for agricultural plant protection is untenable.

9.3.4 Microbial Mixtures

Instead of single-strain PGPRs, a combination of strains holds more promise in agriculture (Nguyen et al. 2017), particularly when the strains exhibit synergistic or additive effects in conferring plant protection (Orozco-Mosqueda et al. 2018), as was shown with *Bacillus* spp. in field trials (Zehnder et al. 2000). Similarly, a group of six endophytes promoted resistance to tobacco wilt disease (Santhanam et al. 2015). A diverse *Pseudomonas* consortium led to greater pathogen suppression and disease protection in tomatoes, likely with the increased survival of the *Pseudomonas* strains (Hu et al. 2016). Strain mixtures including *Bacillus* and *Cutibacetrium* spp. improved growth and biocontrol of pathogens in cucumber (Raupach and Kloepper 1998). In some cases, benefits to the plant were only discernable when two *Pseudomonas* strains were used together resulting in synergistic interactions on chickpea (Meena et al. 2010). Various studies in grapevine (Rolli et al. 2015), maize (Molina-Romero et al. 2017), potato (De Vrieze et al. 2018), and tomato (Berg and Koskella 2018) have demonstrated that multistrain inoculations have the potential to increase plant growth-promoting effects as compared to mono-inoculations. In some cases, bacterial mixtures also improved tolerance to stresses such as drought, as was shown for a cocktail of *Pseudomonas*, *Sphingomonas* sp., *Azospirillum*, and *Acinetobacter* in maize (Molina-Romero et al. 2017).

A diverse set of microbes in a complex inoculum have the potential to occupy different niches in the rhizosphere, expanding plant protection and boosting growth promotion (Finkel et al. 2017). Furthermore, they may confer additive or synergistic benefits, especially when their benefits are afforded through different mechanisms (Timm et al. 2016). While microbial consortia often show greater potential than single strains, sometimes they may be worse than single strains as seen in the case of growth of grapevines during drought (Rolli et al. 2015). In another case, multiple strains of *Pseudomonas* affected community stability and did not improve plant protection (Becker et al. 2012). Other studies also witnessed multistrain inoculations being less beneficial to the plant than single inoculants (De Vrieze et al. 2018; Herrera Paredes et al. 2018). Furthermore, co-inoculation may produce a competitive process that may be subjected to environmental changes, with unpredictable outcomes. Thus, future endeavors with microbial consortia should be driven by knowledge and evidence-based selection of complementary microbial strains.

9.3.5 Disease-Suppressive Soils

With the limitations of current single and multistrain PGPR inoculants, disease-suppressive soils have proved not only to be a treasure trove to identify novel individual PGPR strains but also as sources of beneficial microbiomes in agriculture. Disease-suppressive soils are a great example of microbiome-mediated plant protection from pathogens in the soil (Gomez Exposito et al. 2017). Continual monoculture on agricultural soils can build selective pressures against pathogens to produce disease-suppressive soils enriched in beneficial microbes and microbial and

plant-derived antimicrobial metabolites that mediate disease suppression (Durán et al. 2018; Santhanam et al. 2015), although this can take several years to build (Coque et al. 2020). In disease-suppressive soils, plants can continue to be healthy even in the presence of pathogens (Teixeira et al. 2019) and this partly results from higher microbial diversities than in conventional soils (Garbeva et al. 2006) that can have a protective effect against pathogens. In some cases, disease suppressiveness may also result from changes in the relative abundance and functions of specific bacterial groups rather than their presence or absence (Mendes et al. 2011; Chapelle et al. 2016). Although soil suppressiveness is a complex phenomenon, the ability of a specific plant genotype to gather in the rhizosphere disease-suppressive communities is critical for the transition of the soil from the conductive to the suppressive state.

Within disease-suppressive soils, specific microbes or groups of microbes confer disease protection to plants largely through competition, pathogen antagonism, and the production of antimicrobial compounds (Mendes et al. 2011). For example, *Pseudomonas* spp. obtained from *Fusarium* wilt-suppressive soil conferred resistance to flax (Mazurier et al. 2009). The development of disease suppressiveness involves the selective recruitment of beneficial microbes by the plant roots. For instance, foliar infection with the oomycete pathogen *Hyaloperonospora arabidopsidis* summoned multiple beneficial strains in the soil that functioned synergistically to promote disease suppressiveness and this effect persisted in the following generations (Berendsen et al. 2018). Thus, the development of disease suppression is accomplished through changes in the microbial community and function in the soil. Since the first report by Atkinson of a cotton-grown soil suppressive to *Fusarium* wilt, several bacterial and fungal species conferring DDS have been reported. Typical examples are individual bacterial strains belonging to the genera *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Enterobacter*, *Alcaligenes*, and *Pantoea*; fungal strains of the genera *Trichoderma*, *Penicillium*, and *Clonostachys/Gliocladium*; nonpathogenic *Fusarium* species; and the fungal species *Verticillium biguttatum* and *Pochonia chlamydosporia*. Besides the commonly studied *Bacillus*, *Pseudomonas*, and *Streptomyces*, many other bacterial genera including *Burkholderia*, *Paraburkholderia*, *Enterobacter*, and *Pantoea* show pathogen antagonism (Compant et al. 2019) and are expected to play important roles in the development of disease suppression. Depending on the case, these beneficiaries have been shown to target pathogenic soilborne fungi and oomycetes but also pathogenic bacteria, protists, and parasitic root-knot and cyst nematodes (Gomez Exposito et al. 2017).

In addition to protective strains, disease-suppressive soils also contain microbe- and plant-derived protective compounds that suppress soilborne pathogen growth. This is best exemplified in the case of the wheat take-all disease caused by the fungal root pathogen, *Gaeumannomyces graminis*, which has the potential to wipe out wheat fields (James Cook 2003). The presence of *Pseudomonas*-derived antimicrobial DAPG and oat-derived avenacin in the soil corresponded with the suppression of take-all disease in wheat (Mendes et al. 2011; Huang and Osbourn 2019; Raaijmakers et al. 2009). Thus, in take-all decline, the severity of disease was

reduced with every generation of wheat, consistent with the development of disease-suppressive soil (Turner et al. 2013). Compounds like DAPG and phenazines can also prime the plant immune system, further enhancing disease resistance. *Streptomyces* spp. have also been frequently isolated from disease-suppressive soils and their disease suppressiveness was linked with the production of antifungal volatile organic compounds and thiopeptides (Cordovez et al. 2015; Cha et al. 2016; Newitt and Prudence 2019). The disease suppressiveness of *Paraburkholderia graminis* PHS1 was attributed to the production of sulfur-containing volatile compounds (Carrión et al. 2018). Antimicrobials like DAPG, phenazines, and iturin A can persist in the rhizosphere soil. Therefore, the disease's suppressive nature in soils can persist for generations, particularly if the plant- and microbe-derived compounds are not volatile. Breeding crops for traits related to the recruitment of disease-suppressive microbial communities could be an alternative breeding strategy towards durable disease resistance.

Microbiome studies have broadened our understanding of disease-suppressive soils and revealed that communities constituted by distinct taxonomic groups operate to confer disease suppression. For instance, bacterial species from Proteobacteria (including *Pseudomonas* producing antifungal compounds), Firmicutes, and Actinobacteria were implicated in the development of resistance to *Rhizoctonia* root rot through pathogen antagonism (Mendes et al. 2011). Another report revealed identified Acidobacteria, Actinobacteria, and Firmicutes as keystone groups for resistance to *Fusarium* wilt (Trivedi et al. 2017). In general, a diversity of microbial taxa become more abundant in disease-suppressive soils (reviewed in Gomez Exposito et al. 2017). Collectively, these studies reveal shifts in community composition with the development of disease suppression and the concomitant microbial enrichment may prevent pathogen invasion (Turner et al. 2013). Pathogen- or plant-derived compounds can promote recruitment or growth of new microbial groups; for example, fungal pathogen-derived oxalic acid or plant metabolites encouraged the growth of bacteria from specific families, including *Oxalobacteraceae* and *Burkholderiaceae* that likely served an antagonistic function (Chapelle et al. 2016; Mendes et al. 2011). Many microbial strains have been isolated from rhizospheres and developed as PGPRs for crop protection (Gopal et al. 2013). Disease-suppressive soils can thus be invaluable sources of novel bioactive strains of microbes as well as antimicrobial compounds (Weller et al. 2002). Indeed, the PGPR *Streptomyces* was originally isolated from disease-suppressive soils (Cha et al. 2016). A study of the rhizosphere community in take-all disease revealed *Enterobacter* and *Serratia* as promising candidates for disease suppression (Durán et al. 2018). The complexity of community interactions in disease-suppressive soils, the underlying mechanisms, and the impact of environmental factors remain to be elucidated for many disease-suppressive soils.

Disease suppressiveness can be transferred to new soils by mixing a small portion (1–10% w/w), thus seeding the new soil with a consortium of beneficial microbes (Mendes et al. 2011; Raaijmakers and Mazzola 2016; van der Voort et al. 2016). Similarly, supplementing the soil with siderophore-producing *Pseudomonas* or their siderophores, both isolated from suppressive soil, could suppress disease in wheat

and barley (Gomez Exposito et al. 2017). The organic soil amendments employed in organic farming can also promote disease suppressiveness by increasing soil microbial activity and promoting the recruitment of beneficial microbes. However, the development of disease suppression involves continual monoculture, and crop rotation can accelerate this development of disease suppressiveness (Coque et al. 2020), although in some cases, crop rotation could break disease suppressiveness (Newitt and Prudence 2019), possibly by releasing the selective pressure on the pathogens in the soil. Understanding the mechanisms of disease suppressiveness will be a big step forward in the deployment of plant-protective microbiomes in agriculture.

9.4 Harnessing Microbes for Plant Protection in Sustainable Agriculture

9.4.1 Harnessing Beneficial Microbes for Plant Protection

9.4.1.1 Identification and Selection of Candidate Microbes

While candidate plant-protective microbes can be isolated by screening assays in laboratories, they tend to be laborious. Amplicon-based sequencing methods such as 16S ribosomal RNA offer a relatively cost-effective approach to profile and identify microbial communities, but do not provide information about whether the microbes are beneficial or their relative importance in the community (Levy et al. 2018). Metagenomic sequencing (shotgun metagenomics) can be used to sequence the genomes of the entire rhizosphere community and offer insights into their functional potential and their relative roles. Metagenome sequencing can reveal what genes and functions are enriched in various niches of the rhizosphere (endosphere vs. rhizosphere) as well as dynamic spatiotemporal changes in microbial populations. While elucidation of community structure is a good starting point, the next important step is the functional characterization of promising candidates in the community.

9.4.1.2 Isolation and Functional Characterization of Candidate Microbes

From community profiling, microbial species that are preferentially recruited and/or enriched by the plant may be identified for further characterization. It is estimated that only a small portion of the rhizosphere microbiota is culturable, but recent studies are proving that such estimates are underestimates and more microbes are amenable to culture than previously thought. The ability to grow candidate microbes and explore their functions through plant-microbe experiments is fundamental to the understanding of the plant microbiome and to exploit its full potential. Microbial culture can be employed to test if a plant recruits a microbe or microbial community of interest and can also be used to analyze the underlying mechanisms. Network analysis has been increasingly useful in guiding the selection of representative microbes and identification of hub microbes that are critical to the assembly and function of the microbiome (Gómez Expósito et al. 2017).

If microbial isolates were identified by rRNA profiling, their genomes can be sequenced to further understand their potential. Using the genome, one may explain the organism's observed behavior or trait of interest, examine additional plant growth-promoting traits, and look for genes or gene clusters corresponding to the synthesis of bioactive compounds (e.g., hormones, antimicrobials) and other genes that indicate novel capabilities. For example, genome sequencing of *Streptomyces* S4-7 revealed 35 gene clusters implicated in the biosynthesis of antimicrobial compounds, following which a novel thiopeptide was isolated and showed antimicrobial activity (Cha et al. 2016). Similarly, the genome of *Pseudomonas* sp. contained biosynthetic clusters that allowed the identification of novel antibiotics (Helfrich et al. 2018). Microbes in such cases may be evaluated for antagonistic functions against other microbes or pathogens, although it may be noted that strains that do not show strong bioactivity against phytopathogens in vitro may do so in situ in the presence of root signals (Newitt and Prudence 2019). Good-quality genomes can also serve as reference sequences for the comparison of metagenomics data (Levy et al. 2018). Genome information is not informative of what genes are expressed or functioning in the rhizosphere. This may be accomplished through transcriptomic, proteomic, or metabolomics analysis of the microbe in the rhizosphere. Microbial genes important for plant interaction may be identified through mutational analysis. Recently, transposon sequencing (TnSeq) has turned out to be a facile strategy to create genome-wide mutants of a microbe and systematically test all mutants for a trait of interest (Levy et al. 2018). Such approaches will not only allow the identification of genes important for plant-microbe interaction, but also interactions in the microbiome. Other approaches such as stable isotope probing to assess microbial substrate preferences and metabolic potential are critical to understand the metabolic basis of the plant-microbe interaction (Radajewski et al. 2000).

9.4.1.3 Assembling Synthetic Communities of Candidate Microbes

The representative microbes identified by network analysis can be grown to constitute synthetic communities or SynComs (Gómez Expósito et al. 2017). As microbes function in concert in the microbiome, SynCom scan is employed to study their complex interactions with and impact on gnotobiotic plants in sterile culture (the plant equivalent of germ-free mice). Traditionally, microbial culture in vitro has been a limitation, but recent studies are demonstrating that it is possible to culture as much as 50% of the major members of the microbiome (Bai et al. 2015). SynCom experiments can demonstrate how each species contributes to community assembly and function and how they influence plant fitness (Rodriguez et al. 2019). SynComs also make excellent tools to assess how hub microbiota, which displays a high degree of interaction with other members in the community function as focal points in the community (Hassani et al. 2018). One study showed that the removal of one strain caused five others to disappear, indicating the disproportionately important role of specific members of the community (Niu et al. 2017). Many SynCom studies focus on small communities containing representative strains, but larger synthetic communities involving hundreds of members have also been shown to colonize the rhizosphere reproducibly, making this a powerful approach (Finkel et al. 2017).

Additionally, SynComs are valuable in understanding fundamental aspects of plant-microbial community interactions, for instance, SynCom experiments confirmed the importance of the plant defense hormone, salicylic acid (SA) in gating the endosphere and limiting colonization by certain taxa in *Arabidopsis* (Lebeis et al. 2015).

SynCom experiments have revealed that higher diversity in the synthetic community correlates with better disease suppression (Hassani et al. 2018). More complex *Pseudomonas* consortia afford better protection against *Ralstonia solanacearum*, a root pathogen in tomato, through greater competition and pathogen antagonism (Hu et al. 2016). A simplified SynCom consisting of seven species representative of various taxa from the microbiome was collectively required for resistance to *Fusarium verticilloides* blight in maize (Niu et al. 2017). Generation of SynComs with complementary microbial species with different functions or mechanisms of action may issue additive and synergistic effects, resulting in a resilient microbiome (Gomez Exposito et al. 2017). Some sets of bacterial strains may interact through cohabitation in the same biofilm (Berendsen et al. 2018). The greater plant protection from higher strain diversity has been correlated with a greater diversity of secondary metabolites that can protect the plant through varying mechanisms (Hu et al. 2016). These studies collectively indicate that synthetic communities containing diverse strains, complementary and synergistic with each other, but competitive and antagonistic to other microbes such as potential pathogens, and which can stimulate plant defenses are good candidates for use in sustainable plant protection.

9.4.2 Enabling Plants to Harness Beneficial Microbes for Plant Protection

While soil is the basic source of microbial pool available for plant colonization, host plant genotype also plays an important role in selecting and sustaining the rhizosphere microbiome (Badri et al. 2013; Bulgarelli et al. 2015, 2012; Lebeis et al. 2015; Peiffer et al. 2013). Each plant species, and even different genotypes within the same species, enriches a distinct and selected set of microbes in the rhizosphere and endosphere that are generally beneficial (Perez-Jaramillo et al. 2016), although in some studies the varietal differences were more subtle (Bulgarelli et al. 2015; Peiffer et al. 2013). This selection is primarily dictated by the root exudate composition which also includes selective secondary metabolites, both of which not only serve to cull out certain species can also act as nutrients. A study of *Arabidopsis* accessions found qualitative differences between root exudates that corresponded to differences in rhizosphere microbiota (Micallef et al. 2009). Thus, the differences in microbial communities may be owed to differences in root exudates.

Plant breeding has traditionally focused on traits like yield and disease resistance, but the outburst of microbiome studies in the past decade has prompted consideration that plants may additionally be bred for their ability to recruit preferred partners and PGPR to build optimal microbiomes and disease-suppressive soils (Quiza et al.

2015; Ryan et al. 2009). For example, wheat varieties were selected for their ability to recruit *Pseudomonas* populations for resistance to *Rhizoctonia solani* (Mazzola 2002). Some *Arabidopsis* mutants with altered root exudate composition were also found to recruit beneficial bacteria. Since root exudate composition is critical for microbial recruitment and selection, many studies have focused on modifying exudate composition (reviewed in Quiza et al. 2015) and transferring these traits to crop plants through traditional breeding and genetic engineering, potentially through the CRISPR/Cas9 system (Schaeffer and Nakata 2015) to augment plant protection; however, a detailed understanding of the mechanistic basis of microbial recruitment is the priority.

Identification of plant loci involved in recruiting or supporting the growth of specific bacterial taxa in the roots may be accomplished through quantitative trait loci (QTL) mapping (Collard et al. 2005) and genome-wide association studies (GWAS) with crops and their wild relatives. One study identified several plant QTLs regulating the colonization of *Bacillus cereus* UW85 and the accompanied disease-suppressive effect (Smith and Goodman 1999). Wild relatives of cultivated plants are more effective recruiters of a higher diversity of rhizosphere microbes likely due to a richer root exudate and having coevolved with microbiota that enhances their fitness (Perez-Jaramillo et al. 2016). Plant breeding for improved traits over generations has been successful in improving cultivated plant traits, but often with loss of genes from their wild ancestors (Gopal and Gupta 2016). Some of these genes may have contributed to the synthesis of secondary metabolites, which presumably made the plants more palatable both to humans and, inadvertently, also to insect pests. Revisiting wild varieties to identify genes that promote microbial recruitment is a promising approach to design a fitter rhizosphere microbiome and holobiont (Perez-Jaramillo et al. 2016). A plant engineered to produce a diverse root exudate may be expected to support microbiome diversity in the rhizosphere. Desirable rhizosphere traits in plants could be incorporated into elite breeding programs to enhance crop varieties. Thus, modulation of a plant's ability to attract and retain beneficial microbes is a promising approach to introducing beneficial bacteria in the field. However, it is important to ensure that the soil is equipped with the preferred partners of the plant and supplementing the soil with SynComs could augment the recruitment of the microbiome. Bacterial strains may also be modified for higher responsiveness to plant signals to promote colonization (Cole et al. 2017).

One other way plants can modulate rhizosphere bacterial communities is by targeting quorum sensing (QS), a signaling system used by bacterial species to monitor their population density or those of other species and activate specific coordinated functions (Mohan et al. 2018; Quiza et al. 2015). Plants engineered to produce QS signals or enzymes such as lactonases that can degrade QS signals in the rhizosphere may be able to selectively target certain bacterial groups while retaining others. In addition to improving microbes and plants for better colonization, plant or microbial metabolites could be identified that enhance recruitment by the root. Metabolite profiling and modeling can help identify candidate metabolites that affect community structure and dynamics (Botero et al. 2018). Such metabolites could be used as elicitors to enhance the colonization and retention of preferred beneficial

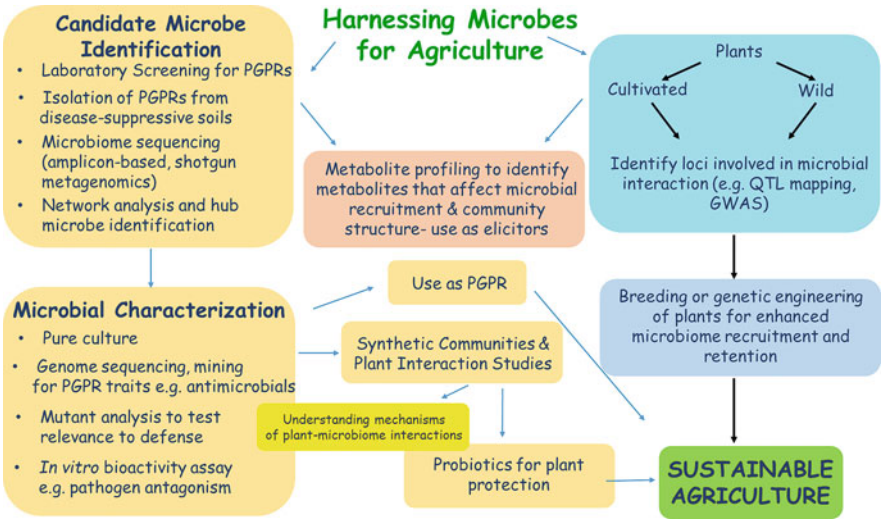

Fig. 9.5 Harnessing microbes for plant protection in agriculture. Can be accomplished via three approaches: identifying microbes ideal for plant colonization and protection (left), identifying metabolites that promote microbial colonization (elicitors), and enhancing the ability of plants to recruit and retain protective microbes

microbes. Thus, a variety of complementary approaches are feasible to enhance the recruitment and enrichment of crop microbiome for enhanced protection; these are summarized in Fig. 9.5.

9.5 Future Considerations for Sustainable Microbiome-Based Agriculture

Rhizosphere microbiomes have coevolved with plants, the local environment, and fluctuating stress conditions serving as a shaping force. The understanding of the microbiome and its dynamic interactions with plants, currently in its infancy, can be potentially applied for sustainable agriculture, particularly in resource-limited environments. An exciting array of opportunities that could transform agriculture await exploration.

9.5.1 Plant Probiotics

While the use of plant bacteria as pure inocula or microbial mixtures is not a new concept to promote plant disease resistance and even though such inoculants showed promising results in laboratory or greenhouse experiments, they fell short in field settings (Glick 2012). With more recent knowledge of the microbiome, thoughtfully selected microbial preparations produced with thorough testing using SynComs are

key to success. Many attributes are ideally desirable in these consortia; these include the ability of the strains to compete and survive in the rhizosphere, protect the plant from pathogens by antagonism, tolerate the plant immune system, and stimulate both local and systemic defenses. The inclusion of hub microbes that are capable of recruiting other microbes to assemble a plant-preferred microbiome in agriculture would be beneficial. However, certain hub microbes such as *Enterobacter cloacae* (Niu et al. 2017) are potential human pathogens and their enrichment in agricultural fields may be considered carefully. The assembling members of the community should preferably show metabolic and functional complementarity with different mechanisms of pathogen antagonism and host defense stimulation so that they combine to afford additive or synergistic protection to the host. The starter community should be representative of the host microbiome, be inherently diverse, or be able to build a diverse microbiome, as diverse microbiomes tend to be resilient; some functional redundancy among the microbes is desirable in this aspect, especially in dynamic environments. Ideally, consortia should include indigenous stress-tolerant microbes that are adapted to the local environment (Mueller and Sachs 2015; Qiu et al. 2019) and capable of assisting plants to withstand fluctuating environmental stresses. Some of these desirable traits could be engineered in the bacteria through recombinant strain production (Quiza et al. 2015), but the risks associated with recombinant strain release and potential gene transfer should be evaluated first.

One challenge in synthesizing ideal consortia is the present limitation in being able to freely grow all microbes in culture. This is particularly true for obligate biotrophs that can only grow on a living host and some of the keystone hub species identified are obligate biotrophs. Bacterial consortia administered as probiotics may be coated onto seeds before sowing (Santhanam et al. 2015), so they can establish the microbial community early on. However, to accomplish this, they need to be competitive to overcome the indigenous microbes already present in the soil. Although fungicide or antibiotic treatments have been recommended to disrupt the existing microbiome in the soil (Quiza et al. 2015), a more sustainable option would be tilling the soil to achieve the same. To ensure invasion of the inocula in the rhizosphere, higher doses may be required, but this may promote undesirable pervasive growth of the microbes in the aerial parts of the plant; for instance, treatment of *Arabidopsis* roots with high doses of *Pseudomonas simiae* (*P. fluorescens*) resulted in the strain spreading to the aerial parts of the plant (Zamioudis and Mohan, unpublished observations). Even if the inoculants establish in the rhizosphere, they may not persist, as in some cases, inocula in the field have been outcompeted by indigenous microbes (van Veen et al. 1997), as has been observed for *Azospirillum* (Ryan et al. 2009; Herschkovitz et al. 2005). To ensure persistence, periodic soil amendments with the inocula may be necessary (Syed et al. 2018).

9.5.2 Mixed Microbiomes

The exclusive focus on bacterial microbiomes in the rhizosphere comes with the cost of inherent bias as the other kingdoms of microbes including fungi and oomycetes can in many cases play a substantial role in community dynamics, particularly in the context of plant protection. PGPRs *Bacillus* and *Pseudomonas* teamed up with mycorrhizal fungi for synergistic suppression of root-knot nematode in chickpea (Akhtar and Siddiqui 2008). Disease suppressiveness in soils is contributed not only by bacteria, but also by fungal genera such as *Aspergillus, Fusarium,* and *Eurotium* (Adam et al. 2014; Giné et al. 2016; Song et al. 2016). Certain rhizospheres such as that of pea are enriched in fungal species in addition to bacterial taxa (Turner et al. 2013). Bacteria and fungi can physically associate as some bacterial biofilms such as that of *Pseudomonas* sp. are formed on the hyphae of fungi like *Laccaria* in the soil (Guennoc et al. 2017; Hassani et al. 2018). Bacteria and fungi could be metabolically interdependent. For instance, fungal enzymes may initiate the breakdown of complex plant-derived substrates such as lignocellulosic material (Baker et al. 2019) and the breakdown products could serve as substrates for bacterial groups. Bacteria, fungi (e.g., *Albugo*), and oomycete species (e.g., *Udeniomyces* and *Dioszegia*) may coordinate to serve as hub microbes that are highly interactive with other microbes in the rhizosphere (Agler et al. 2016). Interkingdom molecular dialogue between bacteria and fungi is possible through quorum sensing (Jarosz et al. 2011). The inclusion of fungi in the bacterial consortium not only diversifies the inoculum but also promotes niche filling and competitive suppression of pathogens (Quiza et al. 2015). However, these interactions have to be evaluated and optimized using SynCom experiments in planta.

9.5.3 Engineered Plants

In addition to better probiotics, plants may also be better equipped to get the best support out of their microbiomes, since plants and their microbiomes function in unison as a holobiont. This is particularly relevant in the context of stress as microbiomes can respond dynamically to confer stress protection. Plant-mediated selection of microbiomes can alter traits such as flowering in *Arabidopsis* and *Brassica* spp. (Panke-Buisse et al. 2017). Genetically engineering plants to be able to modulate their microbiome is one approach as relevant genes could be transferred to crop plants (Qiu et al. 2019). Genes regulating the production of metabolites that attract beneficial microbes can be integrated into or enhanced in a plant. For example, plants releasing volatile organic compounds could attract beneficial bacteria from a distance in the soil (Schulz-Bohm et al. 2018). However, the consequences of change in plant metabolite profiles, their impact on crop quality, and the non-target effects of the metabolites on other organisms have to be carefully evaluated. Comparative genomics of domesticated crops and their wild relatives in combination with metabolite analysis and microbiome profiles can help narrow

down to genes that can enrich crop microbiomes, in what is referred to as the "back to the roots" approach.

9.5.4 Disease-Suppressive Soils

Disease-suppressive soils are gold mines of beneficial microbes and can also be used to inoculate agricultural soils to transplant disease resistance to new soils even if the latter contains pathogens. Such soils can retain the suppressive effect for generations of crops and disease resistance may progress with generations due to enrichment of the microbiome and optimizing selection by the host plants. Undoubtedly, the early studies on suppressive soils focusing on single community members provide valuable insights into the mechanisms involved in disease suppression, yet they neglect the complex interactions among microbial communities as these occur in the root vicinity and within the root interior. Several seminal studies based on metagenomics and metatranscriptomics support that microbial consortia rather than individual strains function synergistically to confer solid protection against pathogens. Thus, multi-omics technologies provide opportunities to dissect disease suppressiveness to an exceptional level of detail and, in this context, may assist in the design of robust synthetic communities of microbes with enhanced disease-suppressive potential. Understanding the mechanisms of how disease suppression evolves in soils can be invaluable in engineering the plants and the soil microbiome to enhance disease suppressiveness. Presently, microbiome engineering is being pursued through artificially selecting a protective microbiome through repeated colonization over multiple generations to achieve an optimal plant-preferred community with protective functions (Mueller and Sachs 2015). This, in effect, creates a disease-suppressive soil. Such microbiomes may, in the future, be mass-cultured and cryopreserved for field application.

9.5.5 Microbiome-Mediated Organic Farming

Cultural practices in organic farming must have a pronounced impact on agricultural microbiomes. The progressive ease of sequencing and microbial community characterization affords the power to characterize the complex and diverse microbiomes that must operate in organic farms. Manure that has been traditionally used to fertilize agricultural fields is enriched in the fecal microbiomes of animals. The substrates used in organic soil amendments are degraded or fermented with microbial action which results in the enrichment of various microbial species. Recently, the microbial composition of traditional organic preparations in rural agriculture is receiving renewed attention; for instance, the compost fermentates named jeevamrutha and beejamrutha, which are made through the fermentation of organic substrates in jaggery and pulse crop flour by microbiota from cow dung, are routinely used as soil amendments in agricultural fields for sustainable crop production (Pattanaik et al. 2020). Microbial profiling of these preparations revealed that

they are enriched in bacteria such as actinomycetes and fungi. Organic farming in itself promotes microbial diversity in the rhizosphere, and organic practices that support the enrichment of beneficial microbes may be explored to promote sustainability in agriculture, especially with increasing ease of microbiome profiling.

9.5.6 Considering Environmental Impacts of and on Microbiome-Based Agriculture

The introduction of a new microbial species into an ecosystem often comes with consequences that may be difficult to quantify (Delgado-Baquerizo et al. 2016). Microbial consortia have to be tested for the effects of their metabolites on nontarget organisms before application in agriculture and whether the metabolic changes in the crop could also affect human health. For instance, the inoculated rhizosphere microbes could enrich antifungal compounds such as polyene macrolide antibiotics, which have the potential to affect human cholesterol metabolism (Zotchev 2003).

Stresses such as drought are expected to aggravate plant disease and herbivory while substantially impacting yield (Bebber et al. 2014; Lobell and Field 2007). Environmental stress, particularly at high temperatures, can modulate the expression of defense genes, increase the transfer of pathogen effector proteins into host cells, reduce pathogen perception, and suppress host defense (Teixeira et al. 2019). Unfortunately, the benefits conferred by the microbiomes on hosts are also threatened by the effects of global climate change (Maclean and Wilson 2011). Microbiomes in agriculture could also be influenced by the environment, particularly climate change, including a rise in carbon dioxide levels, global warming, and altered rainfall patterns (Blankinship et al. 2011). Increased carbon dioxide levels, one of the key components of climate change, can influence rhizosphere structure through the altered root exudation patterns (Drigo et al. 2013). The activity of hub microbes has also been shown to be sensitive to environmental changes (Santoyo et al. 2017; Vacher et al. 2016). Fortunately, plant adaptations to stresses are not only accompanied by rapid compensatory changes in the rhizosphere, typically associated with changes in root exudation profiles, but also with genetic changes in microbes that are beneficial to the host (Rodriguez et al. 2008). Microbial communities have been observed to evolve and adapt faster to environmental changes than the plant itself, helping the plant overcome stress (Lau and Lennon 2012). Understanding the mechanisms of plant-microbiome dynamics during stress may help us design better strategies to harness microbiomes that can rescue plants from biotic and abiotic stresses in a changing environment. Finally, testing microbiome-based agriculture in multiple field trials across distinct locations and over multiple years is critical to overcome limitations in performance under field settings.

In conclusion, steps towards sustainable agriculture are critical to increasing global food security. The application of rhizosphere microbiomes as a sustainable alternative to chemical-based agriculture is gaining ground, thanks to recent advances in non-culture-based characterization of the microbiome and insights into the mechanisms of their interactions with the plant. This may be accomplished

through a combination of microbiome treatments and enhanced recruitment and retention of healthy microbiomes by the plant to create disease-suppressive soils for durable plant protection.

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Endophytic Microbiome-Assisted Drought Tolerance in Plants

10

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Abstract

Agricultural productivity is constantly influenced by several factors such as urbanization, less availability of cultivable land, lack of sufficient automation, and abiotic and biotic stresses. Global climatic conditions significantly influenced the annual precipitation. Thus, decreased rainfalls, increased dry spells, soil salinity, and high temperature considerably affect agriculture productivity. On the other hand, the ever-burgeoning human population demands more food from the limited resources, putting much pressure on agricultural productivity. Drought stress causes several morphological, structural, biochemical, and molecular changes in plants. Plants to endure drought stress synthesize several secondary metabolites, reactive oxygen species, and module hormone production and induce several drought resistance genes. However, not all plants respond to the drought stress in the same magnitude. Most of the cultivable crops are drought-sensitive. Thus, the scientific community adopted several methods to improve drought tolerance in crop plants. Due to the constraints of agronomical practices,

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genetic engineering (GE), and marker-assisted breeding approaches, scientists recently developed a spark of interest in improving the crop plant's performance under extreme environmental conditions with the natural plant-associated microbiome. The plant-associated microbiome may improve drought stress tolerance through multiple pathways, such as improving the mineral nutrition absorption, phytohormone production, ACC deaminase activity, and root system, producing exopolysaccharides, and triggering the systemic induced resistance. The chapter discussed the detailed mechanism of the endophytic microbiome colonization process, functional traits of the microbiome, and drought tolerance mechanism of the endophytic microbiome with some examples.

Keywords

Endophytic microbiome · Drought stress · Drought tolerance · Genetic engineering (GE) · Agriculture productivity

10.1 Introduction

The steady rise in the global population demands 50% more food than the current food production by 2050 (Alexandratos and Bruinsma 2012). Consequently, the gap between food demand and population growth is increasing. On the contrary, the change in the global climate and rise in the atmospheric temperatures make the dry climatic zones drier and the wet climatic zones damper. The lack of precipitation in the dry climatic zones increases the prolonged drought stress. It was estimated that by the year 2030, water shortage due to the prolonged drought in several parts of the globe might affect 40% of the population; as a result, 700 million people, livestock, and crops will be at risk (Seleiman et al. 2021; Shah et al. 2021a). Several abiotic factors also severely affected plant growth and yield (Gull et al. 2019; Kumar 2020; Hossain et al. 2021). Thus, to take up the challenge, there is an imperative need to develop multi-stress-tolerant crops (Mahalingam 2015; He et al. 2018).

Advanced technologies like genetic engineering (GE) and breeding by marker-assisted selection (MAS) have enormously accelerated the generation of high-yielding stress-tolerant crop plants (Zolkin et al. 2021). Due to regulatory issues, several countries have not accepted the cultivation of genetically engineered crop plants. Recently, the scientific community developed a novel eco-friendly, cost-effective strategy using the microbial community to develop stress-tolerant crop plants. Research findings suggest that plant-microbe interaction boosts the plant's natural defense mechanism against environmental and biotic cues. This eco-friendly strategy would overcome the drawbacks of MAS and GE approaches (Lata et al. 2018; Singh et al. 2019; Verma et al. 2021).

Plants colonize with different kinds of microbial complexes known as phytomicrobiome. The microbial complex in the phytomicrobiome includes bacteria, fungi, archaea, and protists (Saad et al. 2020; Trivedi et al. 2020). The host plant chooses the microbial species from the surrounding environment to get maximum

benefits from the colonizer. Some microbes associate with exterior regions of roots and leaves called rhizosphere and phyllosphere, respectively (Abdelfattah et al. 2021), while some microbial species enter the interior regions of plants called "endophytes."

The host plant chooses the microbiome from the surrounding environment to get maximum benefits from the colonizer. According to recent research, the rhizosphere and endophytic bacteria have many variations in their genomic regions, which might explain why endophytic bacteria colonize the interiors of plants. Bacterial endophytes serve the host plant in various ways, including promoting growth, defending against pathogenic organisms, and providing environmental signals. Endophytic bacteria communicate and interact with their host plants by producing signal molecules more effectively than rhizospheric bacteria in some adverse conditions (Jha 2019; Mengistu 2020).

Once colonized, the endophytic microbiome produces a variety of compounds and enzymes that can protect the host plants from the adverse effects of several abiotic factors and have good growth and development. The endophytic microbiome produces antioxidative enzymes like POD, SOD, GR, CAT, and APX and some organic compounds like proline, glycine betaine, and organic acids, along with the ability to fix free nitrogen and produce phytohormones (Chen et al. 2017a, b; Divjot et al. 2020; Al Kahtani et al. 2020; Dubey et al. 2021). The endophytic microbiome also transports the heavy metals across the cell membrane, assists in depositing metals in the intra- and extracellular spaces or within their cell walls, and forms metal complexes and metal redox reactions (Franco-Franklin et al. 2021). Thus, the endophytic microbiome assists the plants in alleviating the effect of abiotic stress and improving plant growth and development. So far, several endophytic bacteria have been successfully employed to ameliorate the plant tolerance to abiotic stress (Khan et al. 2020; Araya et al. 2020; Alsharif et al. 2020; Verma et al. 2021).

10.2 Endophytic Bacteria Definition and History

The Heinrich Friedrich Link (1809), for the first time, described the microorganisms that survive in the plant tissues, not causing any harm to the plants. Later, in 1866, De Bary called these organisms with the Greek phrase *endophyte*, "endon = inside and phyton = plants" (Stone et al. 2000). Endophytic bacteriomes investigated thus far are widespread in several plant species (Ryan et al. 2008). Petrini et al. (1991) noted that "microbiomes (bacteria, actinomycetes, and fungi) are inhabiting the plant organs and can colonize in plant tissue continue their life cycle without causing any apparent disease to their host." Several studies reported the presence of endophytes in various parts of the plant, such as leaves, shoots, roots, and seeds (Chebotar et al. 2015; Santoyo et al. 2016). The plant-accompanying microorganisms could be mutually beneficial, commensals, harmful, and neutralists (Ryan et al. 2007). Early research studies confirmed the microbial colonization in the interior regions of the plants (Laurent 1889; Galippe 1887). Galippe (1887) reported that the fungi moving from the soil into the vegetable crops might benefit the host plant. Further studies

confirmed the role of bacterial-assisted nitrogen fixation in pulse crops through the root nodules (Beijerinck 1888). A Dutch microbiologist, Martinus Willem Beijerinck, isolated bacteria from root nodules of Leguminosae plants (Beijerinck 1888). Later studies identified this bacterium as *Rhizobium leguminosarum* and found that the bacteria can produce ammonia and other nitrogenous substances from the nitrogen (N2) gas available in the atmosphere (Beijerinck 1888; Hellriegel and Wilfarth 1888; Frank 1889). In 1898, Vlog reported the presence of fungal endophyte mycelium in the grass seed *Lolium temulentum*. The German scientist Freeman reported the fungal endophyte in *Persian darnel* (Freeman 1904). Perotti (1926) discussed the nonpathogenic flora of root tissue and also explored the symbiotic relationships of endophytic bacteria with root tissue.

As the number of evidence increases on the beneficial aspects of endophytic microbiomes, more scientific groups focus their research on the plant root-associated endophytic bacteria on promoting plant growth and development. The breakthrough research on identifying endophytic bacteria-assisted nitrogen fixation in the grass species sugarcane triggered more research on identifying such bacterial species (Boddey and Döbereiner 1988), because earlier only legume-associated rhizobacteria were thought to involve in biological nitrogen fixation. The advancements in culture methods and nucleotide sequence-based identification methods further speed up the research progress in the endophytic bacterial field (Compant et al. 2021). Endophytes have been identified in several plant taxonomic groupings, including Angiosperms, Bryophytes, Pteridophytes, and Gymnosperms (Compant et al. 2021).

10.3 Endophytic Diversity

Endophytic microbiome relationships with a plant are not limited to a particular plant host or species, but also they make a habitat in a variety of genera and species. The structure of a plant's endophytic population is dictated by its host species; therefore, various plant species growing in the same soil could have vastly different endophytic diversities (Germida et al. 1998; Ding and Melcher 2016). Different parts of the plant tissues/organs include aerial parts such as shoots, seeds, fruits, leaves, and flowers, and underground parts like roots, both inter- and intracellularly, accommodate a wide range of microbiota (Ryan et al. 2008). Several studies revealed that roots could host the most incredible diversity of microorganisms compared to other plant organs (Amend et al. 2019). The culturable bacterial community number varies in the different parts, and 1 g tissue of roots could host 10^5 to 10^7 cells, whereas in the aerial parts, they might be 10^3–10^4 cells per gram tissue (Compant et al. 2010). A range of factors, including the soil's physical and chemical conditions, have been shown to influence the microbiota of aerial parts (Escobar Rodríguez et al. 2020). A recent study revealed that the colonization of endophytes depends on the growth pattern of the host; in woody plants, affluent growth was observed in the stems, whereas in the grasses, abundant communities were observed in the roots (Harrison and Griffin 2020). The soil environment and plant innate immunity are major

determinantal factors of the bacterial numbers, and they promote only "plant favorable" communities. However, the harmful bacteria overcome these detrimental factors and proliferate their numbers (Liu et al. 2017).

"Acidobacteria, Verrucomicrobia, Bacteroidetes, Proteobacteria, Planctomycetes, and Actinobacteria are the most commonly encountered taxa, and most of them may also be found in the rhizosphere" (Hardoim et al. 2015). "Mundt and Hinkle identified 46 bacterial species from 27 plant species in 1976, while Sturz et al. (1997) identified 25 bacterial species from clover and potato; among them, 18 were common in two plant species." "Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes, Chloroflexi, Firmicutes, and Gemmatimonatedes were among the most common phyla detected in grapevine roots" (Samad et al. 2017). "Proteobacteria, Firmicutes, and Bacteroidetes have also been discovered as prominent phyla inside maize roots" (Correa-Galeote et al. 2018). "Cavalcante and Döbereiner (1988)" isolated *Gluconacetobacter diazotrophicus*, a nitrogen-fixing endophytic bacteria from sugarcane. "Rice plants consist of several genera of endophytic bacteria such as *Pseudomonas* sp. (You and Zhou 1989), *Azoarcus* sp. (Hurek et al. 1994), *Herbaspirillum seropedicae* (Olivares et al. 1996), and *Rhizobium leguminosarum*" (Yanni et al. 1997).

"In general, Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes are dominant root endophytic bacterial communities, whereas Chloroflexi, Cyanobacteria, Armatimonadetes, Verrucomicrobia, Planctomycetes, and Nitrospirae were a lesser ratio dominated communities" (Sessitsch et al. 2012; Edwards et al. 2015).

10.4 Colonization of Endophytic Bacteria

The bacterial endophytic microbiome originates from the rhizosphere environment because plant root exudates and rhizodeposits attract microorganisms. Soil factors, such as lack of nutrients, UV light, and desiccation, drastically reduce the colonization capacity (Compant et al. 2010; Philippot et al. 2013). There are three types of colonization by endophytic bacteria: obligatory, facultative, and passive. The obligate endophytic bacteria cannot survive in soils because these bacteria are derived from seeds. The facultative endophytic bacteria widely exist in the soil. Facultative bacteria colonize plants and carry out the infection throughout the plant when conditions are favorable and suitable. The passive endophytic bacteria cannot colonize plants. It infects and enters the plant through endophytic niches such as wounds and cracks (Christina et al. 2013).

Colonization paths have been explored based on the type of strains. Many distinct routes are involved in migrating bacteria from the surface of the roots to the cortical layer. The plant endodermis turns into an obstacle to further colonization. Bacteria can penetrate the endodermis, enter the phloem and xylem vascular systems, and colonize intercellular spaces for systematic colonization of internal plant components (Compant et al. 2010). Several studies have been carried out in the

last few decades on both plant beneficial and harmful bacteria to identify the path of the microorganism from soil to aboveground parts of the soil. The plant root bacterial endophytes initially attach to the root's surface and enter the root interiors via apoplast in xylem vessels (Compant et al. 2010; Brader et al. 2017). Understanding this transfer of bacteria between root and shoot/leaf is beneficial for developing biofertilizers in agriculture (Bodenhausen et al. 2013; Bulgari et al. 2014; Bai et al. 2015).

Further, advanced technologies in microscopy and marker genes such as GUS and GFP were used to get more insides into the colonization pathway of microorganisms. Based on the research data, various pathways have been proposed to explain the traversing of bacteria from the rhizosphere to the interior root regions. According to Kandel et al. (2017), the microorganism could go into the root through the root tip as well as root hair. *Pseudomonas* spp. enter the interior of olive plants through the root hair (Mercado-Blanco and Prieto 2012; Mercado-Blanco 2015). In another study, the colonization of bacteria through the root cracks has been reported in greater detail. Microorganisms could intrude root tissues through cracks in the secondary root emergence zone, allowing the bacteria to passively enter root tissues (Compant et al. 2019).

Some bacteria produce extracellular enzymes that degrade the plant cell wall at the junctions of the root epidermis and make a path for the entry of the bacteria (Liu et al. 2017). Sometimes, the harmful bacteria or insect path also serves as an entry point for the plant beneficial bacteria (Compant et al. 2010). A comparative study to understand the root colonization of beneficial and harmful bacteria using *Pseudomonas syringae* strains revealed that both strains preferred the colonization of the secondary root emerging location. However, the helpful strain was detected in high density on the surface of primary roots, but the pathogenic strain was discovered more often on secondary roots. The latter strain colonizes severely, injures the roots, and finally settles in xylem zones, but the beneficial strain does not (Passera et al. 2019).

Microscopic analysis of the GFP-tagged PsJN endophytic bacterial strain *Burkholderia phytoformans* in grape roots revealed that endophytic bacteria could colonize the innermost layers of inter- as well as intracellular spaces, in the xylem and phloem cells, and the parenchyma (Compant et al. 2005). Further studies with the model endophytic bacteria *Azoarcus*, *Gluconacetobacter*, *Herbaspirillum*, and *Klebsiella* spp. supported this pathway (Turner et al. 2013). Research on the localization of endophytic bacteria in the leaves revealed that most bacteria reside in the xylem, sub-stomata, and parenchymatic cells. In the stems, bacteria mostly enter through the root xylem cells or the surface (Compant et al. 2021). Bacteria mostly colonize in the epidermis and vascular system of flower parts (Compant et al. 2011). The endophytic microbiome entry into the seeds occurs through the xylem vessels, micropyle, and testa (Mitter et al. 2017).

10.5 Traits for Successful Colonization

Endophytic bacteria are armed with several essential colonization features, such as mobility and the production of cell-wall-disintegrating enzymes, carbohydrate breakdown genes, and signal transduction pathway genes, which enable them to infiltrate, colonize, and translocate within the plant's interior (Piromyou et al. 2015). Several advanced genomics tools coupled with mutation studies confirmed the essentiality of these traits for the colonizing bacteria (Bohm et al. 2007; Straub et al. 2013; Sheibani-Tezerji et al. 2015). The genome sequence analysis of endophytic bacteria of rice roots identified several genes encoding plant cell wall disintegration (Sessitsch et al. 2012). The endophytic bacterial species *Bradyrhizobium SUTN9-2* of rice plants produces the pectinase enzyme, which disintegrates the middle lamella between plant cells and helps in the initial entry and colonization of bacteria (Piromyou et al. 2015). The plant beneficial strain *B. phytoformans* (PsJN) produced oxalate-metabolizing enzymes to metabolize plant-secreted oxalate as a carbon source and attract the plants (Kost et al. 2014). Another essential trait that endophytes required for colonization is siderophore production and synthesis of metabolites that act as biocontrol agents. *Kosakonia* mutant loss together with type 6 (T6SS) secretion system prevents colonization due to lack of siderophore production (Mosquito et al. 2020). Thus, siderophore production is an essential endophytic trait.

10.6 Functional Traits of Endophytes

Endophytic organisms are known for various beneficial functions, such as mineral mobilization, plant defense induction, phytohormone production, production of various secondary metabolites against pathogens, and tolerance to various environmental stresses, some of which are especially significant for the host. The roles of the endophytic organism's traits in host plants have been well studied recently with the help of in vitro assays, metagenome analysis, and functional genomics approaches (Compant et al. 2021). Studies on the functions of endophytic bacteria that are attributed to the natural agriculture field are rare due to their low infection rates and reduced functional activities. Thus, most of existing research data are from experimental laboratory studies (Schenk et al. 2012).

10.7 Nitrogen Fixation

Nitrogen is an essential element, part of several important biological molecules such as nucleic acids and chloroplast. It is relatively common in the environment and presents enormous quantities in the atmosphere, crust, the upper mantle of the earth, and water bodies in the form of dinitrogen (N_2), a nonabsorbable form to the plants. Atmospheric N_2 could be converted into plant-absorbable forms by two natural processes, i.e., lightning and rain and the use of biological organisms (Puri et al.

2018). Biological organisms use the nitrogenase enzyme that breaks down the triple bond of N_2 and produces ammonia. The nitrogenase enzyme is present in just a few bacterial and archaea species (Galloway et al. 2008). Diazotrophs are nitrogen-fixing microorganisms extensively distributed in soil and water as free-living organisms and symbionts associated with the plant root and leaf surface. Initially, all diazotrophs were thought to associate only with legume plants; later on, several research studies confirmed that diazotrophs are also associated with some nonleguminous plants (Döbereiner 1961; Döbereiner et al. 1972).

Further research revealed that these rhizosphere-associated diazotrophs could not produce the adequate N required for plant growth. Later on, research studies identified *Gluconacetobacter diazotrophicus*, a diazotroph from the interior root regions of the nonleguminous plant sugarcane, which involves higher nitrogen fixation rates (Cavalcante and Döbereiner 1988). Endophytic bacteria can improve the accessibility of nitrogen to the plant, which aids in its development. As endophytic bacteria are guarded inside plants, the nitrogenase enzyme protects them from oxygen; they could fix nitrogen more efficiently than rhizosphere bacteria (Sachs et al. 2004). The endophytic bacteria contribute nearly 47% of nitrogen captured from the atmospheric air.

In a study, nitrogen fixation by endophytic bacterial strains *Azospirillum*, *Sphingomonas*, and *Burkholderia* was isolated from traditional rice varieties; further reinfection of *Burkholderia vietnamiensis* to the Arroz 70 rice variety boosted the nitrogen availability and improved the grain yield (Araújo et al. 2013). The endophytic bacterial strains *Burkholderia*, *Klebsiella*, *Novosphingobium*, and *Sphingomonas* improved rice growth, development, and N content (Rangjaroen et al. 2015). In another study, reinoculating the bacterial endophytes *Paenibacillus*, *Bacillus*, *Microbacterium*, and *Klebsiella* to Korean rice cultivars improved the growth and N content (Padda et al. 2017). In another work, inoculation of a well-known N-fixing bacterial strain *Gluconacetobacter diazotrophicus* strain *PAL 5* to sugarcane improved the leaf total N content and drought stress (Aguiar et al. 2016). Four of the 44 endophytic bacterial strains isolated from banana roots exhibited N fixation ability (Andrade-Linares et al. 2013). The pearl millet root nitrogen fixation strain *Pseudomonas aeruginosa* PM389 inoculation to the nonhost plant wheat improved seed germination and development (Gupta et al. 2013).

10.8 Phosphorous Solubilization

One of the most important macronutrients for plant growth and development is phosphorus (P), and it makes up about 0.2% of a plant's dry weight (Azziz et al. 2012; Tak et al. 2012). However, due to its chemical nature, it forms complexes with iron, aluminum, and calcium; thus, only 0.1% of soluble P is available in the soil and is accessible to the plant (Sharma and Agrawal 2013). Thus, phosphorous fertilizers were added to the soils to overcome the phosphorous deficiency in the plant. However, most of the applied phosphorus forms complexes with the soil and becomes unavailable for the plant, and this excess amount leads to ecological

problems (Ezawa et al. 2002; Kang et al. 2012). Thus, there is a need to solubilize phosphorus into the plant's available orthophosphate (Chhabra et al. 2013). Recent research studies revealed that the microorganisms in the rhizospheric soil and colonized in the interior regions could make insoluble phosphate into a plant uptake form (Zaidi et al. 2009; Sharma and Agrawal 2013; Alori et al. 2017). Soil microorganisms use a variety of ways to solubilize P, including pH reduction, organic acid generation, chelation, and exchange processes (Gerke 1992). Microorganisms produce various forms of organic acids, which decrease the pH of the roots adhering to soil; as a result, the chemical bonds in the phosphate complex such as $Ca_3(PO_4)$ (tricalcium phosphate) disassociate; as a result, phosphate turns into a plant-available form.

A stress-tolerant endophytic bacterial strain from the apple rhizosphere exhibited phosphate solubilization and plant growth promotion traits. Biochemical analysis revealed that it produces organic acids such as gluconic and citric acid (Mehta et al. 2011). A total of 106 phosphate-solubilizing bacterial strains were isolated from the sea buckthorn root-attached soil and interior regions of the root. Inoculating five bacterial strains in the tomato seedlings improved their growth and development (Kumar et al. 2015). In a similar study, the *Bacillus subtilis* strain CKT1 from tomato improved the growth and development of tomato seedlings upon reinfection (Walia et al. 2013a, b). Phosphate-solubilizing endophytic bacterial strains *Enterobacter* sp. J49 and *Serratia* sp. S119 from peanuts significantly improved the P accumulation of maize and soybean (Lucero et al. 2021). The endophytic bacterial strains *Pseudomonas fluorescens* from *Miscanthus giganteus* significantly improved growth and P accumulation in pea plants (Otieno et al. 2015). Advanced imaging technologies and biochemical analysis provided significant evidence for endophytic bacteria's role in P uptake. The two endophytic bacterial species from poplar, *Rahnella* sp. (WP42) and *Burkholderia* sp. (WP5), promoted P uptake in wild poplar, as evidenced by X-ray imaging, spectrophotometry, and proteomics analysis (Varga et al. 2020). These investigations suggested that phosphate-solubilizing bacteria may improve phosphate utilization efficiency in various plant species.

10.9 IAA Production

Indole-3-acetic acid (IAA), one of the principal phytohormone auxin, regulates the growth and development of the embryo, photo- and gravitropism, cell division, and differentiation and stimulates extended root growth with the superior number of lateral roots (Teale et al. 2006). Research studies on microorganisms suggest that these organisms also produce IAA (Kazan 2013). The production of IAA in microorganisms might be an evolutionary aspect acquired from ancient symbiosis. Both the plant beneficial and pathogenic microbiome produce IAA. The IAA is mainly produced from the tryptophan-dependent and tryptophan-independent pathways in microorganisms. However, most of the beneficial microorganisms synthesize IAA through the indole-3-pyruvate (IpyA) pathway, while the harmful microorganisms produce it through the indole-3-acetamide (IAM) pathway

(Hardoim et al. 2008). Several IAA-synthesizing beneficial plant microorganisms such as *Pseudomonas, Rhizobium*, *Azospirillum*, *Enterobacter*, *Azotobacter*, *Klebsiella*, *Alcaligenes*, *Pantoea*, *Acetobacter*, *Herbaspirillum*, *Burkholderia*, *Bacillus*, *Rhodococcus*, and *Streptomyces* were identified in several research studies (Ali et al. 2017).

Precise modulation of plant IAA levels with the help of plant beneficial microorganisms is one of the fundamental approaches for the crop improvement. In a study, a bacterial culture supernatant from the stationary phase growth of endophytic bacteria isolated from the terrestrial orchid induced root formation and growth (Tsavkelova et al. 2007). The endophytic bacterial isolates *Sphingobacterium thalpophilum* AS34, *Pseudomonas aeruginosa* AS36, and *Enterobacter aerogenes* AS75 isolated from the *Withania somnifera* induced a high percentage of direct somatic embryogenesis and regeneration (Soundar Raju et al. 2020). The *Moringa peregrina endophytic bacteria and Bacillus subtilis* LK14 exhibited significantly higher IAA levels and improved tomato's biomass and chlorophyll content (Khan et al. 2016a, b). The amount of IAA produced by the endophytic bacteria is also crucial for the bacteria-plant association. The engineered bacteria *Pseudomonas putida* GR12-2 for the production of elevated levels of IAA reduced the growth in mung beans (Patten and Glick 2002). This is because elevated levels of auxins trigger the production of the stress hormone ethylene (Woodward and Bartel 2005). The bacterial IAA production is considered an essential trait in selecting beneficial plant bacteria. Moreover, plant IAA levels can also determine whether bacterial IAA stimulates or suppresses plant growth, as bacterial IAA production usually benefits those plants with low levels of endogenous IAA (Glick 2012).

10.10 ACC Deaminase Production

The enzyme ACC (1-aminocyclopropane 1-carboxylate) deaminase is a microbial multimeric enzyme that cleaves the phytohormone ethylene biosynthesis precursor ACC to α-ketobutyrate and ammonia, hence inhibiting the synthesis of ethylene (Glick 2014). The phytohormone ethylene plays a regulatory role in various physiological and developmental pathways. Primarily, it regulates root initiation, nodule formation, fruit ripening, cell elongation, auxin transport, and leaf senescence. Ethylene is also a stress-responsive hormone; several biotic/abiotic stresses trigger ethylene production (Sun et al. 2016a, b). Research studies on plant-associated endophytic bacteria revealed that several endophytes could produce ACC deaminase (Zhang et al. 2011; Rashid et al. 2012; Afzal et al. 2019). Thus in the endophyte-plant association, endophytic bacteria lower the ethylene levels and alleviate the stress-induced effects in plants.

The ACC deaminase-producing endophytic bacterial isolates from tomatoes significantly decreased the production levels of ethylene of canola compared to the noninfected plants with endophytic bacteria (Rashid et al. 2012). Similarly, ACC deaminase-producing *Pseudomonas migulae* 8R6 strains increased the biomass and

yield in tomato plants compared to uninoculated tomato plants (Ali et al. 2014). The salt-tolerant and ACC deaminase-producing endophytic bacteria *Achromobacter xylosoxidans* from *Catharanthus roseus* alleviated the plant's salt stress-induced effects by lowering the ethylene levels and triggering the antioxidant defense mechanism (Qin et al. 2014). The ACC deaminase and IAA producing the endophyte *Bacillus subtilis* LK14 from *Moringa peregrina* exhibited significantly higher IAA levels and lower ethylene accumulation, displaying improved biomass chlorophyll content in tomato (Khan et al. 2016a, b). The ACC deaminase-producing endophytic bacteria strain *Pseudomonas* spp. OFT5 in the tomato plant reduced the ethylene production levels. This resulted in tomato plants alleviating salt stress-induced effects and improving their growth and biomass (Win et al. 2018). The endophytic bacterial *Bradyrhizobium* strain SUTN9-2, capable of producing ACC deaminase upon infection in rice, decreased ethylene production under drought stress conditions, maintaining the cell membrane integrity, high leaf relative water content, and yield under field drought stress conditions (Sarapat et al. 2020). All this evidence suggests that the endophytic bacteria with ACC deaminase production activity reduce the ethylene production in plants and protect the plants from stress-induced damage.

10.11 Drought Stress in Plants

Drought stress or water shortage in plants is a critical abiotic stress caused by low precipitation, high light intensity, high/low temperatures, and more salts in the soil. The lack of precipitation in the dry climatic zones increases the prolonged drought stress. It was estimated that by the year 2030, water shortage due to the prolonged drought in several parts of the globe might affect 40% of the population; as a result, 700 million people, livestock, and crops will be at risk (Seleiman et al. 2021; Shah et al. 2021a, b). Crop output must be enhanced under drought stress to provide sufficient clothes, food, and housing to the growing population. Numerous strategies were adopted to promote drought resistance in plants with greater yield (Takahashi et al. 2020).

Drought as multifaceted stress triggers various morphological, anatomical, biochemical, and molecular changes and yield. The plant's foremost visual symptom of the drought is reduced growth rates in the shoot and leaf (Farooq et al. 2012). Water deficit affects the nutrient and water uptake, reducing the leaf's size, stem, and root development. The loss of water from the plant cell decreases the water potential, causing the lowering of turgor pressure. The reduced turgor pressure and photoassimilation rates further decreased cell elongation and root proliferation (Mahmood et al. 2019).

Based on their genetic makeup, plants overcome drought stress through three different strategies, i.e., drought avoidance, tolerance, and escape (Chaves et al. 2003; Basu et al. 2016). Plants avoid the damages caused by drought stress via regulating the water uptake and loss by dint of anatomical modifications such as leaf rolling, increasing the trichome number, wax content, and dropping older leaves.

Plants try to escape the drought by completing the life cycle with a hasty growth and marginal seeds before encountering drought stress. Some plants synthesize metabolites and sugar to maintain the cellular water potential through the osmotic adjustment and tolerate drought stress (Chaves et al. 2003).

One of the key activities impacted by drought stress is photosynthesis. The significant factors that inhibit photosynthesis during the drought are early leaf shedding, reduced leaf surface area, limitation of CO_2 availability due to the stomatal closure, rise in leaf temperatures, and impaired activities of dark reaction and Calvin cycle enzymes (Salehi-Lisar and Bakhshayeshan-Agdam 2016). The imbalance between the dark and light reactions triggers the production and accumulation of reactive oxygen species (ROS) in the chloroplasts (Raghavendra et al. 2010). To protect themselves from the photoinduced damage during the drought stress, plants deflect the absorbed light into the thermal dissipation through the xanthophyll cycle or activate the photorespiration process (Demmig-Adams and Adams 1996; Chaves et al. 2003). Simultaneously, plants also trigger the production of antioxidative enzymes to quench ROS. Scavenging systems include a variety of antioxidants and enzymes, counteract ROS entities, and convert them to less hazardous compounds in the cell (Hasanuzzaman et al. 2020).

One biochemical mechanism that protects the plants from drought stress is an osmotic adjustment (OA). Upon drought, plants synthesize and accumulate specific compatible solutes such as glycine betaine; sugars, i.e., sugar alcohols and sorbitol; and amino acid proline. These compatible solutes enhance the cellular osmotic force and increase the intake of water from the surroundings, and also they provide protection to the cellular membranes, proteins, and enzymes (Sanders and Arndt 2012). In response to drought stress, plants synthesize several drought-induced proteins, including ABA-responsive protein RAB17 and dehydration-induced proteins (dehydrins). They interact with biological macromolecules, stabilize protein folding as chaperones, have more excellent oxidation properties, and protect cellular membranes (Graether and Boddington 2014).

Plant hormones such as auxins, abscisic acid, and ethylene, as chemically active substances, act as chemical signaling molecules and transduce the signals among different parts of the plant and across the cell. Drought stress increases the production of plant hormones IAA, ABA, and ethylene and decreases the production of cytokinins (Yang et al. 2021). The membrane receptors of the plants sense the drought and osmotic chemical signals and convert them into an intracellular signal. Further, these signals were transduced downstream as secondary messengers, which trigger the expression of various regulatory and functional genes in the nucleus. Based on their function under drought stress, the gene products are categorized into four groups: (1) signal transduction proteins (mitogen-activated protein kinases [MAPK], calcium-dependent protein kinases [CDPK], receptor protein kinases, ribosomal protein kinases, and transcription regulation protein kinases), (2) protein phosphatases (phosphodiesterases and phospholipase), (3) regulatory proteins including the "transcription factors" (AREB, AP2/ERF, NAC, bZIP, MYC, and MYB), and (4) functional proteins which are involved in water uptake and

macromolecule protection (water channel proteins, osmoprotectants, heat-shock proteins, and dehydrins) (Hura et al. 2022).

Millions of microorganisms inhabit the plant root system, creating a complex biological community and promoting plant development and production (Mondal et al. 2020). A research report identified that pepper plants colonized with some endophytic bacterial isolates displayed a greater tolerance level to drought stress than the uninfected plants (Schmidt et al. 2014). The microorganisms, upon infection, improved the root system of plants as the water and nutrient absorption were enhanced (Ullah et al. 2019). "The plant-associated bacteria improved the plant stress tolerance by secretion of bacterial exopolysaccharides, reduction in the level of ethylene in plant roots by ACC deaminase, production of volatile compounds, accumulation of osmolytes, activation of the antioxidant defense system, production of plant growth regulators such as abscisic acid (ABA), indole-3-acetic acid (IAA), cytokinin, gibberellins" (Ali and Khan 2021).

The endophytic bacteria *Pantoea alhagi*, isolated from the desert legume plant *Alhagi sparsifolia*, improved the drought tolerance of nonhost plant wheat. The endophyte also exhibited several PGP traits (Chen et al. 2017a, b). In a similar study, the endophyte *Burkholderia phytofirmans* PsJN improved the wheat water use efficiency, photosynthetic rates, and chlorophyll content under drought stress (Naveed et al. 2014). The endophytic bacterial strains *Bacillus aquimaris* 3.13 and *Micrococcus luteus* 4.43 enhanced Jerusalem artichoke's inulin levels and drought tolerance (Namwongsa et al. 2019). The endophytic bacterial strain *Bacillus thuringiensis AZP2* improved wheat's drought tolerance and biomass under drought stress (Timmusk et al. 2014). Under drought stress, the endophytic bacterial strain *Pseudomonas fluorescens*-primed maize seedling accumulated more proline, phytohormones than the non-inoculated seedlings (Ansary et al. 2012). In another study, the *Pseudomonas* sp. endophytic bacteria increase the production of soluble sugars and subsequently osmotic adjustment under drought stress conditions in maize (Bano and Fatima 2009). Several endophytic bacteria exhibited drought tolerance, improved growth, and enhanced yields, as tabulated in Table 10.1.

Thus, applying endophytic bacterial inoculations to host and nonhost plants improved the growth and agronomic efficiency by reducing production costs and environmental pollution.

10.12 Conclusion and Future Perspectives

The use of microbiome is an eco-friendly approach that minimizes the use of chemical fertilizers and pesticides and helps maintain the ecological balance in the agroecosystem. Recent research studies suggest that microbiome biostimulants have a more significant potential to be a long-term and practical approach to mitigate abiotic stress caused by global climate change. Based on the ability to produce phytohormones, absorb nutrients, and produce secondary metabolites by the endophytic microbiome, several studies have highlighted their potential use in drought stress tolerance in crop plants. However, most of the studies were confined to

Table 10.1 Endophytic bacteria used for the stimulation of drought stress tolerance in plants

S. No.	Endophytic bacteria/ action-bacteria	Host plant	Plant part used for isolation	Inoculated plants	Beneficial features related to drought tolerance	Citations
1	*Azospirillum lipoferum*	Sugar cane (*Saccharum officinarum*)	Roots	Maize (*Zea mays*)	ABA, IAA, and gibberellic acid maintained RWC and alleviated drought stress	Cohen et al. (2009), Reinhardt et al. (2008)
2	*Streptomyces coelicolor*, *S. olivaceus*, and *S. geysiriensis*	Bui (*Aerva tomentosa*), Keeker (*Acacia nilotica*), Kheep (*Leptadenia pyrotechnica*), Phog (*Calligonum polygonoides*), and Bajra (*Pennisetum glaucum*)	Roots	Wheat (*Triticum aestivum*)	Enhanced auxin and IAA production, higher seedling vigor and yield	Yandigeri et al. (2012)
3	*Gluconacetobacter diazotrophicus*	Sugarcane (*Saccharum officinarum*)	Roots and shoots	Sugarcane (*Saccharum officinarum*)	Activation of drought stress-responsive genes, ABA, and ethylene signaling pathways	
4	*Pseudomonas azotoformans*	*Alyssum serpyllifolium*	Leaves	*Trifolium arvense*	Increased relative water content, chlorophyll content, SOD, POD, CAT, proline, and plant biomass	Timmusk et al. (2014)
5	*Burkholderia phytofirmans*	Onion (*Allium cepa*)	Roots	Wheat (*Triticum aestivum*)	Improved photosynthetic rate, water use efficiency, chlorophyll content, grain yield, ionic balance, and antioxidant level	Naveed et al. (2014)
6	*Burkholderia phytofirmans* and *Enterobacter* sp.	Onion (*Allium cepa*)	Roots	Maize (*Zea mays*)	Increased leaf area, shoot and root biomass, photosynthesis, chlorophyll content, and photochemical efficiency	Naveed et al. (2014)

7	*Bacillus subtilis*	Switch grass (*Panicum virgatum*)	Leaves	*Brachypodium distachyon*	Increased root and shoot biomass, yield, total soluble sugars, and starch content and upregulated drought-responsive genes	Gagne-Bourgue et al. (2013), Gagné-Bourque et al. (2015)
8	*Burkholderia vietnamiensis*, *Rhizobium tropici*, *Acinetobacter calcoaceticus*, *Rahnella* sp., *Burkholderia* sp., *Enterobacter asburiae*, *Sphingomonas yanoikuyae*, *Pseudomonas* sp., and *Curtobacterium* sp.	Poplar (*Populus* sp.)	Stem	Poplar (*Populus* sp.)	Increased biomass, total plant nitrogen, and chlorophyll content and reduced stomatal conductance and damage caused by ROS	Khan et al. (2016b)
9	*Burkholderia phytofirmans*	Onion (*Allium cepa*)	Roots	*Panicum virgatum*	Higher biomass and photosynthetic level	Sessitsch et al. (2005), Wang et al. (2015)
10	*Bacillus amyloliquefaciens*	Grapevine	Roots	Grapevine	Secreted melatonin and reduced MDA, H_2O_2, and O_2^-	Jiao et al. (2016)
11	*Pantoea alhagi*	*Alhagi sparsifolia*	Leaves	Wheat (*Triticum aestivum*)	Increased soluble sugars, IAA, EPS, siderophore, ammonia, and protease production and decreased proline and MDA accumulation and chlorophyll degradation	Chen et al. (2017a, b)
12	*Sphingomonas*	*Tephrosia apollinea*	Leaves	Soya bean (*Glycine max*)	Enhanced ABA and jasmonic acid content and increased plant biomass (dry) glutathione, glutamine, photosynthetic pigments, glycine, and proline	Asaf et al. (2017), Khan et al. (2017)

(continued)

Table 10.1 (continued)

S. No.	Endophytic bacteria/ action-bacteria	Host plant	Plant part used for isolation	Inoculated plants	Beneficial features related to drought tolerance	Citations
13	*Flavobacterium* (50%), *Pseudomonas* (37%)	*Medicago polymorpha*, *M. lupulina*, *M. littoralis*, *Vicia benghalensis*, *Ostorhinchus compressus*	Roots	*Arabidopsis thaliana*	Nitrogen fixation and produced IAA, siderophores, and volatile organic compounds	Cardoso et al. (2018)
14	*Bacillus subtilis* and *Paenibacillus illinoisensis*	Peppers (*Capsicum annuum*)	Roots	Peppers (*Capsicum annuum*)	Increased total plant biomass and root length and improved net photosynthetic rate, transpiration, cell turgor, and proline content	Vigani et al. (2018)
15	*Bacillus pumilus*	*Glycyrrhiza uralensis*	Not mentioned	*Glycyrrhiza uralensis*	Increased root length, CAT, GPX, and GR and decreased H_2O_2 and MDA content and O_2^- production	Xie et al. (2019)
16	*Bacillus amyloliquefaciens*	*Euphorbia trigona*	Not mentioned	*Solanum lycopersicum*	Altered oxidative status and stomatal and photosystem II functioning and induced growth promotion	Eke et al. (2019)
17	*Bacillus aquimaris* and *Micrococcus luteus*	Jerusalem artichoke	Leaf and stem	Jerusalem artichoke	Increased height and shoot and root weight, photosynthesis, insulin levels	Namwongsa et al. (2019)
18	*Ochrobactrum* sp.	Sorghum bicolor	Roots	Sorghum bicolor	Plant growth promotion as well as induced stress	Govindasamy, et al. (2020)
19	*Staphylococcus* sp.	*Curcuma longa*	Rhizome	*Vigna unguiculata*	Modulated both macro- and micronutrients and drought stress	Jayakumar et al. (2020)

20	*Pseudomonas*	*Alhagi sparsifolia*	rhizosphere and root endosphere	*Alhagi sparsifolia*	Improved drought resistance	Zhang et al. (2020)
21	*Endophytic bacterial microbes*	*Wheat*	Plant tissues	wheat	Drought stress varietal characteristics	Žiarovská et al. (2020)
22	*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Paenibacillus polymyxa*	*Phleum pratense*	Rhizosphere	*Brachypodium distachyon*	Strong chemotactic behavior, the alginate and (EPS) contents were evaluated, biofilm formation	Saleh et al. (2020)
23	*Bacillus qingshengi*	*Oryza sativa*	Roots	*Oryza sativa*	Enhanced N fixation ability, phosphate solubilization ability, ACC deaminase activity, and siderophore production	Pang et al. (2020)
24	*Bacillus subtilis* Dcl1	*Curcuma longa*	Rhizome	*Vigna unguiculata*	Produced the catalase, superoxide dismutase, peroxidases, gamma-glutamyl transpeptidase, glutathione, and glycolate oxidase	Jayakumar et al. (2020)
25	*Gluconacetobacter diazotrophicus* strain Pal5	–	–	*Oryza sativa*	Increased in the root area and higher levels of osmoprotectant	Silva et al. (2020)
25	*Burkholderia phytofirmans*	–	–	*Chenopodium quinoa*	Stimulated the growth and yield of quinoa in highly salt-affected soils	Yang et al. (2020)
27	*Kosakonia cowanii*	*Lactuca serriola*	Seeds	*Arabidopsis thaliana*	Drought tolerance	Jeong et al. (2021)

(continued)

Table 10.1 (continued)

S. No.	Endophytic bacteria/ action-bacteria	Host plant	Plant part used for isolation	Inoculated plants	Beneficial features related to drought tolerance	Citations
28	*Bacillus cereus*, *Pseudomonas otitidis*, and *Pseudomonas* sp.	Soya bean (*Glycine max)*	Root	Soya bean (*Glycine max*)	Mitigated the effect of drought as well as fungal diseases on the early seedling growth of soybean	Dubey et al. (2021)
29	*Gluconacetobacter diazotrophicus*	–	–	*Zea mays* L.	Increased plant biomass, chlorophyll content, plant nitrogen uptake, and water use efficiency	Tufail et al. (2021)
30	*Stenotrophomonas* sp.	Poplar	–	Poplar	Production of indole-3-acetic acid and promoted plant growth	Ulrich et al. (2021)
31	*Azotobacter chroococcum* Avi2	–	–	*Oryza sativa*	Determined photosynthetic efficacy (chlorophyll fluorescence-imaging), antioxidants, and plant growth promotion (PGP)	Kumar et al. (2021)
32	*Pantoea agglomerans* ANP8	*Medicago sativa*	Root nodules	Legumes	Production of IAA, solubilization of phosphates, glucose dehydrogenase	Noori et al. (2021)
33	*Herbaspirillum* (AP21, AP02), *Azospirillum* (D7), and *Pseudomonas* (N7) strains	–	–	*Lolium perenne*	Increased plant dry biomass, production of siderophores and EPS	Cortés-Patiño et al. (2021)

controlled laboratory conditions. Research data from the laboratory conditions should be shared with policymakers and stakeholders through extension services and publicized, and the technology spread broadly in a wide range of crops, geographies, and environmental circumstances. Compared to the synthetic compounds, the adoption of microbial biostimulants in agroecological and biological research still has several downsides, mostly due to their lower efficiency and increased environmental sensitivity. The absence of a standard global regulatory framework poses a barrier to product commercialization and may discourage the development of innovative goods.

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The Cellulosome: A Fiber-Degrading Strategist of the Rumen Microbiome

11

Srijana Mukkala, Pallaval Veera Bramhachari, and Y. Harish Kumar Reddy

Abstract

Microbes in the rumen of herbivores are responsible for effective plant biomass decomposition and digestion. Recent efforts to transform cellulosic biomass into biofuels have heightened interest in the bacterial fibrinolytic processes used by these bacteria. In ecology, plant cell wall material is used to transmit energy between the host and parasitic organisms. Herbivores eat plant material and digest it via symbiotic stomach microbiota (protozoa, fungi, and bacteria). Much anaerobic lignocellulose and hemicellulose-digesting bacteria populate the rumen. Cellulosome is a plant cell wall destroying bacteria's strategic arsenal. Raphael Lamed identified this complex protein in 1983 in an extremophile *Clostridium thermocellum*. The cellulosome complex and its actions were also being studied as "Swiss knife" shape and protein complex, the cellulosome protein complex (carbohydrate-binding modules (CBM), cohesin, dockerin, enzymes, and scaffoldings. Scientists discovered these compounds in rumen microbes. A

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constant study has helped us learn more about rumen bacteria and how their cellulosomes break down plant cell walls. The rumen community depends on cellulolytic *Ruminococcus* spp. Cohesin-dockerin molecules combine to form cellulosome complexes. Designer cellulosomes are chimeric-tailored cellulosomes that function in a cell-free system. They improve the hydrolysis of cellulosic substrates to create value-added products. For this purpose, recombinant constructions and artificial self-assembling chimeric proteins are produced. The capacity of rumen microbes to digest refractory cellulose is of great industrial importance, and metagenomics research is helping to understand and determine the quantities and kinds of cellulolytic bacteria found in the bovine rumen complex ecosystem. This chapter explains the cellulosomal machinery's extensive function in lignocellulose-degrading bacteria.

Keywords

Cellulosomes · Cellulosic substrates · Rumen microbes · Lignocellulose-degrading bacteria

11.1 Introduction

Microorganisms are the most versatile and adaptable forms of life on Earth, and they have been around for over 3.5 billion years. Bacteria governed the biosphere during the first 2 billion years of its existence, populating every biological niche from glacial ice to deep-sea hydrothermal vents. Bacteria from this period established the primary metabolic pathways that are now found in all living species, in addition to a variety of metabolic activities including nitrogen fixation, which is only seen in bacteria today. As bacteria ruled the world for so long, they reshaped the planet's anaerobic environment into one rich in oxygen while also creating a vast quantity of organic chemicals. They eventually succeeded in creating a habitat that could support more advanced forms of life.

There are billions of years' worth of genetic adaptations to an ever-changing world in the biochemistry and physiology of bacteria and other microbes right now. At the same time, microbes are more resistant to the ravages of human technology than larger, more sophisticated forms of life because of their physiologic and metabolic diversity and their capacity to exist in narrow niches. As a result, probably, most of the pre-human microbial species are still available for study.

A new strategy was devised by humans to identify new sources of bulk organic compounds. Plants create an enormous amount of carbohydrate-rich compounds. Lignocellulose is the primary structural component of wood; starch in grains, potatoes as well as sugars in molasses and syrup of maize which are all examples of these compounds (Lynd 1990). A mix of microbial fermentation and chemical processes might be used to transform plant materials into primary feedstock compounds in concept (Fig. 11.1). Oxygen generation, alcoholic drinks, and food

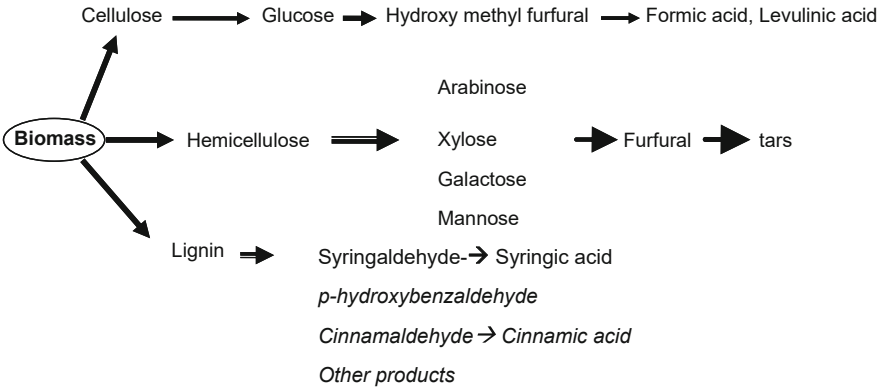

Fig. 11.1 Composition of ligno-cellulosic biomass and potential degradation products. Schematic shows a sequence of the conversion of biomass polymers to monomeric sugars and degradation products

generated from fermentation have all relied on the metabolic activity of particular bacteria (Lynd 1989). Direct bacterial or fungal breakdown of biomass yields a wide range of "oxy-chemicals" as a byproduct. Small molecules to complex molecules such as plastic, rubber, and solvents are all examples of feedstock chemicals that may be used to synthesize a wide variety of other chemicals. The incorporation of genes for degradative enzymes, genetic manipulations of metabolic pathways, and recombinant microorganisms with favorable growth characteristics is the tool for an applied microbiologist to obtain the most efficient conversion of the feedstock to the desired end product. Unfortunately, the accumulation of microbial cell mass represents competition for end products and frequently poses problems of disposal as well. Modern genetic engineering techniques make it possible to manipulate cells to maximize the formation of a product while permitting only a low level of cell growth.

There are hundreds of species of fungi; bacteria that can digest and use cellulose and hemicellulose in lingo-cellulose plant material. Aerobes, anaerobes as well as mesophiles and thermophiles, are among these creatures (Cavedon et al. 1990; Duong et al. 1983). They can be found in large numbers throughout the natural world. Even while many bacteria can thrive on cellulose or develop enzymes that can break down amorphous cellulose, only a handful can produce the whole complement of extracellular cellulases capable of degrading crystalline cellulose in vitro. Among the latter organisms, the most extensively studied sources of cellulolytic enzymes have been the fungi *Trichoderma* and *Phanerochaete* and the bacteria *Cellulomonas* (an aerobe) and *Clostridium thermocellum* (an anaerobe).

Every cursory perusal of current scientific literature shows cellulose hydrolysis by cellulases (EC 3.2.1.4, EC 3.2.1.91, and other enzymes) to be among the most intensively studied topics. Research and development on these enzymes and their production, properties, and applications are important if their actions are to be controlled and better utilized. By catalyzing the decay of forest and agricultural residues, these enzymes recycle nutrients and so serve to maintain soil fertility and mechanical properties.

11.2 Biomass

However, in the biotechnology context, "any organic matter that develops through photosynthetic conversion of solar energy" is widely considered to be "biomass." Green plants, algae, and photosynthetic microorganisms turn the sun's energy into biomass, which may be used for food, fuel, and other purposes. Over a year, photosynthesis on land and at sea generates biomass with an energy content estimated at three times that of humans on the planet.

11.2.1 Major Components of Plant Biomass

Higher land plants' vascular tissues include cellulose fibrils embedded in an amorphous matrix of lignin and hemicelluloses in their cell walls (Table 11.1). Non-covalent forces, as well as covalent cross-links, hold these three polymers together, forming a composite substance known as lingo-cellulose, which accounts for more than 90% of the dry weight of a plant cell. The amount of each polymer varies depending on the species and age of the plant, as well as across different parts of the plant. Compared to hardwoods, softwoods often include a larger percentage of lignin. Grass has the greatest concentration of hemicellulose.

11.2.2 Cellulose

The most common carbohydrate polymer is cellulose, which is found in the walls of plant cells. While plentiful, cellulose is a tough polymer to degrade due to its inability to dissolve and the presence of hydrogen-bonded crystalline threads that are difficult to remove. Iα and Iβ are the two main cellulose isomers found in plant cellulose. When it comes to the triclinic form of cellulose (Iα), there is only one chain per unit cell, and it has a greater energy density than the more stable monoclinic Iβ form (Sugiyama and Suh 2011, Atalla and Vanderhart 1984). Plant cell wall cellulose is mostly made up of the stable Iβ form, which is more sensitive to hydrolysis than the Iα form. All of the glycosyl hydroxyl groups in cellulose are located in the equatorial plane, while the axial plane is filled by nonpolar aliphatic protons (which do not form hydrogen bonds). Because of this, the "sides" of elementary microfibrils are hydrophobic, while the "tops and bottoms" are polar and hydrogen bonding.

The cellulosome, a huge extracellular enzyme complex composed of a scaffolding protein and numerous associated cellulases, has developed in anaerobic microbes

Table 11.1 Composition of lignocellulose in vascular plants (percentage)

Source	Cellulose	Hemicellulose	Lignin
Grasses	25–40	25–50	10–30
Softwoods	45–50	25–35	25–35
Hardwoods	45–55	24–40	18–25

to break down plant cell walls (Bayer et al. 1985). Cellulosomes might be used in biotechnological applications such as the synthesis of ethanol or organic acids from affordable renewable resources by converting cellulosic biomass into sugars. In vitro and in vivo cellulosome research is advancing rapidly, allowing for the creation of systems that can fulfill these objectives (Levy and Shoseyov 2002; Shoseyov et al. 2003).

Microorganisms have been shown to have two distinct enzyme systems for degrading plant cell walls. Unlike cellulose, hemicellulose, pectin, and lignin are easier to break down. Endoglucanases, exoglucanases, and accessory enzymes are released by aerobic fungi and bacteria and can work together to assault plant cell walls. The glycoside hydrolases of *Trichoderma reesei* are the most investigated of these enzymes. Another sort of system has developed in anaerobic microbes that include the creation of a huge extracellular enzyme complex known as the "cellulosome," which is composed of numerous bound enzymes (Warren 1996; Bayer et al. 1985; Coughlan et al. 1985).

11.2.2.1 Degradation of Cellulosic Biomass by Microorganisms

In addition to providing a sustainable source of mixed sugars, plant biomass—the most common biopolymer—has long been recognized as a possible source of alternative energy. But innovative technologies are still required to overcome the numerous obstacles to establishing premium systems for turning biomass into "fuels and chemicals" (McBee 1950; Ljungdahl and Eriksson 1985; Canganella and Wiegel 1993; Beguin and Aubert 1994; Himmel 2008; Moraïs et al. 2010). When compared to the current study strategy, we know very little about the deconstruction and conversion of plant cell walls by enzymatic hydrolysis and/or microbial hydrolysis and/or fermentation. More research is needed to fill in the gaps in our knowledge. Microorganisms play a vital part in the carbon cycle of the Earth by degrading plant cell walls. Cellulose, hemicellulose, and pectin make up 30–40% of most plant cell walls, while lignin and cellulose make up the remaining 15–40%. Oligomers and other tiny carbon molecules are broken down into glucose and other sugars by enzymatic decomposition before being converted into CO_2 by the body.

Carbon and energy from plant cell wall polysaccharides may be used by a wide range of microbes, making them an important part of the carbon cycle. Sometimes free-living bacteria exploit such polysaccharides from decaying plant materials, such as compost piles and sewage sludge; in other circumstances, the microbes aid higher animals (e.g. ruminants) in the conversion of the polysaccharides into digestible parts.

When compared to aerobic bacteria, which create a large number of enzymes like cellulases and hemicellulases, anaerobe biosynthetic apparatuses are far more parsimonious in their enzyme production. When it comes to the extracellular breakdown of polymeric substrates like the plant cell wall's refractory crystalline components, anaerobic environments are thought to be more conducive to this type of machinery's development. It is therefore not surprising that anaerobic bacteria have developed new methods for breaking down plant stuff, and the cellulosome structure appears to be the most impressive of them. Ongoing research has shown

that (Robson and Chambliss 1989; Shimada et al. 1994; Bayer et al. 2004; Valenzuela-Ortega and French 2019).

Bacteria, protozoa, fungi, and archaea all live in the gastrointestinal tract of animals (Rosenberg and Zilber-Rosenberg 2018). Most often found in the rumen system, these synergistic microbial communities (bacteria and fungus) have impressive metabolic capacities and durability. In response to this successful degradation and utilization of plant biomass, there has been a rapid increase in the development of synthetic microbial consortia for biotechnology (Minty et al. 2013). In this review, we emphasized bridging the effective strategies applied by various microorganisms and rumen microbiota to degrade the plant biomass. This context mainly focuses on cellulosome-containing microorganisms, in which first we need to discuss *Hungateiclostridium thermocellum* and its importance. This *Hungateiclostridium thermocellum* bacterium is considered one of the best laboratory grown microorganisms to study the cellulosome and its applications (Felix and Ljungdahl 1993). Bio-engineering principles approach was applied to explore the cellulosome and its strategies in rumen consortia and their mutualistic ability in efficient biodegradation of lignocellulosic feedstocks (Fontes and Gilbert 2010; Gilbert 2007).

11.3 Rumen Microbiota

Ruminants are hoofed mammals that have a unique digestive system (rumen) that allows them to better use energy from fibrous plant material than other herbivores. The taxonomic origin of the rumen's microbiome components based on the gene sequences encoding CAZymes implied the presence of 19 phyla of microbes. Metagenomics analysis of the rumen's microbiome, by Gharechahi and Salekdeh (2018) exclusively identified the species belonging to the *Bacteroidetes* (56.3%), the *Firmicutes* (32.8%), the *Spirochaetes* (4.0%), the *Fibrobacteres* (2.2%), the *Proteobacteria* (1.4%), the *Lentisphaerae* (1.3%), the *Euryarchaeota* (0.4%) and the *Verrucomicrobia* (0.3%) collectively represented 98.7% of sequences (Gharechahi and Salekdeh 2018). The symbiotic organisms present in the rumen of ruminant animals facilitate the digestion of plant-based fiber. The diverse rumen microbiome contributes to the nutrition of the host animal by converting non-digestible biomass into readily absorbable compounds. This symbiosis in the rumen is particularly important for herbivores, which are unable to produce hydrolytic enzymes required for the degradation of recalcitrant lignocellulosic plant biomass endogenously that form a major component of their regular diet.

11.3.1 Physicochemical Properties of the Rumen

Ruminant herbivores such as sheep and cattle have reticulums (rumens), omasums (stomachs), and abomasums. In ruminants, fermentation takes place mostly in the rumen (Tharwat et al. 2012). Factors like pH, temperature, osmotic pressure, redox potential, and buffering capacity impact the rumen microbial community. Enzymes

produced by rumen microbes help ruminants digest cellulosic material (Aschenbach et al. 2011). Due to the fermentation heat created by the rumen microbiota, ruminant temperatures range between 39 and 41 °C (Wahrmund et al. 2012), rumen's pH is 5.5–7.0 (Krause and Oetzel 2006). Several factors affect the rumen's pH, including the feed consumed, saliva production, and absorption of short-chain fatty acids, as well as the ruminal epithelium's bicarbonate and phosphate exchange (Aschenbach et al. 2011). Approximately 250 mOsm/kg, the osmolality of ruminal fluid is controlled by the composition of the animal's food, as well as the fermentative products that are produced (Lodemann and Martens 2006). Under absolutely anaerobic circumstances, a wide variety of rumen microorganisms, including bacteria, protozoa, and fungus, coexist in symbiotic relationships with ruminants (Ozutsumi et al. 2005). Bacteria are the most sensitive to the rumen's physical and chemical features of the microbiota (McAllister et al. 1990).

11.3.2 Ruminal Bacteria

Ruminants like cattle, sheep, goats, and cervids have an enlarged gastrointestinal tract often called the forestomach. The forestomach comprises the rumen, reticulum, and omasum. The rumen of the ruminants is enriched naturally with anaerobic microorganisms like bacteria, fungi, and protists. The presence of these cellulolytic microorganisms enables rumen to function efficiently as a bioreactor for lignocellulosic conversion. Various bacterial genera are associated with the rumen ecosystem. The microbiome of rumen varies from host to host and largely depends on the diet of the animal; however the majority of the microbiome includes *Prevotella*, *Butyrivibrio*, *Ruminococcus*, *Bacteriodales*, and *Clostridiales* (Henderson et al. 2015). The competitive environment in the rumen is dependent on bacterial preference for certain substrates, energy requirements for maintenance, and resistance to toxic metabolic products (Russell et al. 1979). Rumen microorganisms ferment various substrates to release volatile fatty acids which in turn act as a major energy source for all the rumen microbiome. Various cellulose degradation strategies were identified which are followed by the rumen bacteria in a ruminal ecosystem (Flint 2008).

Nevertheless, the ruminant, (sheep, goats, and cervids) have an enlarged gastrointestinal tract often called the forestomach. The forestomach comprises the rumen, reticulum, and omasum. The rumen of the ruminants is enriched naturally with anaerobic microorganisms like bacteria, fungi, and protists. The presence of these cellulolytic microorganisms enables rumen to function efficiently as a bioreactor for lignocellulosic conversion. The microbiome of rumen varies from host to host and largely depends on the diet of the animal; however the majority of the microbiome includes *Prevotella*, *Butyrivibrio*, *Ruminococcus*, *Bacteriodales*, and *Clostridiales* (Henderson et al. 2015). Rumen microbiomes ferment various substrates to release volatile fatty acids which in turn act as a major energy source for all the rumen microbiome. Various cellulose degradation strategies were identified which are followed by the rumen bacteria in a ruminal ecosystem (Flint 2008).

Recent advancements in enzymatic studies helped find the cellulolytic functions in many bacterial species (Naas et al. 2014; Mackenzie et al. 2015; Dassa et al. 2014) including rumen bacteria. An extracellular multienzyme complex known as a cellulosome is produced by the firmicute *Ruminococcus flavefaciens*, one of the most researched rumen bacteria in terms of its production. The catalytic domains of this extracellular cellulosome are exposed to the substrate since it is connected to the bacterial cell surface. Dockerin-bearing enzymes often bind to numerous cohesin domains on a scaffolding subunit of a cellulosome (Bayer et al. 2004).

There are few rumen bacteria whose cellulolytic mechanism is not well understood. Cellulose depolymerization is a less understood system; it mostly involves attaching to the substrate through an unknown protein followed by cleavage of the cellulose polymer by a distinct set of cellulases (Wilson 2009; Suen et al. 2011; Ransom-Jones et al. 2014).

The rumen possesses a natural degradative environment composed of a genomically diverse set of microbiomes. However, rumen microbes are not easy to grow in pure form in the lab media, and hence the genetic information and other characteristics are not clear. Often, the underrepresented rumen microorganisms are missed in the culture, but they may contribute significantly to other surrounding microbiomes in these complex environments (Ley et al. 2008).

Despite the profound scientific research on rumen microbiota, still, a lot is unknown about their complex natural fiber utilizing engineering mechanism and their distribution. The rumen microbiome cellulolytic process has been the focus in this era. Multiple bacteria in the rumen form a complicated network that aids in the destruction and use of plant biofibers, resulting in the formation of fermentation products that are beneficial to the animal's health and well-being (Qi et al. 2010; Mizrahi 2013; Dassa et al. 2014). Researchers found a strategic player in the rumen ecosystem, which behaves similar to the most studied complex proteins (cellulosome) found in *Clostridium thermocellum*. The complex protein contains scaffoldin subunit which has modular proteins like CBM, which mediate interaction with plant fibers. Mainly in anchoring to the substrate and digestion, microbes in the rumen ecosystem break down plant material by hydrolyzing polysaccharides in the local environment using specific enzymes (Flint 1997; Flint et al. 2008; Mizrahi 2013). The principal degraders of plant fiber in this environment haven't yet been found among the few rumen plant cell wall-degrading bacteria.

In the rumen, the most active cellulolytic *Ruminococcus* and *Fibrobacter* species degrade cellulose (Qi et al. 2010). There have been in-depth studies on two *Ruminococcus* species: ScaA, ScaB, ScaC, and ScaE are all found in a single gene cluster in the genomes of multiple *R. flavefaciens* strains, whereas only one scaffolding is found in *R. albus* strains 7 and SY3 and none in strain 8 (Ding et al. 2001; Rincón et al. 2004; Rincon et al. 2005). This is in contrast to only one scaffoldin in *R. albus* strains 7 and SY3 and none in strain 8 (Ding et al. 2001).

About 22 amino acid residues of each Ca^{2+}-binding loop helix motifs are joined by a linker inside the dockerins, a small protein module. Bayer et al. (2004, 2013) and Haimovitz et al. (2008) provide comprehensive explanations of dockerins.

Ruminococcus species make up just a tiny percentage of the rumen ecology and are currently the only ones that transport cellulosomal components inside the rumen environment. The rumen ecology has a very effective fiber-degrading microbiome, as well as a few well-characterized cellulosome-producing bacteria (Dassa et al. 2014).

11.3.3 Rumen Protozoa

For 40–80% of the biomass-degrading microorganisms, protozoans are to be found, the majority of them belong to the Entodiniomorphida and Holotricha orders (Firkins et al. 2007; Yáñez-Ruiz et al. 2004). As most protozoa in the rumen are held in the diet, the rumen abomasum is unable to move these organisms readily (Hook et al. 2011). More than 90% of all cellulolytic protozoa come from a order known as Entodiniomorphida, which is very effective in both hydrolyzing and fermenting celluloses. The in vitro degradation of crystalline cellulose by cellulolytic protozoa of the genera *Polyplastron* and *Eudiplodinium*, and to a lesser extent by *Epidinium* (Fondevila and Dehority 2001), is quick and effective.

11.3.4 The Ruminal Fungi

Only 8% of the rumen microbiome is made up of rumen fungus; however, these organisms are critical to the digestion of the rumen (Nam and Garnsworthy 2007). It was discovered that some of the fungi have rhizoids that attach to the meal particles (Denman et al. 2008). Fungal populations in the rumen can be increased by feeding high-lignified fodder. When ruminants are fed a large amount of quickly fermentable carbohydrates, fungi in the duodenum, cecum, and feces are promptly eradicated (Grenet et al. 1989).

11.3.5 Cellulolytic Fungi

Hydrolytic enzymes, such as those produced by *Neocallix* species, *Piromyces* species, and orpinomyces species, are found in ruminant cellulolytic fungus. Compared to cellulolytic bacterial species, they were shown to break down structural polysaccharides more effectively in monoculture (Bernalier et al. 1992). To rapidly digest non-lignified tissues and fracture zones of lignified tissues by mechanical action, zoospores produced by cellulolytic fungi can adhere quickly to feed particles (Bernalier et al. 1992; Grenet et al. 1989). Ruminal fungi, therefore, play an important role in the digestion of lignin. Among the plant cell wall solubilizers that can open the cellulose to bacterial breakdown is the species *N. frontalis* (Borneman et al. 1991).

The cellulosome, a huge extracellular enzyme complex composed of a scaffolding protein and numerous associated cellulases, has developed in anaerobic microbes

to break down plant cell walls. Cellulosomes might be used in biotechnological applications such as the generation of high-value goods such as ethanol or organic acids from affordable renewable resources by converting cellulosic biomass into sugars. In vitro and in vivo cellulosome research is advancing rapidly, allowing for the creation of systems that can fulfill these objectives. Another sort of system originated in anaerobic microbes and involves in the creation of a huge extracellular enzyme complex termed the *"cellulosome"*, which includes a scaffoldin like protein and several attached enzymes.

11.4 Cellulosome

Raphael Lamed and Ed Bayer met at Tel Aviv University in the early 1980s and began working on the cellulosome idea. The first cellulosome was identified in the anaerobic thermophilic bacteria *Clostridium thermocellum (Current name: Acetivibrio thermocellus and homotypic synonyms: Clostridium thermocellum, Hungateiclostridium thermocellum, Ruminiclostridium thermocellum)*. This microbe *Clostridium thermocellum* had been isolated in 1920's by Viljoen et al. (1926) from manure and later described by McBee (1948) and completely sequenced at the DOE Joint Genome Institute. The *C. thermocellum* contains a unique extracellular enzyme system capable of breaking down insoluble cellulose into ethanol which is vital for biomass energy.

Cellulose, the most prevalent organic polymer on Earth, is degraded efficiently by these enzymes. A multi-functional integrating component (named scaffoldin) organizes the numerous cellulolytic subunits (e.g., enzymes) into the complex. Multiple endoglucanases, cellobiohydrolases, xylanases, and other degradative enzymes target diverse, insoluble cellulose substrates inside a cellulosome.

Before the cellulosome was discovered, cellulase systems of cellulolytic bacteria were seen as a collection of various enzymes in a free state (Stutzenberger 1990). This idea was based on prior research on fungus cellulases. For the past 50 years, aerobic cellulolytic fungi have been widely investigated for their free cellulases. Attempts to isolate free cellulases from anaerobic bacteria have previously failed. Several cellulosome-related signature sequences (i.e., cohesins and dockerins) have been identified in diverse bacteria and fungi, but most are dockerin-tagged enzymes. The principal cellulolytic ruminal bacteria and ruminal fungus have glycoside hydrolase (GH) genes (Flint 1997; Flint and Forsberg 1995; Selinger et al. 1996).

11.4.1 Cellulosome Components

- One or more cohesin modules can be found in the scaffoldin subunit and are linked to other functional modules. Scaffoldins may contain modules such as the CBM, dockerin, X modules of unknown function, the S-layer homology (SLH), or the sortase-anchoring motif, depending on the scaffoldin.

- Cohesin modules are the major building blocks of scaffoldins, which are accountable for organizing the cellulolytic subunits into the multienzyme complex.
- Dockerin modules link the catalytic enzymes to the scaffoldin, which is the primary building component of scaffoldins. Internally, the dockerin has a twofold symmetry consisting of an F-hand pattern that is repeated (a calcium-binding loop preceding a helix). Scaffoldins' C-terminus contains dockerin as well.

Catalytic subunits contain dockerin modules that serve to incorporate catalytic modules into the cellulosome complex. These catalytic modules include glycoside hydrolases (GH), polysaccharide lyases (PL), and carboxylesterases (CE) (https://www.weizmann.ac.il/Biomolecular_Sciences/Bayer/research-activities/cellulosome-systems; https://www.weizmann.ac.il/Biomolecular_Sciences/Bayer/research-activities/enzymes Accessed on 12 Dec 2021 Courtesy Ed Bayer's Group, Dept. of Biomolecular Sciences, Weizmann Institute of Science; and Demain et al. 2005).

Cohesin-dockerin interactions can be viewed as a kind of plug-and-socket mechanism in which the dockerin plugs into the cohesin socket. In general, the interaction is inter-species and intra-species (type) specific, although some cross-reactivity has been found in a few cases. The cohesin-dockerin interaction is one of the most potent protein-protein interactions known in nature, in most cases approaching the strength of high-affinity antigen-antibody interactions ($K_a \sim 10^{11}\ M^{-1}$). So far, cohesins have been phylogenetically distributed into three groups according to sequence homology; type I cohesin, type II cohesin, and the recently discovered type III cohesin. The dockerins that interact with each cohesin type are, by definition, of the same type.

11.4.2 Cellulosome Systems

Cellulosomes were discovered in other cellulolytic bacteria early on (Demain et al. 2005; Beguin and Lemaire 1996) and were not exclusive to *C. thermocellum*. A basic cellulosome system consists of a single scaffoldin, whereas a complex cellulosome system consists of many scaffoldins that interact with each other. The structure of the cellulosome depends on the arrangement of modules on the scaffoldin subunit and the specificity of cohesins and dockerins for their modular counterparts. The dockerin-bearing subunits are directly incorporated into the cellulosome complex by primary scaffoldins, adapter scaffoldins enhance the repertory or number of components in the complex, and anchoring scaffoldins link the complex to the bacterial cell surface by anchoring scaffoldins.

Cellulosome systems may be divided into two categories based on their level of complexity (simple and complex type). There is a single carbohydrate-binding module (CBM), one or more X2 modules and up to nine cohesins in the scaffoldins of simple cellulosome systems. It is the dockerin-bearing enzymes into the complex that are integrated by these major scaffoldins. The chemical mechanism behind the

cell surface association of simple cellulosomes is still a mystery in some situations. Cell wall attachment may be facilitated by the X2 module.

Different bacterial species have been shown to have complex cellulosome systems (Accessed 2020-12-12; Ding et al. 1999; Ding et al. 2000; Ding et al. 2001). The chromosome has "enzyme-linked gene clusters" that contain the genes for several cellulosome components. A complex cellulosome architecture is created when more than one scaffoldin interacts with one another in different ways. The cellulosome complex integrates enzymes directly into at least one kind of primary scaffoldin. The cellulosome complex is attached to the cell surface by a specific module or sequence of scaffoldins in each species. The "many scaffoldin gene clusters" are found on the chromosome in complicated cellulosome systems.

11.4.2.1 Regulation of Cellulosomal Genes

At the microscopic, physiological, and transcriptional levels, the variables that control cellulosomal gene expression have been studied. Polycellulosomes were found in protuberances in the early scanning electron microscopic examinations (Lamed et al. 1983). It was found that protuberances appeared in cellulose-grown cells, but not glucose-, fructose-, *cellobiose*-, or CMC-grown cells in research (Blair and Anderson 1999). When cells were cultured in cellulose rather than in cellobiose, *engB*'s relative levels of mRNA were greater, according to early transcription research (Attwood et al. 1994), indicating that the gene was transcribed as a single transcription unit.

11.4.2.2 Rumen Cellulolytic Bacteria and Fungi, Along with the Presence or Absence of Cellulosome

There are several cellulosome-producing anaerobic bacteria currently known, including *Acetivibrio*, *Bacteroides*, *Clostridium acetobutylicum* (a suspected but not proven cellulobacterium), *Clostridium acetobutylicum* (a cellulobacterium that has been shown to produce cellulosomes), *Clostridium cellobioparum* (a cellulobacterium that has been shown to produce cellulosomes), and *Clostridium josui* (a suspected but not proven *cellulobacterium*). If they're not linked to bacteria's cell wall, cellulosomes are free-floating extracellular complexes capable of degrading non-soluble substrates and transporting them to cells. *C. thermocellum*, *C. cellulolyticum*, and *C. cellulovorans* are some of the best-characterized cellulosomes, but their huge size and variability have hampered efforts to understand cellulosome structure and function. Others (such as *Acetivibrio* and *Ruminococcus flavefaciens* cellulosome systems) appear to be much more complex.

The following is a comprehensive list of all known cellulolytic bacterial species that utilize crystalline cellulose as their only carbon source in the process of hydrolyzing (Table 11.2). Continuous hydrolysis of at least microcrystalline cellulose like Avicel or better filter paper, cotton linters, and bacterial cellulose is required for "substantial" hydrolysis (e.g., release of reducing equivalents). A new taxonomy system published in *Bergey's Manual of Systematic Bacteriology* is superimposed on the phylogenetic tree produced from 16 S rRNA sequence calculations with the ARB software package (Garrity et al. 2001 and Garrity 2001).

Table 11.2 List of rumen bacteria and their cellulosomal presence or absence

Phylogeny	Genus species	Temperature	CS	References
Family: Lachnospiraceae	*Butyrivibrio fibrisolvens*	M	+	Berger et al. (1990)
	Ruminococcus flavefaciens	M	+ (a), (b)	Aurilia et al. (2000)
	Ruminococcus succinogenes	M		Fields et al. (2000)
	Ruminococcus albus	M	+ (a), (b)	Ohara et al. (2000a, b)
Family: Eubacteriaceae	*Eubacterium cellulolyticum*	M		Anderson and Blair (1996)
Family: Clostridiaceae	*Clostridium chartatabidum*	M		Kelly et al. (1987)
	Clostridium cellobioparum	M	+ (b)	Lamed et al. (1987)
	Clostridium-like species (T30)	H	+ (b)	Courtesy: PhD thesis work of Harish Kumar Reddy Y
	Clostridium-like species (CT2)	H	+ (b)	Reddy et al. (2010a, b)
Family: Fibrobacteriaceae	*Fibrobacter succinogenes*	M	+ (c)	Schellhorn and Forsberg (1984)

Note: Temp, growth temperature; m, mesophilic; h, thermophilic (growth optimum above 50 °C). m, mesophilic; h, thermophilic; CS, the presence of cellulosomes; evidence: +, present (a) presence of dockerin of cohesin sequences, (b) biochemical evidence (multienzyme complexes), and (c) presence of cell protuberances in electron microscopy

In rumen environments, fungi play a critical role in the conversion and consumption of plant biomass because of their powerful enzymes that degrade fibers and their invasive proliferation. Fungi that decompose cellulose in an anaerobic environment are the most common. Since the paradigm-shifting work in the 1970s concentrated mostly on rumen fungus, when he first defined anaerobic fungi, Colin Orpin discovered strange fungal phyla. According to Orpin (1975), due to the presence of the host's food components, the rumen fungus population density increases as a result of this stimulation. Many ruminant fungi, such as *Neocallimastix* sp., *Piromyces* sp., *Caecomyces* sp., *Orpinomyces* sp., and *Anaeromyces* sp., are involved in the breakdown of plant biomass. Cellulosome-bearing anaerobic fungi have been extensively explored in metagenomics studies with high-quality genomic assemblies (Haitjema et al. 2017; Youssef et al. 2013).

Anaeromyces robustus, *Neocallimastix californiae*, *Orpinomyces* sp., and *Piromyces finnis* are the most investigated rumen fungus with cellulosome. Fungi microorganisms have a similar role in anaerobic gut environments to their aerobic counterparts in soil and water. Fungi create colonies and produce extracellular enzymes that mobilize structural plant polymers to be accessible to other

microorganisms and the host, i.e. symbiosis, by holding themselves (fungi) to plant-based materials. Cellulolytic, hemicellulolytic, glycolytic, and proteolytic enzymes are all produced by anaerobic fungi, which are the microbes that use the most fiber (Ljungdahl 2008; Raghothama et al. 2001; Williams and Orpin 1987). The following sections deal with fungus cellulosomes. Fungal cellulosome enzymes and their domains (CAZyme) likely arose from bacterial enzymes via horizontal gene transfer (HGT). Similar structures in anaerobic fungi have been documented for many years by molecular biologists, which are known to assemble through sequence-divergent non-catalytic dockerin domains (NCDDs) (Haitjema et al. 2014). Many researchers are still interested in the cellulosome's components, modular assembly method, and functional purpose.

11.4.2.2.1 *Ruminococcus* Cellulosome

Bacterial cellulosomes are organized employing a special type of subunit, the scaffoldin, which is composed of an array of cohesin modules. The cohesin interacts selectively and tenaciously with a complementary type of domain, the dockerin, which is borne by each of the cellulosome enzyme subunits (Fig. 11.2). The integrity of the complex is thus maintained by the cohesin-dockerin interaction (Xu et al. 2004). The scaffoldin usually contains a module termed carbohydrate-binding module (CBM) which is responsible for the binding of the complex to the substrate. All cellulosomal components which are present on the same subunit are separated by linker sequences (Doi and Kosugi 2004).

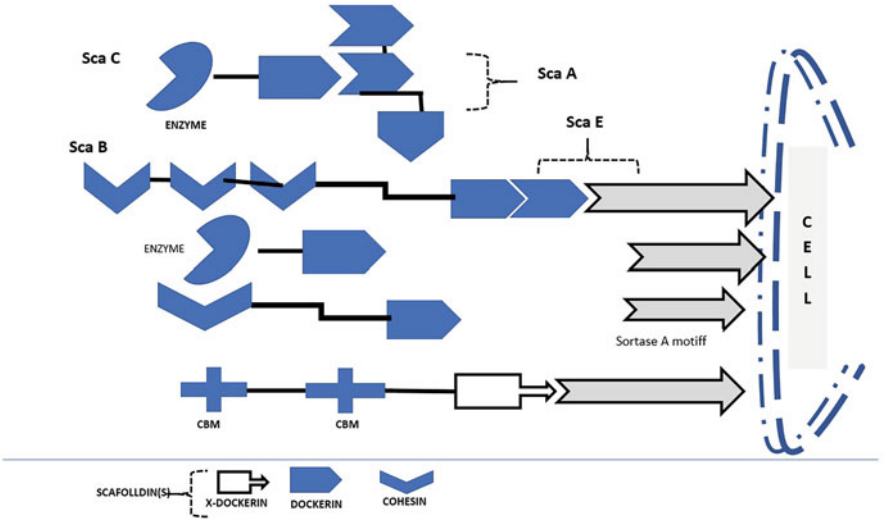

Fig. 11.2 Illustration of cellulosome architecture of *Ruminococcus* spp.: Overview of the modular interactions in the cellulosome system and outline of the cellulosome-related proteins in the designated strains of *R. flavefaciens* and *R. albus*

When scaffoldins were first characterized (Bayer et al. 1994), their cohesins were called type I cohesins based on sequence homology and were involved in binding cellulosomal enzymes containing dockerins known as type I dockerins (Bayer et al. 1998). Further studies showed that there were other cohesins, non-homologous to the type I cohesins, and these are known as type II and type III cohesins. Type II cohesins are found on anchoring proteins and bind to type II dockerin that are found on a scaffolding (Doi and Kosugi 2004; Leibovitz and Béguin 1996; Leibovitz et al. 1997; Lemaire et al. 1995).

The cohesin-dockerin interaction is crucial for cellulosome assembly. It mediates a set of interactions among the enzymes, scaffoldin, and anchoring protein which results in cell surface attachment of the cellulosome (Xu et al. 2003). The interaction between the cohesin and dockerin domains provides the definitive molecular mechanism that integrates the enzyme subunits into the cellulosome complex (Salamitou et al. 1994a, b; Lytle et al. 1996; Tokatlidis et al. 1993, 1991).

Cohesin and dockerin interactions within a species are not limited to specific pairings, evident from biochemical data (Gal et al. 1997; Yaron et al. 1995). It appears that the dockerins are all recognized in the same way by various cohesins. As a result, a single bacterium can produce a wide variety of cellulosomes, each with a unique makeup (depending on which enzymes are bound to the scaffolding). Likely, the increased functional display and the potential for enzyme synergism provided by this variety of cellulosomes may allow the bacterial cell to attack a wider range of substrates.

An anaerobic environment in the rumen is one of the most active places in nature for the digestion of plant cell wall components (Hungate 1966). Glycoside hydrolase genes have been discovered in the cellulolytic ruminal bacteria, ruminal fungus, and ruminal protozoa (Flint 1997; Flint and Forsberg 1995; Devillard et al. 1999). However, little is known about how ruminal microbes break down lignocellulosic material through the organization of enzyme systems. Prior biochemical and ultrastructural research has shown that *Ruminococcus* species have protuberances on their cells' surfaces that mimic cellulosomes in *C. thermocellum*, one of the most significant ruminal bacteria in the rumen (Lamed et al. 1985; Lamed et al. 1991; Lamed et al. 1987). In enzymes from *Ruminococcus*, dockerin sequences similar to those reported in *Clostridium* spp. were discovered more recently. *R. flavefaciens* 17 xylanases, cellulases, and esterases (Aurilia et al. 2000; Kirby et al. 1997) and *R. albus* cellulases are some examples (Ohara et al. 2000a, b; Ohmiya et al. 2003; Ding et al. 2001). A scaffolding-like protein and/or anchoring proteins may be present in *Ruminococcus* species that arrange enzyme subunits into such complexes, as evidenced by the discovery of dockerins in these enzymes.

Microbes in the rumen use specific enzymes to hydrolyze polysaccharides in the plant cell wall, breaking them down (Flint 1997; Flint et al. 2008; Mizrahi 2013). Rumen cell wall-degrading bacteria have only been found to be a secondary plant fiber degrader a few times. In the rumen, the most active cellulose-degrading bacteria are the cellulolytic *Ruminococcus* and *Fibrobacter* spp. (Qi et al. 2010). Researchers have studied *R. flavefaciens* and *R. albus* genomes in depth, finding that *R. flavefaciens* strains FD-1, 007c, 17, and others encode numerous interacting

scaffoldins, such as ScaA, ScaB, ScaC, and ScaE, which are contained in a single gene cluster, whereas *R. albus* strains 7 and SY3 encode only one scaffolding and strain 8 encodes none (Ding et al. 2001; Rincón et al. 2004; Rincon et al. 2005; Jindou et al. 2006; Miller et al. 2009; Dassa et al. 2014). Only a tiny portion of the rumen ecology is occupied by these species, and they are presently thought to be the only ones that transport cellulosomal components inside the rumen habitat.

11.4.2.3 Fungal Cellulosome

To decompose lignocellulose, bacteria, and fungi that are anaerobic produces cellulosomes, which are a protein scaffold bonded together by numerous enzymes working in concert (cohesin and dockerin). Large herbivorous mammals generally have anaerobic fungus in their rumens and hindguts, where up to 30% of their CAZymes are cellulosome-associated CAZymes (Henske et al. 2017).

To further understand cellulose depolymerization, I'm turning to fungus to see if they have cellulosomes that are similar to those seen in bacteria. To put it in perspective, the anaerobic fungal cellulosome is more comprehended than the bacterial one, which is why questions about its composition, design, and method for tethering enzymes persist to this day. Cellulosomes formed by bacteria and fungi are examined by Gilmore et al. (2015), who compare the present state of knowledge in this area, as well as their use in synthetic enzyme-tethered systems for tunneled biocatalysis. There are still many unanswered questions about the potential of fungal cellulosome-inspired systems, which have been emphasized by Gilmore et al. (2015) and Haitjema et al. (2017).

A family of repeat-rich, non-catalytic scaffolds in the genomes of five anaerobic fungi has recently been discovered by comparative genomic and proteomic confirmation (Haitjema et al. 2017). Gene sequences encoding components of fungal cellulosomes differ greatly from those of bacteria (Sunna et al. 2000; Haitjema et al. 2017; Gilmore et al. 2015); hence the structure of these domains is likewise different from that of bacterial cellulosomes. The predominant colonizers of plant material in the rumen microbiome are anaerobic gut fungus; however, they are rarely investigated due to a dearth of defined isolates in the field. Although most gut fungi have large rhizoidal networks, which are likely involved in the breakdown of plant cell walls, fungi of the *Caecomyces* genus lack these rhizoids. When it comes to plant cell wall hydrolysis, *Caecomyces churrovis* is one of the most diversified CAZyme-producing fungal isolates known to date, according to Henske and co-authors in a recent study (Henske et al. 2017; Gilmore et al. 2015).

Elucidating the type and location of fungal dockerins is essential for designing synthetic enzymes or synthetic fungal cellulosomes, as fungal dockerins exist at either the N- or C-terminus of proteins and in tandem repeats. In addition, compared to bacterial cellulosomes, fungal native cellulosomes offer a much wider variety of cellulases with dockerin domains, including GH3, GH6, and GH45 (Haitjema et al. 2017). As a result, the incorporation of novel enzymes into cellulosomes, such as those that increase activity and thermal stability, might be achieved by using fungal cellulosomes as templates for chimeric enzymes. The protease inhibitory effect of serpins led to their discovery as a protein superfamily with homologous structures

throughout all kingdoms of life. There are several different types of extracellular protease inhibitors, some of which have never before been seen in the *Dikarya* (such as serpins, which have been found in eukaryotic metazoa, but not previously in fungi) (Youssef et al. 2013). Serpins with dockerin domains have been found, confirming their cellulosomal location and their possible involvement in fighting plant proteases, as previously suggested. *Piromyces* sp. strain E2 cellulosome contains celpin (538 amino acids), a serpin protein with an unclear function (Steenbakkers et al. 2008). The fungal serpin is likely to have a role in protecting the cellulosome from plant proteinases because of the cellulosome's restricted location inside the plant tissue and the rumen's auto-proteolysis of plant material. *Piromyces* sp. strain E2's celpin protein is the first non-structural, non-hydrolytic component of a fungus cellulosome. In addition, the celpin protein of *Piromyces* sp. strain E2 is the first fungus to possess a serine proteinase inhibitor (Steenbakkers et al. 2008). Cellulosomes are structures extracellularly produced by an anaerobic fungus, which include scaffoldin-bound extracellular enzymes (Orpin 1994). The fungal dockerin domain (FDD), which is similar in structure to the carbohydrate-binding module family 10 (CBM10), is found in anaerobic fungi's cellulosome-bound genes (CBM10). According to Ljungdahl, incorporating GH3, GH6, and GH45 enzymes into anaerobic fungal cellulosomes increases their biocatalytic activity (Ljungdahl 2008). Fungal cellulosomes may be an evolutionary chimeric structure—a fungal complex that co-opts helpful functions from its bacterial neighbors in the gut microbiome. Fungal cellulosomes may directly convert cellulose to fermentable monosaccharides thanks to the extra β-glucosidase provided by GH3, whereas clostridial cellulosomes create low-molecular-weight oligosaccharides (Steenbakkers et al. 2003). Fungal cellulosomes may therefore be a mixture of enzymes from a variety of gastrointestinal fungi in their natural habitats (e.g., the microbial community of the herbivore rumen). This contrasts sharply with the great species specificity of bacterial cellulosomes (Bayer et al. 2004).

11.5 Metagenomic Approach for Biomass Utilization

By analyzing genetic material from environmental samples, metagenomics may access this biogenetic diversity without the need to culture cells. As a result, biotopes with high turnover rates of recalcitrant biomass, like lignocellulosic plant cell walls, have become a major resource for bioprospecting; further, this material is a major asset in the quest for alternative biocatalytic (enzymes) for various industrial processes, such as the production of biofuels from plant feed stocks. Metagenomics technologies have made significant contributions as a result of the identification of novel enzymes, although this young venture still needs a lot of work. These are the two most common strategies for screening metagenomes: function-based and sequence-based.

By cloning a gene and over-expressing it in a suitable host, scientists have traditionally found organisms that exhibit the required activity and then used that organism to find new enzyme diversity. Genomics has opened up a new avenue of

research (Ferrer et al. 2009). More and more people realize that the microbial world holds the most biodiversity in the biosphere; hence it will be microorganisms that will supply most of our enzyme diversity and novel uses. Microbes are notorious for their inability to be cultured (Amann et al. 1990; Zengler et al. 2002) which limits the use of standard methods for enzyme discovery. The "metagenomics" or "environmental genomics" techniques have been developed in response to the predicted rich enzymatic selections from the uncultured microbial community because they are based on novel genomics-based discovery methodologies. It is common for these methods to be referred to as "culture-independent" and "mined" organisms.

The race is on to discover the full extent of biocatalytic variety and usefulness. Accessing natural enzyme diversity, exploring enzymes' wider catalytic potential, and customizing and fine-tuning promising activity for applications are all part of this process (Ferrer et al. 2009).

Biocatalysts with improved performance and lower cost are needed in industrial processes to efficiently break down resistant plant biomass into fermentable sugars. In the metagenomes of natural microbial biomass decay communities, there may be enzymes that can break down biomass. For the discovery of new enzymes that degrade biomass and the evaluation of cellulolytic enzyme activity, metagenomics is a useful tool. Research into novel glycoside hydrolases (GHases) from microbial biomass breakdown communities, particularly those from previously unknown or farmed microorganisms, is becoming increasingly common (Edwards et al. 2006; Breitbart et al. 2003; Breitbart et al. 2002; Wegley et al. 2007; Angly et al. 2006; Breitbart and Rohwer 2005; Fierer et al. 2007).

Genome sequences from an actively decomposing biomass may be used to generate a metagenome expression library, which can then be used to test for new and promising GHases. The cloning that was efficiently expressed in *E. coli* was the outcome of these joint efforts. The cloned GHases that were effective and selective might be useful in the breakdown of biomass.

Cellulosome and glycoside hydrolase genes were compared and found that early colonization of fiber in the rumen microbiome appears to be driven by organisms that contain enzymes that target the more resistant main chains of complex plant polysaccharides, particularly cellulose. It is also possible that a diet-dependent differential in glycoside hydrolase content exists between the bovine rumen (forages and legumes) and the termite hindgut microbiota in terms of glycoside hydrolase concentration (wood). It has been shown that the fiber-adherent rumen microbiota of the bovine shows forage-specific glycoside hydrolases (Brulc et al. 2009).

Biomass degradation genes and genomes were discovered in cow rumen microbiota using 268 GB of metagenomic sequencing data produced in recent investigations. Biomass-degrading genes and genomes have been discovered in the rumens of cows (Jami and Mizrahi 2012; Hess et al. 2011). To maintain the rumen microbiome's equilibrium, each of the rumen's viral communities has a specific role. Rumen viral communities, on the other hand, are less well understood. Because of their sheer abundance (an estimated 1031 viral particles per square kilometer on our globe), viruses often go unnoticed despite their potentially devastating effect on human health (Breitbart and Rohwer 2005; Rohwer and Thurber 2009).

11.6 Future Perspectives and Conclusions

Amorphous and crystallized topologies of cellulose can be found, even if its chemical makeup is simple. Native cellulose is a difficult substrate for enzymatic hydrolysis because of its insolubility and variability. Altogether more than 71 genes code for cellulosomal components in the genome of *C. thermocellum*, almost all containing catalytic modules with some exceptions of proteins with structural or unknown functions. Most of the genes can be assigned a putative function. The list includes cellulases (both endo- and exoglucanases), xylanases/xyloglucanases, mannanases, pectinases, pectate lyases (PL), carbohydrate-esterases (CE), glycosidases, chitinase, and a mixed-linkage β-glucanase (Zverlov et al. 2006; Lynd and Zhang 2002).

A high number of endo-xylanases, xyloglucanase, putative β-xylosidases, α-arabinofuranosidases, and glucuronidase could be responsible for the effective degradation of the hemicellulose enwrapping the cellulosic crystals. Depolymerization is supported by esterases and debranching glycosidases located in the cellulosome. The structure of the genes shows some regularity, and almost half of the putative components bear a carbohydrate-binding module (CBM). Cellulose crystals are hydrolyzed by the synergistic action of processive and nonprocessive β-glucanases of GH families 5, 8, 9, and 48. Unexpectedly Cel8A seems to play a key role in cellulose hydrolysis (Schwarz et al. 1995; Zverlov et al. 2006).

To some extent, the rumen bacteria's ability to convert biomass into ethanol can alleviate some of the world's dependency on petroleum. Cellulolytic and saccharolytic *Clostridium* species bacteria may be co-cultured with agricultural and industrial waste to provide alternative energy sources that are both environmentally friendly and economically viable. When Reddy and colleagues experimented with agricultural leftovers, particularly banana waste with newly identified *Clostridium* sp. (CT2) and co-culture with the anaerobic bacteria in 2010, they were able to support this method. An ethanol-tolerant cellulolytic mesophilic strain was found in a decomposing paper by the author's team as well. There are many ways to maximize yields of bio-compounds and ethanol from rumen microbiota, and this chapter focuses on the cellulases of rumen microbiota, their presence in extracellular complexes or organelles (the cellulosomes), the binding of the cellulosome to cellulose, cellulosome genetics, regulation of their synthesis and co-culture, and other methodologies.

The nature's most abundant carbohydrate polymer—cellulose—is found in plant cell walls. Hydrogen-bonded crystalline fibers make it incredibly difficult to break down, although it is plentiful. The cellulosome, a huge extracellular enzyme complex composed of a scaffolding protein and numerous associated cellulases, has developed in anaerobic microbes to break down plant cell walls. There are several biotechnological uses for cellulosomes, including the manufacture of high-value products like ethanol or organic acids from cheap renewable resources via cellulosome-mediated sugar conversion (Carreira and Ljungdahl 1993). To attain these objectives, new in vitro and in vivo systems are being developed thanks to rapid advancements in cellulosome research.

Our understanding of the fundamental structure and function of the cellulosome system is now at the point where cellulosome researchers may make rapid steps toward the creation of several valuable biotechnological applications for cellulosomes. Plant fiber breakdown, which is a key function of the cellulosome machinery, opens up several possibilities for the development of novel recombinant molecules that may be used to create value-added products. Several factors have contributed to our understanding of cellulosomal mechanisms, including advanced biotechnological techniques, metagenomics data, and new database management systems. These factors, combined with the rumen microbiota's mutualism, have led to a paradigm shift in the study of the plant biomass utilization process in ruminants (Tringe and Rubin 2005).

Cellulosome-producing bacteria have been extensively investigated in this rumen habitat, which has a fiber-degrading microbiome. In this ecosystem, all of the modular proteins were allocated and participated in catabolic functions, as well as microbial interactions to a certain level. As the microbiomes store a great deal of information on mutualism and the use of refractory cellulose-based biomass, this sort of study is helpful to the next generation of researchers trying to understand evolutionary changes.

To date, studies have shown that the cellulosome has a wide range of complementary parts, all of which can interact and be involved in many extracellular functional activities in the rumen ecosystem. There is still much to learn about how these enzymes interact with other proteins from a physiological perspective in the targeted ecological niche. The rumen microbiome's operation can be better understood by looking at the role played by the cellulosomal machinery utilized by the rumen microorganisms. Fungal cellulosomes may have a selection advantage over bacteria in these conditions because of their plasticity, which suggests that fungal cellulosomes have numerous scaffoldins. As a result of this fundamental understanding of these unique components, biotechnological cellulosomes may be designed with greater efficiency.

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Metagenomic Approaches for Studying Plant–Microbe Interactions

12

S. Murali Mohan and Pola Sudhakar

Abstract

Plant microbiome from environmental samples, including soil rhizosphere, consists of all microbial genomes and plays an essential role in maintaining plant growth and health in addition to tolerating biotic and abiotic stresses and climate change. Plant microbiome is beneficial to the plant in many ways, such as nitrogen metabolism and enhancing plant growth-promoting (PGP) effects. It is also believed that plant growth-promoting microbes (PGPM) enhance plant growth by a variety of mechanisms such as enhancing soil nutrient bioavailability, disease resistance, damage due to herbivores, and improving water acquisition. However, the microbial composition is mostly influenced by soil factors, plant genotype, and exudates from the plants as well.

Moreover, the plant microbiome depends on the plant–microbe interactions and cultivation practices as well. Recently, more emphasis is on the study of underlying genes affecting plant–microbe interaction by high-throughput methodologies, including 16S rRNA marker gene sequencing and metagenome approaches for studying plant-microbiome interaction and microbial community in the plant surroundings. The metagenomic studies offer the possibility to explore the taxonomic composition of plant microbiome and its functional properties as well. Taxonomic analysis for amplicon sequencing is carried out using bioinformatics tools such as QIAMI and Greengenes database to identify operational taxonomic units (OTUs); however, in the case of whole-genome

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shotgun (WGS) sequencing, taxonomic classification is achieved using tools such as "Kraken." Recent advances in metatranscriptomics characterize members of the microbial community that are responsible for specific functions and identify the genes playing an essential role in plant–microbe interaction. Therefore, the present chapter focuses on reviewing the above molecular methodologies in detail for studying plant microbial community.

Keywords

Metagenome · Microbiome · Plant–microbe interaction · Sequencing

12.1 Introduction

Diverse microorganisms live in association with agriculturally important crop species and play an essential role in the survival, growth, and development of the host plant. The microorganisms enhance the productivity of crops in various ways, such as protection from phytopathogens, and serve as essential biocontrol agents (Kaushal et al. 2017). Microbes exist in all plants (Partida-Martinez and Heil 2011), and many of the plant characters such as nutrition optimization, resistance to biotic stress, and abiotic stresses associated with hormonal regulation are dependent on microorganisms.

There is also evidence that the microorganisms can optimize the endogenous hormonal balance of plants and activate mechanisms of systemic resistance to stresses (Kumar et al. 2012). The rhizosphere, soil around plant roots, forms an essential component of soil and harbors microbes, which influence through their biological, physical, and chemical interactions with the plant. Besides, the microbial community present in the soil is beneficial to the plants, and the interaction between plant and microbe can be beneficial to plant development. Therefore, it is required to study the microbial community of rhizosphere, as an understanding of plant microbiome (although plant microbiome includes entophytic bacteria as well), and plant–microbe interaction can provide valuable information.

On the other hand, the microbial community structure of plant rhizosphere seems to be dependent upon the strategies adopted by plants to combat pathogen attack as well as to overcome nutritional deficiency, for example, the legumes overcome nitrogen deficiency by releasing flavonoids that attract rhizobia which establish a symbiotic relationship with the host plant for fixing molecular nitrogen (N_2) for the plant. Similarly, *Zea mays* releases 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one that attracts beneficial rhizobacterium *Pseudomonas putida*. In the case of *Arabidopsis*, the plant releases malic acid from the roots when it is attacked by foliar pathogen *Pseudomonas syringae* and triggers host defense responses against *P. syringae* (Rudrappa et al. 2008).

Optimization of endogenous hormonal balance of plants and activation of systemic responses to stress has also been reported by Kumar et al. (2012). Therefore, plant microbial associations can be seen as mutualistic. For the first time, in

Arabidopsis thaliana, the microbial populations that were in contact with the rhizosphere region were studied using the metatranscriptomics approach (Chaparro et al. 2014). Chaparro et al. (2014) reported the correlation between root exudation pattern and that of the functional capacity of the soil microbiome and also the effect of root exudation pattern on soil microbial community. In this report, a metatranscriptomic analysis was carried out to establish a relationship between the functional properties of microbes in the rhizosphere region and different developmental stages of plant showing particular root exudation patterns.

A strong association between rhizobial microbial communities and root exudate composition was also reported in another study by Broeckling et al. (2008). Recently, Deng et al. (2019) also studied bacterial diversity using amplicon sequencing and shotgun metagenomic sequencing approaches and provided incites about the dynamics of community structure in strawberry. However, many of these microbial populations are not easily culturable through routine culture techniques, and culture-independent methods have to be developed to study diversity present in the environmental samples. In this context, next-generation sequencing (NGS) technology is considered a potential alternative to microbial culture techniques for studying microbial communities present in the environmental samples.

Nevertheless, the advances in sequencing technology have thrown challenges at the researchers in dealing with millions of reads produced from different sequencing platforms such as Illumina, Pacific Biosciences, and Oxford Nanopore that are available at present. However, a large number of bioinformatics tools were also developed in parallel to deal with these large datasets, and these tools helped immensely derive meaningful biological information from the enormous sequence data.

In this background, the present chapter focuses on reviewing three presently available molecular approaches (Table 12.1), such as amplicon sequencing (16S rRNA marker gene, for instance), and recently emerged metagenomic approach, which is an advanced method for studying microbial community occupying plant-soil interface (PSI). Besides, a brief overview of metatranscriptomic analysis is also provided in this chapter considering its potential for understanding bacterial communities in terms of regulation of complex molecular and physiological processes.

To begin with, amplicon sequencing approaches are frequently applied for studying plant microbiome structure and their distribution based on environmental samples (Knief 2014). The word "metabonomics" is also used to refer to marker gene sequencing (Breitwieser et al. 2017). The marker genes are invaluable tools for phylogenetic profiling as their distribution is uniform across the microbial world. The use of a phylogenetic marker was first explored by Stein et al. (1996), and the variable regions in the 16S rRNA gene serve as classification candidates (Claesson et al. 2010).

The conserved regions, flanking hypervariable regions, are targeted by primers and help in differentiating among microbial species. Besides, these variable regions are also uniform in their distribution in microbial populations (Bates et al. 2011). For primer pair details, please see a recent review by Lucaciu et al. (2019). The

Table 12.1 Molecular approaches for microbiome applications

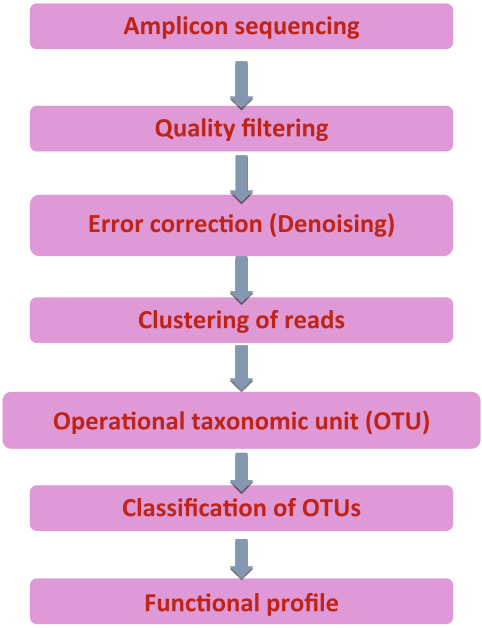
S. no.	Molecular approach	Advantages	Limitations	Important applications
1	Amplicon sequencing for 16S rRNA and 18S rRNA	Specific provides more accurate species identification, cost-effective, allows multiplexing, data interpretation is manageable	Targets 16S rRNA genes only, large bias in read counts, gene copy number, and primer mismatch	Species identification and taxonomic classification
2	Shotgun metagenomics	Sequencing of all genomic sequences facilitates functional profiling, identifies closely related species	Interference of host DNA depends heavily on a reference database, requires more number of reads	Microbiome diversity studies
3	Metatranscriptomics	Captures all of the RNA, representative of transcribed genes	Bacterial gene expression profile and understanding the regulation of complex processes	Gene expression profile including actively transcribed genes

significant advantage of amplicon sequencing is that it can efficiently target distinct groups of microbes such as archaea; however, the contamination poses a challenge to amplicon sequencing (Glassing et al. 2016). Apart from 16S rRNA for identifying bacterial species, fungal profiling is also carried out by a genomic region known as the internal transcribed spacer (ITS) region using improved primer pairs, as reported by Nilsson et al. (2019). A few other functional genes that are considered as phylogenetic markers used for amplicon sequencing studies include *dsrB*, *nifH*, *mcrA*, and *nxrB* (for more details, please see Lucaciu et al. 2019).

A few databases such as Greengenes (DeSantis et al. 2006), RDP (Cole et al. 2005), and SILVA (Carlton et al. 2002) serve as an additional resource for candidate marker genes for phylogenetic profiling.

The steps of marker gene profiling (Flowchart 12.1) briefly involves amplicon sequencing, quality filtering and error correction (denoising), clustering of reads into operational taxonomic units (OTUs) based on sequence similarity, classification of OTUs (Callahan et al. 2016), and defining the functional profile. In addition to amplicon sequencing, the microbial composition of the plant microbiome is also determined by high-throughput methods such as metagenome sequencing.

The metagenome represents the aggregate genomes of a community. The metagenomic analysis is an advanced whole-genome approach, otherwise referred to as shotgun metagenomic sequencing, which serves as an essential strategy to identify rare microorganisms in the environmental samples.



Flowchart 12.1 Steps showing amplicon sequencing

The first metagenomic approach dates back to 1991 when Schmidt and collaborators generated a metagenomic library from marine picoplankton (Schmidt et al. 1991). In the case of plants, several studies reported the use of metagenomic approaches for studying plant microbiome. For example, Mendes et al. (2014) carried out a study to explore the microbial populations in the rhizosphere region of soybean.

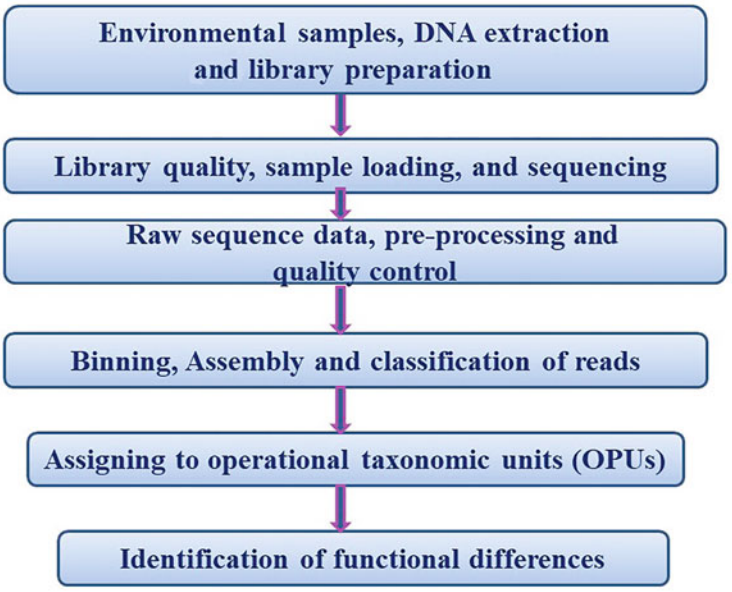
Kumar et al. (2018) "reported metagenomic analysis of rhizosphere microflora of oil-contaminated soil planted with barley and alfalfa; however, this study reported the hydrocarbon-degrading capabilities of microorganisms"). In another study (Chen et al. 2017), the role of the microbes in minimizing soil contamination was studied using a metagenomic approach. Similarly, Unno and Shinano (2013) reported the importance of bacterial populations in the rhizosphere and its effect on plant growth when there is a change in microbial composition by using a metagenomic approach. However, different plant species are inhabited by different microbial populations in the rhizosphere region due to a diverse range of root exudates (Haichar et al. 2008; Neeru et al. 2009; Saleem et al. 2018). Several other studies also reported that the rhizosphere microbial community could be shaped by plant host habitat, root exudates, and root architectural or phenotypic traits (Yurgel et al. 2017, 2018). However, there are certain limitations to the whole metagenome approach, i.e., very few datasets may be suitable for estimating taxonomic abundance (Kwak and Park 2018). As reported by Zaheer et al. (2018), sequencing depth also shows an impact on scientific inferences. The actual metagenome analysis also poses a challenge in relating gene repertoire of the whole metagenome with microbial

Table 12.2 Different software and databases used for metagenomic analysis

S. no.	Bioinformatics tools available	Action	References
1	NGS QC toolkit	Removal of low-quality reads	Patel and Jain (2012)
2	QIIME	Taxonomic assignment of amplicon	Kuczynski et al. (2011)
3	KEGG MGENES	Functional composition	https://www.genome.jp/mgenes/
4	Kraken	Taxonomic classification	Wood and Salzberg (2014)
5	MetaVelvet	Assembly	Namiki et al. (2012)
6	SOAPdenovo	Assembly	Li et al. (2010)
7	MegaHit	Assembly	Li et al. (2015)
8	Genovo	Assembly	Laserson et al. (2011)
9	MetaGeneMark	ORF prediction	Wenhan et al. (2010)
10	RAPSearch	Functional annotation	Yuzhen et al. (2011)
11	UPARSE	Operational taxonomic unit generation	Edgar (2013)
12	PIPITS	ITS amplicon processing	Gweon et al. (2015)
13	Greengenes	16S rRNA gene classification	DeSantis et al. (2006)
14	Silva	16S rRNA gene classification	Quast et al. (2013)
15	RDP	16S rRNA gene classification	Cole et al. (2014)

community structure. Therefore, a thorough understanding of metagenome analysis is required for establishing a relation between the microbial community and aggregate genomes coming from these microbes present in environmental samples. Moreover, the association between plant and microorganisms was also reported to be asymptomatic (Nissinen et al. 2019), which may complicate the understanding of plant–microbe interaction at times. Nevertheless, the metagenomic studies have been able to uncover the microorganisms which cannot be cultured in routine microbial culture techniques.

Several bioinformatics tools used for metagenomic analysis were reviewed in a recent report (Dubey et al. 2020); however, a few bioinformatics tools routinely used for the analysis of metagenomic data are provided (Table 12.2) in the present chapter. It is noteworthy that a few other molecular methodologies such as metatranscriptomics, metaproteomics, and metabolomics would also complement a metagenomic approach. The metagenomic approach aims to determine the composition of the microbial community in the targeted environment (i.e., the rhizosphere region in this case). The collection of soil samples from the rhizosphere region of the plants present in a temporary location and filed plot may be pooled to make a composite sample and consists of biological replicates. The workflow of metagenome analysis (Flowchart 12.2) involves the extraction of environmental DNA, NGS of environmental samples, and generating sequence reads from which microbial community profile can be elucidated using bioinformatics approaches. The raw reads obtained are initially pre-processed using tools such as FastQC, which



Flowchart 12.2 Showing steps in metagenome sequencing approach

trims low-quality sequences. The pre-processing also involves the removal of adapters and filtering the reads by quality and length and prepares the sequence data for subsequent analysis. After pre-processing, the next step involves classifying each read based on taxa using microbial genomes in the public domain as a reference.

Further downstream analysis explores the diversity of microbial taxa in the sample. Binning and assembly are two essential steps employed during the classification of reads. Binning is the process of genomic clustering sequences into groups for identifying each subset as a taxon. Besides, it may also be investigated about the functional profile of the genes present and expressed in the microbial community. Finally, functional differences between microbial communities are identified. However, as reported by Wu et al. (2018), the information related to the functional capabilities of root-associated microbes was limited. An alternative approach to the above process given in the workflow is that the reads are obtained after quality control is directly sent for taxonomic classification, which avoids assembly process (for more details, please see Breitwieser et al. 2017); however, this depends on the researcher choice.

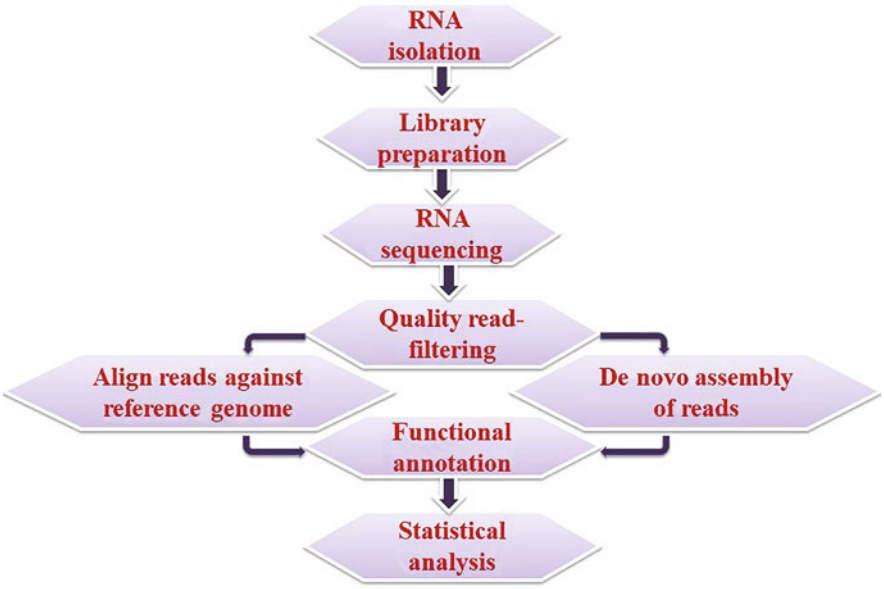
A metagenomic pipeline consisting of bioinformatics tools (Table 12.2) may be helpful in different stages right from improving raw sequences generated initially by the sequencing machines, subjecting them to quality control, and then assembling the sequences and annotation of assembled genomes. When marker genes (16s rRNA) are used for metagenomic studies, these marker genes identify clade-specific bacterial taxa across all samples.

The bacterial population belonging to phylum *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Cyanobacteria*, *Methylobacterium*, and *Flavobacterium* using (Suhaimi et al. 2017; Kumar et al. 2018; Wu et al. 2018; Fuentes et al. 2020; Manoj et al. 2020). However, comparative metagenome studies are useful in revealing environmental perturbations on community structure and community-wide shifts, as reported by Luo et al. (2014) and Simon and Daniel (2009).

The metabolomic profile associated with the microbiome may be a piece of evidence for dependence on environmental factors and provides valuable information about interactions of the microbial community with the host plant. The metagenomic approach not only offers a way to carry out culture-independent genomic analysis of microbial community but also allows identification of novel organisms that are uncultivably present in the environmental samples. The traditional laboratory culture techniques may not identify the microbes as many of them are non-cultivable (Chen and Pachter 2005) and are of anonymous genomic sequences (Aguiar-Pulido et al. 2016). The sequence reads used in metagenomic data analysis can also be downloaded from genomic resources available in the public domain (https://www.ncbi.nlm.nih.gov/genbank/metagenome/). The metagenomic datasets coming from different projects can also be submitted in this portal; for instance, users can register initially as "Environmental BioProject" before preparing the sequence submission to GenBank. If the metagenomics project involves the sequencing of 16S rRNA, it has to be designated as a Targeted Loci BioProject. The information about the project includes a description of the isolation source and the scope of the project. Later, in future correspondence, the user has to use the assigned BioSample ID(s) regarding the metagenomics project. The project allows the submission of either unassembled sequences to NCBI Sequence Read Archive (SRA) or contigs (longer sequences produced by short read assembly) as WGS project. As mentioned above, the raw data used for metagenomic analysis may include partial genomes, or in some cases, it includes supporting sequences such as 16S ribosomal RNAs.

Finally, the metatranscriptomics approach for drawing inferences between functional aspects of microbial communities and their relative abundance is briefly discussed. Metatranscriptomics provides a clue about the dynamics of plant–microbe interactions to characterize active microbes (Bashiardes et al. 2016), discovers novel microbial interactions (Bikel et al. 2015), and also gives an insight of how changes in the environmental conditions shape the microbial community. Metatranscriptomics is also used to characterize members of the microbial community that are responsible for specific functions and identifies the genes playing an essential role in plant–microbe interaction. The main steps involved in this method proceeds with mRNA enrichment, library preparation, and RNA sequencing (Flowchart 12.3). The quality of the data (reads) is improved further by filtering reads in terms of length and sequence quality to obtain quality reads for subsequent downstream analysis. Different workflows used for metatranscriptomics work were provided in a recent review (Shakya et al. 2019).

In summary, the knowledge related to recent molecular approaches, including amplicon sequencing, high-throughput metagenome sequencing and,



Flowchart 12.3 Showing workflow of metatranscriptome

metatranscriptomics, is routinely being utilized to study microbial population associated with different plant species and their functional role/significance at different plant developmental stages. However, this knowledge has to be combined with emerging aspects of the microbiome, such as metaproteomics and meta-metabolomics, to yield fruitful results in understanding various dynamics of the plant microbiome. Besides, it is also essential to leverage information from different training, workshop, and public outreach programs conducted in the field of the plant microbiome.

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13 Nitty-Gritty into the Plant Microbiomes: Understanding Microbial Niche Associations and Dynamics in Various Plant Parts

Gandham Sandeep Kumar, Sholapuri Payani, Pallaval Veera Bramhachari, and G. V. Swarnalatha

Abstract

The plant microbiome, which can be found in every accessible tissue of healthy plants, is made up of a diverse collection of microorganisms that are classified according to their taxonomy. Plants with plant-associated microbiomes have improved growth, nutrient uptake, stress tolerance, and disease resistance, among other characteristics. Microbes associated with plants show several genetic, biochemical, physical, and metabolic links, all of which have implications for plant health. Beyond identifying information gaps and potential initiatives, we are investigating how a plant's microbiome can be influenced by these interactions, which can then alter the plant's ability to absorb nutrients and stay healthy.

Keywords

Plant microbiomes · Microbiota · Abiotic · Agriculture

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13.1 Introduction

The composition of the plant microbiome is bacteria, fungus, protists, nematode worms, and viruses. In their natural habitats, these bacteria create complex connections with plants, increasing plant productivity and health. Some argue that plants and bacteria constitute a "holobiont," with plant and bacterial evolution symbiotic. This is yet to be confirmed. The bulk of species in complex plant microbiological communities has deep branching lines and poor phylogenetic resolution. High-throughput sequencing allows us to better understand plant and environmental microbes. The phrase "core microbiota" refers to a set of bacteria that are constantly found around a single host in varying conditions (Lemanceau et al. 2017). Plant-associated microorganisms include archaea, algae, and nematodes, as well as bacteria and fungi. Plant-associated microbiome genes have aided our understanding of bacterial adaptation to plants. Many questions remain unresolved about how plants and microorganisms interact in communities.

The microbiome of a plant contains beneficial, neutral, and even harmful microbes. Plant development, nutrient uptake, and disease resistance have all been linked to microbial populations (Gouda et al. 2018). Microbial communities related to plants have beneficial features, but these benefits cannot be anticipated from individual member traits. Many elements are at play here, including the dynamics of pathogen populations and their interactions with the plant microbiome environments (Trivedi et al. 2017).

Microbes can assist their host plants in several ways, including nitrogen fixation, water conservation, disease protection, antibiosis, and hydrolytic enzyme production. However, improving a plant's resistance responses gives an indirect benefit. Researchers believe that plant-associated microbe interactions are a way of producing new phenotypes that can survive in a variety of situations (Trivedi et al. 2020). This is because the microbiome of plants influences several plant properties. It is possible to create synthetic communities (SynComs) of microorganisms that assist plant growth in greenhouse or field conditions. A brief overview of plant microbiomes is provided in this chapter, as well as information on microbiome types appropriate for each plant part and also on cultivational, abiotic stress factors on the molecular structures of plant microbiomes.

13.2 The Microbiome Composition of Plants

The microorganisms in microbiota are found in tissues that are intimately linked to plants. In terms of mineral and nutrient uptake, tolerance to stress, immunological regulation, and resistance to disease, plant microbiomes promote the genetic and metabolic performance of the host plant (Liu et al. 2020). Algae, bacteria, fungi, oomycetes, and archaea are important plant microbiomes. The majority of research data is accessible on bacteria (bacteriome) and to a lesser extent on fungi (mycobiome). Plant-microbiome interaction offers both merits and disadvantages. Some microbiomes promote plant growth, whereas others are harmful and neutral

(Glick 2020). The plant microbiota comprises a diverse range of species that are transported horizontally and vertically through the soil environment and seeds, respectively (Trivedi et al. 2020). Plant productivity enhancement mediated by microbiomes is a superb platform for a modern green revolution.

Plant species have a vast range of microbiome compositions (Chaparro et al. 2014), genotypes (Bressan et al. 2009), and even in highly similar plants with minor genetic changes, such as transgenics (Badri et al. 2009). An organism's microbiome can be significantly altered by abiotic stress (drought, salinity, temperature, and UV radiation, among other things) (Lin et al. 2016).

13.3 Diverse Microbial Niches in Various Plant Parts

Plants are connected to the majority of microbes in the indigenous ecosystem, and they interact with a wide variety of systems. The highly varied plant system seems to have a better degree of microbial diversity (Mahnert et al. 2015). Decomposition of leaves and branches, as well as the discharge of root exudates, can affect microbial diversity (Millard and Singh 2010). Plants regulate multiple processes in this environment to attract or deter microorganisms from the bulk soil microbiome. The microbiological diversity of the bulk soil is directly related to the effectiveness of these selective mechanisms. The differences in properties of plant-microbe interactions in natural and agricultural ecosystems are depicted in Fig. 13.1.

Plant diversity represents varied root systems and exudate composition along with high microbial diversity. In a natural ecosystem, plants rely on microbiomes for nutritional supplements and protection. The agricultural field, on the contrary, has a low diversity of species of plants and a monotonous exhibition of roots and exudates. This lowers the microbiological diversity. A greater number of investigations are there on the microbiomes of plants found in forest ecosystems of extreme environments (Jorquera et al. 2016), in normal forest trees (Bonito et al. 2014), or in plants with different strengths such as *Arabidopsis thaliana* (Zolla et al. 2013) and medicinal plants. Recent data indicate that some core phytomicrobiome species are present in the majority of samples of a specified plant species, despite geographical and environmental situations (Hamonts et al. 2018). Plants are highly focused on

Fig. 13.1 Differences in plant and microbe traits and microbial interactions in natural ecosystems and agricultural fields

selecting the microbiota from the atmosphere (flower environment), the troposphere (external environment of fruits), and the phyllosphere (external environment of aerial plant parts and germinated seeds (Hardoim 2015). *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* appear to predominate in aboveground portions (branch, leaf, flower, and seed) (Steven et al. 2018). The largest reservoir of microbial habitats is the rhizosphere microbiome. *Planctomycetes*, *Verrucomicrobia*, *Acetobacteria*, and *Gemmatimonadetes* are the dominant bacteria in the root microbiome, according to previous research.

13.4 Microbiomes of Plant Parts in the Rhizosphere

13.4.1 The Microbiome of the Roots

Root-associated microbiomes have an important function in providing nutrition, immunity, and long-term viability in a tough environment (Fitzpatrick et al. 2018). Root microbiomes are thought to serve a similar and broad function in plant health and fitness as gut microbiomes do in humans, including the supply of nutrients, defense from pests and diseases, and adaptability to stress (Singh et al. 2018). The plant primarily selects the root microbiomes by structural reforms and by secretion of carbohydrates, amino, and organic acids through the root exudation. The rhizobiome serves as a magnet for soil bacteria, attracting or repelling them to the plant. The relationship between soil bacteria and roots is regulated by root exudates. Monosaccharides (fructose, mannose, and glucose), disaccharides (maltose), five carbon (arabinose), and oligosaccharides are among the sugar molecules found in root exudates. Microbes, on the other hand, select plants based on their expandability and capability for resources, as well as their potential to offer tolerance to abiotic and biotic challenges (Trivedi et al. 2020). The amino acids; organic acids such as benzoic, ferulic, and ascorbic acids; and some tannins, flavonoids, steroids, terpenes, etc. are found in exudates of roots and play an imperative role in microbiome selection and recruitment.

Because of their role as a microbial seed bank for plants and interactions with soil, root-associated microbiomes (e.g., endosphere) are extraordinarily diverse (e.g., endosphere). In-plant roots, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*, are prominent microbial components (Hacquard et al. 2015). Several studies of root microbiomes have confirmed the enrichment reports mentioned above and the reduction reports on plant root microbial taxa including maize, *Arabidopsis*, lettuce, barley, sugarcane, and rice (Cordero et al. 2020). The schematic representation of communities of microbe fluctuation within plant root and different parts of soil is shown in Fig. 13.2 (Glick and Gamalero 2021).

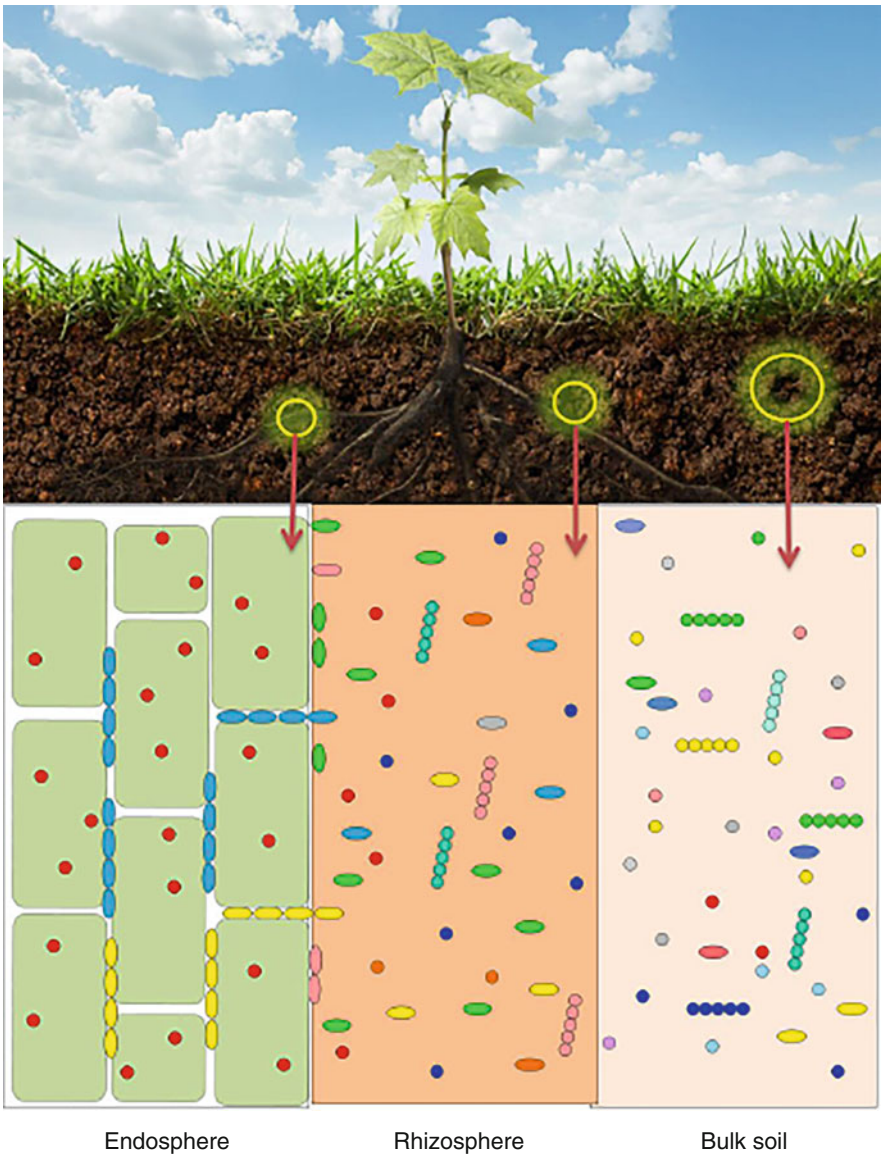

Fig. 13.2 A schematic representation of several microorganisms found in the endosphere, rhizosphere, and bulk soil of plants (Glick and Gamalero 2021)

13.4.2 The Microbiomes of Aboveground Plant Components

The phyllosphere of plants has diverse microorganisms. This diversity depends on the exudation of trichomes, wax layers, and secondary metabolites of the plant (Remus-Emsermann and Schlechter 2018). The exterior portion of the leaf, flower,

stem, and their endosphere makes up the microbial habitats. The nectar microbiomes, which interact with pollinators, are shown to impact pollinator behavior and health and are thus gaining more study interest (Liu et al. 2019). Surface phyllosphere microbiome cultivar-dependent selection can be affected by abiotic variables such as humidity, increasing radiation levels, temperature fluctuations, nutrient accessibility, and genetic composition of plants in both direct and indirect ways (Singh et al. 2019). Enzymes, phytohormones, biocontrol agents, and other metabolic products generated by seed-borne endophytes enhance the productivity and efficiency of a plant under stressful circumstances (Mukherjee et al. 2020b). The restoring seed microbiome results in the improvement of plant growth (Mukherjee et al. 2020a).

13.4.3 Can Seed Microbiomes Influence Plant Health?

Seeds are the most important products in most farmed crops, beneficial for human consumption as well as for crop cultivation. The seed microbiome contains a vast range of microbial species that can influence plant health through symbiosis, interspecific cooperation, commensalism, and harmful interactions (Links et al. 2014). Individual plant species have particular microbial species on a surface within the seed that act as a microbiome pool for the plant's endophytic microbiome. The predominant phyla of microbial groups mentioned above can colonize seeds. Seed microbiomes vary in richness of microbes and composition based on numerous environmental factors, including abiotic and biotic factors (Klaedtke et al. 2016).

Phyla *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* evolved a vast spectrum of microbial taxa from their original seeds (Johnston-Monje et al. 2016). Alfalfa and *Cucurbita pepo* seed microbiomes are dominated by *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, according to other studies (Adam et al. 2018). Plant species, soil, and environmental conditions all influence seed microbiome formation (Nelson 2018). Endophytic mycobiota for germinating seeds is derived from seed fungal endophytes, which are dominated by *Ascomycota* (Raj et al. 2019). According to Vujanovic et al. (2019), seed microbiome components can be transferred to direction perpendicular from one generation to the next, but the mechanism of seed microbiome congregation and the ecological process of seed microbiome remain a mystery.

Enzymes, phytohormones, biological controls, and other byproducts generated by seed-borne endophytes improve plant productivity and effectiveness under stressful environments. Previous research has shown that restoring seed microorganisms in plants with different plant growth-promoting treatments can boost the plant development in chickpeas (Mukherjee et al. 2020a). A great deal of potential exists in the use of seed microbiome to manage abiotic or biotic challenges and, as a result, boost crop output. According to a recent discovery, flower inoculation is a well-developed method for testing scientific questions and in the future also for exploiting microbiomes of seed for long-term agriculture (Mitter et al. 2017).

13.4.4 Flower Microbiomes in Plant Reproductive Success

The environment of flower microbiomes has a critical role in the persistent viability of essential ecosystem facilities, as well as with reproductive potential of plant and communication with useful insects. Flower-associated microbial communities, on the other hand, are far less linked to the microbiome of the phyllosphere (Massoni et al. 2020). The flower microbiome's heterogeneity and diversity are far lower than that of the phyllosphere microbiome. Owing to the nutrient-rich and excessive osmotic potential of the nectar present in the flower, *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* are the most common taxa detected in floral microbiomes, which differ from root and leaf microbiomes. Aside from that, the flower has an open habitat that allows microorganisms, both pathogenic and plant probiotics, to spread through insects and other animals (Kim et al. 2019).

Floral microbiomes also provide insight into the overall ecology of microbial community which can be used as models to study microbiological construction and survivorship. Finally, knowing the repercussions of flower microbial communities throughout the plant's entire lifecycle, from inflorescence to fruit to endosperm and thereafter to a normal adult, will help researchers determine whether vertical flower microbiota transmission is feasible and prevalent, as well as which floral communities behave as native reservoirs for microbiota of plant in general.

13.4.5 Leaf Microbiome Modulates the Host Plant Immune System

The microbiome found in the leaf is an important element of the plant that regulates transpiration and photosynthesis which inturn on plant development and expansion. As a result, the microbiome on the leaf can modify the plant's functional qualities to protect it from changing surroundings and weather patterns. Toju et al. (2019) discovered that *Alphaproteobacteria* (*Sphingomonas* and *Methylobacterium*) exhibited more controlling leaf-associated bacterial associations in the tomato plant, while Bai et al. (2015) discovered that *Gammaproteobacteria* (*Pseudomonas* sp.) proved more controlling bacterial leaf associations in non-cultivating and cultivating plants. In most leaf microbiomes, the phylum *Proteobacteria* has been found. Leaf microbiomes of rice plants are identified as *Burkholderia*, *Pseudomonas*, *Xanthomonas*, and *Mycoplasma* (Roman-Reyna et al. 2020). *Epicoccum* was discovered to be the most abundant genus among ancient plants. In the microbiome of tomato leaves, ascomycete and basidiomycete fungi were shown to be more colonized. Similarly, the fungus taxa *Moesziomyces*, *Hannaella*, *Cladosporium*, and *Dioszegia* were found in abundance in the tomato leaf microbiome (Toju et al. 2019). Leaf microbes are resistant to abiotic stress factors (Crombie et al. 2018). However, the overstressed and degraded state of the leaf renders these environments selective to specific bacteria. Colonization development may be dependent on a range of microbial activities, including the ability to take nutrients and creation of biofilm from the environment (Streletskii et al. 2019). Plant fitness is influenced by leaf microorganisms under abiotic stress circumstances such as harmful UV

radiation, oxidative stress, and dehydration, and they can use vitamins on the surface of leaves (Yoshida et al. 2019). The aboveground tissue plant growth and immune system of the host plant are modulated by leaf microbial communities (Stone et al. 2018).

13.5 Root-Shoot Circuit Microbiota Promotes Plant Stress Resistance

Endophytic bacteria have been found in the shoot material of several plants, according to research. Communities of *Sphingomonas*, *Methylobacterium*, and *Curtobacterium* are more limited in sugarcane stalks, according to Hamonts et al. (2018). The biomass of *Sedum alfredii* shoots increased twofold after vertical transmission in irradiated soil, according to Luo et al. (2019). *Sedum alfredii* shoot biomass is significantly connected to a dominant set of microbial linkages from *Streptomycetaceae*, *Nocardioidaceae*, and *Nocardioidaceae*. To spread vertically, these endophytes can grow primarily in the shoot meristem of newly generated shoot tissue (Shahzad et al. 2018). Bidirectional microbiota-root-shoot interactions are expected to play a substantial impact on plant health because plant root microbiota governs the host development and immunological systems (Hacquard et al. 2015). Researchers (Stassen et al. 2021) predict that by year 2020, there will be a prominent enhancement in the number of people with autism spectrum disorder (ASD). Root-shoot microbiota circuit helps plants cope with stress to get a better grasp of how signals from microbes in the soil and the environment aboveground interact to influence plant behavior.

13.6 Plant and Microbe: Molecular Interactions

There are both living and nonliving elements in bulk soil that interact frequently to preserve ecosystem equilibrium. Rhizosphere shows interactions between two plants or two microbes and between a plant and microbe (Richardson and Simpson 2011). Decomposition of organic debris, recycling of nutrients, toxicity removal, suppression of pathogens, and noxious species are all facilitated by the soil microbiome (Singh 2015a, b). A symbiotic and defensive link between plants and bacteria is commonly regarded as a result of molecular signaling. Both plant- and microbe-derived signaling molecules are implicated in plant-microbe interactions. As a result, the signaling molecule consists of both primary and secondary metabolites (e.g., carbohydrates, proteins, and organic acids). Bacteria and fungi release substances like auxins and cytokines that affect cell proliferation and root system architecture, resulting in increased water absorption and lateral root hair growth (Ortíz-Castro et al. 2009). Acyl-acetoin and 2,3-butanediol are the bacterial volatile chemicals that act as the signaling molecules for plant-microbial communication and hence stimulate plant growth promoters (Ortíz-Castro et al. 2009). Bacterial exudates emit citrate, oxalate, and malate, which work as detoxifiers in the rhizosphere to remove

aluminum toxicity. The plant's ability to endure aluminum toxicity is enhanced by the organic acids produced by the bacterial community (Ma et al. 2001). Lipopolysaccharides are produced by the plant as a result of the release of flavonoids, which communicate with the rhizobia-legume signal transduction pathway (2010). *Fusarium oxysporum*, a soil-dwelling plant pathogen, grows in the roots of the tomato host plant when class III peroxidases are active, as shown by Turrà et al. (*Solanum lycopersicum*). There are a wide variety of signaling molecules and their specific roles in microbial communities, which enhances the communication between plants and microorganisms.

13.7 Impact of Plant Microbiomes on Biological and Non-Biological Variables

13.7.1 Pathogens

Plant phytoconstituents reduce plant infections, acting as antibiotics that inhibit the diseases' growth and reproduction. Many bacterial and fungal diseases, larvae, and insects are vulnerable to phytohormones and phytoconstituents in recent times. By reactivating dormant microbiota and producing phytohormones, these plant microbiomes are involved in offering protection against infections and biotic stress (Figuerola et al. 2015). Resistance to the fungus *Gaeumannomyces graminis* was established by inoculating barley with *Pseudomonas* species, which function as antagonists and protect the plant from infections (Rodriguez et al. 2019). *Rhizophagus irregularis*, an AM fungus, increased *Medicago truncatula*'s resistance to *Xanthomonas campestris*, and rhizobia enhanced its resistance to *Erysiphe pisi* (Smigielski et al. 2019).

13.7.2 Abiotic Stress

Microbial communities subjected to natural environmental conditions affect the evolution of microbiomes in varied forms (Tripathi et al. 2017). There were distinct microbial communities in the rhizospheres of plants grown in different climates. Arid ecosystems, on the other hand, lack diversity in taxonomic and functional variety. The microbiota of the rhizosphere differs significantly among soil types and nations, and these differences in taxonomic richness and structure were found to be exacerbated by the effects of environmental alterations on diverse microbiomes. The pH of the soil was found to be a significant factor in taxonomic diversity and structure (Simonin et al. 2020). Abiotic factors that affect microorganisms, such as drought, are critical. As a result of soil osmotic stress, nutrients are unable to move freely, and oxygen cannot get to the soil. *Actinobacteria* and *Chloroflexi* are among the microbes that are more prevalent in the rhizospheres of drought-resistant plants, but *Acetobacteria* and *Deltaproteobacteria* are less prevalent. A rise in the number of *Acetobacteria* was seen in the root microbiome when drought circumstances

prevailed (Fitzpatrick et al. 2018). In part, it is due to a decrease in the affluence of antagonistic microbes such as *Streptomycetes*, *Micrococcaceae*, and *Mycobacteriaceae*, which change their microbe community and reduce their disease suppression rate. The bacterial community in the microbiome is profoundly affected by temperature variations, which aids in disease suppression (van der Voort et al. 2016). Radiation changes leaf bacterial populations by damaging the DNA of microbes. Some bacteria can withstand high levels of ultraviolet radiation due to the existence of pigments and mucopolysaccharides and the production of spores (Kumar et al. 2019).

13.8 Sustainable Agricultural Practices for Enhanced Productivity

Plant microbiomes are constantly changing as a result of regular agricultural practices. Changes in soil properties are to blame for this phenomenon. Directly stimulating or inhibiting the microbiota's activity, depending on dietary preferences, is one way it can influence it while trying to interfere with the way plants select microorganisms is another (Cai et al. 2016). The protection of microbial flora is becoming increasingly important as sustainable technology gains traction around the world. Plant microbial communities need to be studied extensively to understand their importance and influence on their structure. The bacterial microbiome of Yebra mate is altered by the use of agroforestry and the application of the green manure system, whereas monoculture cultivation results in an abundance of fungal microbiome development (Bergottini et al. 2017). Plant microbiomes are frequently altered as a result of routine agricultural practices. Soil characteristics change as a result of this effect. Depending on the diet, it can either stimulate or impede the microbiota's activity, or it can interfere with the way plants select microorganisms indirectly (Cai et al. 2016). There is a pressing need to protect microbial flora as sustainable technology takes the root in agriculture around the world. Plant microbial community structure must be studied extensively to understand its importance and influence. While the Yebra mate bacterial microbiome grows in abundance when grown in a monoculture, the agroforestry systems and green manure usage change the bacterial microbiome structures (Bergottini et al. 2017).

In comparison to the usual cultivation system, the use of organic compost and rotational cultivation for crops enhances the phylogenic, microbial, and bacterial diversity of the soil. *Fusarium* pathogens are inhibited by the use of mulch in the potato's rhizosphere, which increases fungal diversity (Qin et al. 2017). Nitrogen fertilizer application is a common practice in agriculture, and understanding the microbiome's response to nitrogen fertilizer application is critical. *Bacillales*, *Rhodocyclales*, and *Nitrosomonadales*, all of which play a role in nitrogen cycling, are impacted by nitrogen fertilizer use (Zhu et al. 2016). For more than a decade, long-term monoculture alters the structure of soil microbiomes. A decline in *Firmicutes* in non-rhizospheric soil was observed in studies on black pepper conducted over a four-decade period that showed monoculture increased bacterial

phyla levels in the rhizosphere (Li et al. 2016). Beneficial bacterial population reduction is depicted to have increased rhizosphere *Fusarium* population. Crop rotation between black pepper and banana has been shown to reduce *Fusarium oxysporum* pathogens while increasing the microbiome structure composed of *Gemmatimonas*, *Sphingobium*, *Sphingomonas*, *Penicillium*, and *Chaetomium*. Seed microbiomes alfalfa (Lopez et al. 2017) and *Cucurbita pepo* (Adam et al. 2018) were found to be predominated by *Proteobacteria*, *Actinobacteria*, and *Firmicutes* in other studies (Fig. 13.2). Soil, environmental conditions, and plant species all influence the seed microbiome's composition (Nelson 2018). Sugar beets have a very limited microbial genetic diversity, whereas wild plants have a much more diverse bacterial community (Zachow et al. 2014).

13.9 Future Perspectives and Conclusions

We can boost a plant's productivity by inoculating the soil with a microbial community that we know everything about, thanks to our detailed knowledge of its microbiome. Cultivating a variety of plant species in the same location is one traditional method of increasing microbial diversity. The microbiota of plants is essential for their survival in addition to the basic nutritional needs of plants. When cultivating and harvesting plants, the plant mycobiome is one of the most important aspects to consider. Under both living and nonliving stress conditions, the plant's microbial community provides protection. To better understand and help plants, more research into the microbiome interactions is required. Plants need microbiota in addition to their basic nutritional and other needs to thrive. Cultivated and wild plants alike must consider the plant mycobiome. Plant defenses are assisted by the microbiome in both biotic and abiotic stress situations. To better understand and improve the lives of plants, researchers need to study the interactions between plant microbiomes in greater detail.

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14 A Conceptual Framework to Explore the Functional Implications of Coral-Associated Microbiomes and Their Role in Promoting Plant Growth

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Abstract

Bringing food to the plates of 7.8 billion people is a challenging task to accomplish, especially in the present situation with limited land resources, water resources, and implications of global climate change due to depletion of soil and environment. Soil health management is an important agenda for preserving the biotic components of terrestrial ecosystem as it is for sustainable agriculture. A key for sustainable soil management is to minimize the use of chemical fertilizers and pesticides, practice crop rotation, and fortify soils with organic matter and microorganisms. The use of organic fertilizers and biofertilizers are highly appreciated in agricultural practices than chemical fertilizers. Today, biofertilizers derived from microorganisms are available in the markets across globe because of the large input of research invested in studying plant growth-promoting microorganisms. Though much interest has been spent on assaying microorganisms of terrestrial origin for land-based applications, the latest studies

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have come up with findings advocating the remarkable potential of microorganisms from extreme ecosystems like mangroves and oceans. Coral reefs are home to a variety of useful microorganisms that have important ecological functions and can synthesize biologically active molecules. Compounds derived from corals and their microbiota are being extensively studied for biomedical applications. Microorganisms isolated from corals have been reported to hold a plethora of land-based applications including bioremediation, alleviation of saline stress in plants, antimicrobial activity, and antibiofilm activity against phytopathogens. This book chapter elucidates the applications of coral microbiota for improving soil quality and plant growth.

Keywords

Coral · Microbiota · Antimicrobial · Composting · Bioremediation

14.1 Introduction

Soil is an essential component of the biosphere that supports the growth of plants and microorganisms eventually, supporting the existence of all life forms in the terrestrial ecosystem. Anthropogenic activities have inflicted a decline in soil productivity, mainly due to land clearing, desertification, extensive farming, environmental pollution, and salinization of soil (Oldeman 1994). The use of chemical fertilizers was a chief contributor of nitrogen and phosphorous in soil, consequently increasing global food production in the past 50 years (Abd-Alla et al. 2014). The rigorous use of chemical fertilizers to enhance productivity has not only reduced the fertility of soil but also has threatened the existence of certain beneficial insects and contaminated water basins, ultimately damaging the ecosystem (Mishra et al. 2013). Poor management of land cultivation has even caused changes in hydrological cycles and increased greenhouse gas emissions culminating in ozone depletion and global climate change (Bengtsson 1998). With increased human population by 2050, it is predicted that the availability of fertile land becomes lower with resultant food security (Ibarrola Rivas and Nonhebel 2016). Thus, it is of utmost importance that we preserve soil quality by boycotting chemical substances for agricultural practices and adopt biological measures promoting soil sustainability.

Many researchers have found biofertilizers derived from microorganisms of rhizosphere soil effective to prevent infectious diseases of crops and improve soil quality (Kloepper 1978; Penrose and Glick 2003; Kang et al. 2014). The use of biological fertilizers has also been proven to improve saline stress response, photosynthesis, and mineral absorption of plants (Golpayegani and Tilebeni 2011). The mechanism of action behind the plant growth-promoting effects of biofertilizer includes production of indole acetic acid (Tsavkelova et al. 2006), decrease in ethylene production in plants (Glick et al. 1998), phosphate solubilization (Jorquera et al. 2008), and enhancement of nitrogen fixation (Zahran 2001), synthesized hydrogen cyanide (Rezzonico et al. 2007), siderophores (Carrillo-Castañeda et al.

2002), and antibiotics against pathogens (Whipps 2001). The implementation of biological control methods in agriculture is safe, environment-friendly, and most importantly cheaper, making it available for small-scale farmers (SubbaRoa 2001).

Composting is another environmentally benign choice to replace chemical fertilizers. Composting can be used to replenish organic matter of degraded soil and improves carbon sequestration (Pergola et al. 2018). Composting influences the environment in a positive manner by decreasing greenhouse gas emissions (Luske 2010) and reducing carbon footprint (Schwarz and Bonhotal 2018).

Several pieces of research have investigated the beneficial roles of microorganisms isolated from marine grooves to marine environment for crop cultivation (Rashad et al. 2015; Suksaard et al. 2017; Gong et al. 2018; Nafis et al. 2019). Very few reports in the literature have stated the potential application of microorganisms belonging to the coral microbiota for land cultivation.

14.2 Coral Reefs and Their Microbial Community

Corals are regarded as the tropical rainforests of marine ecosystem since they harbor one of the world's divergent ecological communities. They are crucial components of the marine environment as they maintain nutrient cycling and carbon-nitrogen cycle (Rädecker et al. 2014). The coral reefs accommodate a vast number of microorganisms which can regulate the ecosystem as well as physiology of the hosts. Microorganisms such as bacteria, fungi, algae, and protists together make up the microbiome of corals.

14.2.1 Bacterial Community

The bacterial composition of corals varies from species to species and remains similar in the same coral species isolated from different spaces (Rohwer et al. 2001) and at different time (Meenatchi et al. 2020). 16S rDNA sequencing studies revealed that bacterial groups in corals were in the order: γ-*Proteobacteria*, α-*Proteobacteria*, *Bacillus*/*Clostridium*, *Cytophaga-flavobacterium*/*Flexibacter-Bacteroides*, cyanobacteria, and some other bacterial groups which were not identified (Rohwer et al. 2002). Bacteria of corals regulate nitrogen flux as they have experimentally identified to own genes for nitrogen fixation (Lesser et al. 2004). Coral bacterial community interactions are crucial for the health of corals, and factors that interrupt these interactions will help find new ways to preserve corals.

14.2.2 Archaeal Community

Archaea from coral most notably participates in conversion of ammonia to nitrite (Francis et al. 2007). Archaea belonging to phylum *Crenarchaeota* and

Euryarchaeota are distributed among several species of corals without exhibiting any species-specific diversity (Wegley et al. 2004). Members of archaeal community such as *Halococcus salifodinae* can carry out denitrification of nitrite into nitrogen under anoxic conditions at night (Siboni et al. 2008). The sequences of ammonia monooxygenase subunit A (*amoA*) genes in archaea of corals were phylogenetically distantly related to those procured from coastal sediments (Beman et al. 2007). Culturable archaea from marine ecosystem capable of oxidizing ammonia was reported by Könneke et al. (2005) which gives hope in identifying culturable archaea from corals too.

14.2.3 Fungal Community

The fungal microbiota of coral reefs is classified into obligate marine fungi and facultative marine fungi. The former group of fungi grows exclusively in marine environment, whereas the latter grows in both marine and freshwater habitats (Kohlmeyer and Kohlmeyer 1979). The obligate marine fungi belong to the class of *Ascomycetes* which includes *Corallicola nana* (Volkmann-Kohlmeyer and Kohlmeyer 1992), *Lulworthia calcicole* (Kohlmeyer and Volkmann-Kohlmeyer 1989), *Halographis runica* (Kohlmeyer and Volkmann-Kohlmeyer 1988), and *Koralionastes* sp. (Kohlmeyer and Volkmann-Kohlmeyer 1987). These fungi are non-culturable under lab conditions and have been suggested to depend on corals to derive nutrients (Kohlmeyer and Volkmann-Kohlmeyer 2003). Fungi of terrestrial environment observed in corals consist of species of *Aspergillus* spp. and *Penicillium* spp. (Morrison-Gardiner 2002). 18S rDNA metabarcoding studies have revealed new members of coral fungi such as *Geranomyces* (*Chytridiomycota*), *Flammulina* (*Basidiomycota*), and *Ophiosphaerella* (*Ascomycota*) (Góes-Neto et al. 2020).

14.2.4 Algal Community

Symbiodinium, a dinoflagellate colloquially known as zooxanthellae, is a critical component of reef ecosystem as they can cause mass mortalities of corals due to stress-associated bleaching (Baker 2003). Corals and *Symbiodinium* exhibit a symbiotic relationship where the algae provide nutrients to corals via photosynthesis and corals acts as a shelter for the algae (Muscatine and Porter 1977). The genome analysis of *Symbiodinium* confirms the existence of gene families which are mandatory for mutualistic relation with corals (Liu et al. 2018). The reefs of corals also harbor algae which are protected from harmful radiations by filtering them out before reaching the algal cells (Shashar et al. 1997).

14.2.5 Protistal Community

The eukaryotic microbiota of coral mucus is dominated by stramenopiles of family Thraustochytriaceae (Siboni et al. 2010). The protists of coral microbiome carry out important functions which enable the survival of coral holobiome. The protists biosynthesize some important metabolites which relieves the corals from stressful events such as bleaching (Harel et al. 2008). An ideal example for this would be the production of carotenoids and polyunsaturated fatty acids by Thraustochytrids in corals (Carmona et al. 2003). *Vitrella brassicaformis* and *Chromera velia* belonging to the phylum Chromerida are photosynthetic protists seen in corals (Linares et al. 2014; Oborník and Lukeš 2015). The exact role of these protists is unclear, although it has been hypothesized that they either act as a parasite or as a photosymbiont of corals (Cumbo and Baird 2013).

14.3 Application of Coral Microbiota for Soil and Plant Health

Initially the coelenterates and other marine invertebrates were exploited to obtain secondary metabolites with biological activity. Most of these metabolites have medicinal properties such as anticancer, antioxidant, antibacterial, and antiviral activity (Sang et al. 2019). Only few bioactive compounds suitable for agricultural purposes have been procured from corals (Table 14.1). The anthropogenic activities have become a threat to the coral system because of which more interest is directed toward developing bioactive compounds from microbial sources of these environments (Radjasa 2004). Coral aquaculture practice has been introduced since then, to preserve corals, avail certain economic benefits, and continue research studies in them. Corals are cultivated either in situ in aquariums or ex situ in mariculture systems (Sheridan et al. 2013).

The biochemical processes carried out by the microorganisms can influence even the life cycle of the host organism and food webs in the ecosystem. An example for

Table 14.1 Metabolites synthesized by corals with potential agricultural applications

Name of the compound	Source of the compound	Application	References
AmCyan, ZsGreen, ZsYellow, and AsRed fluorescent proteins	*Anemonia manjano*, *A. sulcate*, *Zoanthus* sp., and *Discosoma* sp.	Microscopic visualization of phytopathogens *Magnaporthe grisea* and *Fusarium verticillioides*	Bourett et al. (2002)
Crude extracts	*Pseudopterogorgia* and *Pseudoplexaura*	Antifungal activity against *Aspergillus flavus*	Kim et al. (2000)
Cembrene-C	*Sinularia* sp.	Antifungal activity against *A. flavus*	Lei et al. (2014)
Bioactive metabolites	*Sinularia capillosa*	Antibacterial activity against *S. marcescens*	Cheng et al. (2010)

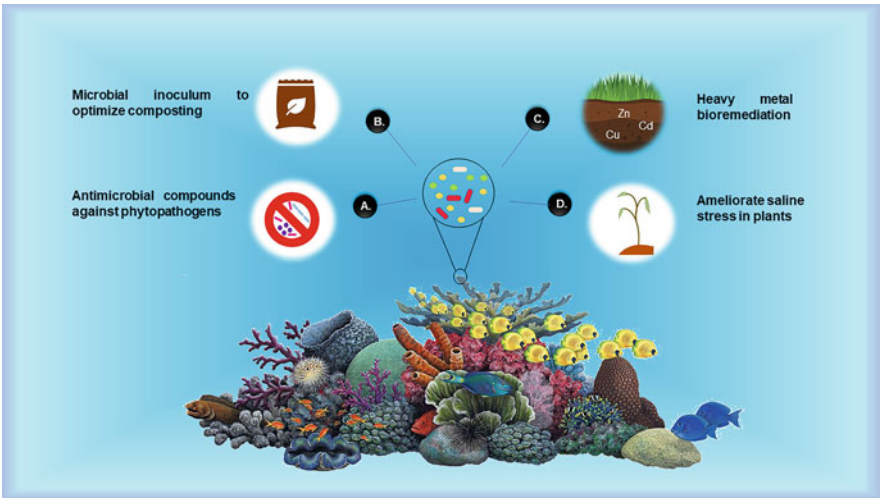

Fig. 14.1 Applications of coral microbiota to promote soil and plant health. (**a**) Antimicrobial compounds against phytopathogens. (**b**) Microbial inoculum to optimize composting. (**c**) Heavy metal bioremediation. (**d**) Ameliorate saline stress in plants

this complex relationship is the settlement of larvae of sea urchins and metamorphosis of corals under the regulation of benthic microorganisms (Webster et al. 2004; Huggett et al. 2008). The microorganisms in coral reefs can fix nitrogen, carry out photosynthesis (Lesser et al. 2004), oxidize ammonia (Wegley et al. 2007), and even produce antibiotics (Ritchie 2006). This multitude of biological functions carried out by coral reef microbiome attracted scientific community to explore biologically important compounds from coral ecosystems. Microorganisms in the marine environment are adapted to saline and high-pressure conditions due to which their biosynthetic pathways differ from microorganisms of terrestrial environment (Namikoshi et al. 2000). Bioactive compounds biosynthesized by coral microbiota have antimicrobial activity against pathogens of humans as well as aquatic animals (Kamel and Slattery 2005; Radjasa and Sabdono 2008). The following section discusses various applications of coral-associated microbes in agriculture (Fig. 14.1).

14.3.1 Antimicrobial and Antibiofilm Compounds from Coral Microbiota

The use of harmful pesticides for controlling phytopathogens has costed the environment drastically because of which biological compounds have been considered as a good alternative. Biosurfactants are bioactive compounds involved in plant-microbe interactions, and they can enhance the bioavailability of nutrients for the beneficial microbes of the plant (Sachdev and Cameotra 2013). Bacteria isolated

from corals have been found to synthesize potent biosurfactant molecules (Padmavathi and Pandian 2014). *Pseudomonas aeruginosa* is a plant pathogen reported to colonize roots forming biofilms resistant to antibiotics and cause plant mortality (Walker et al. 2004). Coral-associated microorganisms from the Gulf of Mannar capable of producing biosurfactants were *Bacillus anthracis*, *Providencia rettgeri*, *Psychrobacter* sp., *Bacillus pumilus*, and *Bacillus flexus*. Among the biosurfactants synthesized by these bacteria, *Providencia rettgeri* and *Psychrobacter* sp. produced biosurfactant active against biofilms of *P. aeruginosa* even at high temperatures (Padmavathi and Pandian 2014). These biosurfactants being active in spite of such temperature fluctuations are more advantageous since they can be utilized for plants growing at different temperatures.

Insects act a vector in transmitting certain phytopathogens. For example, *Anasa tristis* commonly known as squash bug can transmit *Serratia marcescens* which results in cucurbit yellow vine disease in *Citrullus lanatus* and *Cucurbita pepo* (Bruton et al. 2003). Microorganisms seen on the mucus layer of corals are an important source of antimicrobial compounds. Eighty-three percent of bacteria isolated from Brazilian coral mucus exhibited antibacterial activity against *S. marcescens* (Pereira et al. 2017). *Aspergillus brevipes* RK06, a coral-associated fungus of Randayan Island, Kalimantan Barat, synthesized an antimicrobial product active against *P. aeruginosa* (Nofiani et al. 2011). Actinomycetes isolated from the mucus of the coral *Acropora digitifera* from the Gulf of Mannar also synthesized antimicrobial compounds against *P. aeruginosa* (Nithyanand et al. 2011). *Pseudoalteromonas* sp. from coral microbiota synthesized an antifungal compound alteramide A, at dark conditions. The compound interfered the production of mycotoxin citrinin and citrinadins of *Penicillium citrinum*, which is a coral pathogen (Moree et al. 2014) as well as a seed-borne pathogen (Marcenaro and Valkonen 2016). However, characterization of these antimicrobial compounds is necessary to conduct application studies in agriculture.

14.3.2 Coral Reef Microorganisms for Composting

Practices like composting are highly encouraged because it not only decreases the waste but also acts as organic fertilizer for agricultural purposes and improves the quality of soil. The extent of degradation of waste components in compost depends on the microbial inoculum added to it. Addition of ammonia-oxidizing bacteria was found to reduce the loss of nitrogen linked with emission of ammonia substantially enhancing the level of nitrogen content in compost (Zhang et al. 2016). Corals such as *Alcyonium gracillimum* and *Tubastraea coccinea* harbor phylogenetically distinct members of microorganisms which can oxidize ammonia and help in denitrification (Yang et al. 2013). The ascidian microbiota also contains ammonia oxidizing archaeal community which regulates the flux of nitrogen in reef ecosystems (Erwin et al. 2014). These microorganisms can be used for preparation of inoculum in waste composting. However, only very few microorganisms are cultivable from corals (Sun et al. 2016). Hence, development of new techniques and tools to culture

these microorganisms will improve exploration studies of coral-associated microorganisms. Besides there are no reports available regarding the use of ammonia-oxidizing bacteria from corals for terrestrial agriculture applications.

14.3.3 Coral Reef Microbiota for Soil Bioremediation

Soil is polluted by accumulation of heavy metals due to human activities. Heavy metals in soil obstruct the microbial activity in soil further affecting the health of plants (Jiang et al. 2008). Bacteria can be used to remove toxic metals from soil (Iyer et al. 2005), as their cell wall consists of functional group that can bind to metals (Daughney et al. 2002). Due to chemical precipitation, phosphorous remains immobilized in soil (Gyaneshwar et al. 2002), and phosphate-solubilizing microbes solubilize such insoluble phosphorous in soil which plants can utilize (Chandler et al. 2008). Phosphate-solubilizing bacteria can be implemental in agricultural practices for removal of heavy metals as well as solubilization of phosphate in soil. The bacteria *Cronobacter muytjensii* KSCAS2 isolated from coral reefs displayed excellent phosphate-solubilizing properties. The bacteria were found to synthesize exopolysaccharides which enabled the sequestration of heavy metals such as zinc, cadmium, copper, and chromium (Saranya et al. 2018). There is a very scarce information regarding the application of coral-associated microbes for bioremediation purposes and requires further exploration. More studies into the characteristics of metal-chelating compounds produced by coral microbial community can provide more insights regarding their bioremediation potential.

14.3.4 Coral Reef Microbes to Combat Salt Stress in Plants

Increased salt content in soil affects the soil health leading to crop failure (Rengasamy 2006), and studies estimate that the global saline soil generation is 7% (Tester and Davenport 2003). The high levels of salt in soil not only interfere photosynthetic productivity of plants (Cramer and Nowak 1992) but also wreck the microbial community of the soil. Halophilic microorganisms from saline soils have been used as plant growth-promoting bacteria to relieve saline stress in plants (Orhan 2016). Since corals survive in saline environments, their associated microbiota can be used for ameliorating saline stress in plants. *Salinispora arenicola* and *Actinobacteria* seen isolated from corals like *Porites lobata* and *Porites panamensis* from central Pacific exhibited a wide range of plant growth-promoting activities such as photoprotection and enhanced seed viability under saline conditions. *Actinobacteria* acted as an endosymbiont in the roots of wild tobacco plants and also produced 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme which could have inhibited the ethylene-mediated stress in plants (Ocampo-Alvarez et al. 2020). The potential of *S. arenicola* to fight saline stress in other important food crops needs to be inspected.

14.4 Conclusion

The use of chemical pesticides and fertilizers has more deleterious effect than benefit, and they are no longer reliable options in agriculture. The use of microorganisms in agriculture for heavy metal bioremediation, for plant growth promotion, and to control phytopathogens is much safer than the conventional methods used. The goals of sustainable agriculture should not focus only on increasing plant productivity to meet consumer demands. Instead, it should also aim for production of organic and safe food products. Microorganisms from terrestrial and marine environments have been analyzed for many agricultural and environmental applications. But the available information in literature elucidating agricultural applications of microorganisms from corals are insufficient. Coral reefs are no doubt a giant source of beneficial microorganisms which can aid in achieving sustainable agriculture. There are many emerging studies which focus on exploring the microbial community of corals to develop probiotics in order to promote coral health (ados Santos et al. 2015; Rosado et al. 2019). Such explorations can be coupled with application studies to discover their hidden potential. Initiatives for coral aquaculture to facilitate drug discovery have been suggested (Leal et al. 2013); a similar initiative for soil and agricultural applications needs to be proposed. Many techniques are arising in the research field to cultivate unculturable microorganisms, and we can expect many studies unveiling the potential of coral microbiota for terrestrial agriculture applications in the future.

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Soil Microbiome: Characteristics, Impact of Climate Change and Resilience

15

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Abstract

Soil contains a variety of biotic and abiotic substances and serves as a medium for plant growth as well as a habitat for microorganisms, all of which help maintain and contribute to ecosystem services, because they are involved in organic matter breakdown, nutrient cycles and soil health. Microorganisms in the soil are clearly an important part of both natural and managed ecosystems. Despite the difficulties of surviving in soil, a gram of soil may include hundreds of different microbial taxa, including viruses and organisms from all three domains of life. As soil environmental factors are so different, soil microbial populations are extremely diverse. There are many different microbial habitats in a single soil, each with its own microbial composition. The spatial variability of soil microbial community structure is generally greater than the temporal variability. Soil and site characteristics, such as soil pH, temperature and organic carbon availability,

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may frequently predict the composition of soil bacterial communities and the abundances of individual species. Recent advancements in marker gene, genomic and metagenomic studies have substantially increased our capacity to characterize the soil microbiome and uncover the factors that change soil microbial populations with time.

Keywords

Soil · Metagenomics · Microbiome · Climate · Resilience

15.1 Introduction

Soil contains an interacting population of bacteria, archaea, viruses, fungus and protozoa, collectively known as the 'soil microbiome'. The abiotic environment of soil is also highly diverse, with disconnected air-filled and/or water-filled pores, as well as patchy resources that might serve as microbial hotspots (Kuzyakov and Blagodatskaya 2015). The soil microbiome plays an important role in global nutrient cycle and plant nutrition, as well as supports a wide range of ecosystem processes that fluctuate depending on the environment. Soil is composed of minerals, organic matter, biological organisms, gas and water, all of which provide nutrition and shelter for microbes to thrive in (Needelman 2013). Thus, soil is a highly heterogeneous environment on both the micro and macro scales, which is reflected in the diversity of microorganisms that thrive there. Bacteria and fungi are the most common microorganisms in soil, with archaea being less abundant, followed by protists and viruses (Fierer 2017).

Soil microbiomes vary widely around the globe, with taxonomic and functional (gene) diversity of soil bacteria being highest in the mid-latitudes and lowest at the poles and equator (Bahram et al. 2018). The total amount of microbial biomass found in a soil at any given time can be influenced by a variety of biotic and abiotic factors, including the abundance of microbial predators (such as protists or nematodes) and the amount of accessible carbon. Soil moisture availability is the strongest predictor of total soil microbial biomass on a worldwide scale; wetter habitats (e.g. tropical rainforests) often have more standing microbial biomass (Serna-Chavez et al. 2013). For bacteria, pH, along with carbon and oxygen quality/quantity, soil moisture and N and P availability, is the most important environmental variable that influences community composition (Fierer 2017; Bahram et al. 2018). Researchers have been able to explore the full extent of soil microbial diversity and develop a more thorough understanding of specific microbial influences on soil processes thanks to recent methodological advancements. DNA- and RNA-based studies of the soil microbiome, in particular, have considerably expanded our understanding of the phylogenetic and taxonomic structure of soil microbial communities (Fierer et al. 2009; Torsvik and Øvreås 2002).

In this chapter we describe the characteristics of soil microbiome and factors influencing soil microbiome. We have also the discussed role of soil microbiome in

global nutrient cycling and ecosystem functioning. Types of soils and their microbial composition, plant-microbe interaction, significance of soil microbiome in bioremediation, impact of climate change on soil microbiome and metagenomic tools for soil microbiome studies have been delineated comprehensively.

15.2 Characteristics of Soil Microbiome

The major determinants that influence the establishment of soil material are climate, time of soil formation, parent material and organisms (Jenny 1994). One of the studies addresses that the microbial composition of soil not only depends on temperature; nevertheless it is more diverse because of moisture and nutrient content of soil. Moisture availability is considered as one of the best indicators of abundant microbial diversity all over the world. The geographical distribution which has the highest moisture content soil has an enormous microbial load when compared to others (Serna-Chavez et al. 2013). Uniform distribution of all microbial groups cannot be observed in the case of soil. Among the most prevalent ones are bacteria and fungi, and they are 10^2–10^4-fold higher in biomass when compared to protists, archaea, etc. If the structure of a particular community is only taken into consideration, nearly all bacterial and archaeal species are limited, and hardly one or two of them are ample (Lynch and Neufeld 2015). Moreover, a study conducted in the USA analysed 500 plus soil samples from the Central Park in New York and concluded that more than 80% of bacterial and archaeal groups were present in the samples (Ramirez et al. 2014). Predominance of a smaller number of fungal and protist groups is observed in soil, and majority of the specific groups are uncharacterized. Mahé et al. (2017) demonstrated that parasitic members like Apicomplexa are the preeminent species in Neotropical rainforest even though the ecosystem comprises distinct species. The studies by Rosenthal et al. (2017) also emphasized the above-discussed facts. Their studies on corticioid fungi accentuated fungi as geographically structured groups. Their work provided insights on the diversity of fungi over North American pine forests. However, biology and taxonomy of these fungal groups are hardly known.

The diversity and coexistence of different microbes in soil differs with respect to soil type. Surprisingly this point is valid although the sampling sites are very near ranging from centimetres (O'Brien et al. 2016). This difference in distribution of microbiota is mainly due to geographical irregularity in the soil surroundings and particular features of sites selected for sample collection. Alteration in soil microbiome structure can be identified from plant species distribution on that area. Various plant species may possess divergent microorganisms in the soil on which they grow, and these microorganisms particularly tend to form associations with distinct plant communities. For example, certain mycorrhizal fungi, fungal plant pathogens and nitrogen-fixing bacteria tend to form associations with peculiar plant groups (Berg and Smalla 2009, Van Der Heijden et al. 2008). Furthermore, Peay et al. (2013) demonstrated that fungal species distribution and richness are extensively associated with plants. By evaluating the composition of soil microbiota, the

alterations in plant community structure grown on the same soil can be identified (Barberán et al. 2015). Nevertheless, some studies showed that certain plant species do not influence soil microbiota of soil where they are grown (Lekberg and Waller 2016; Nunan et al. 2005). Bulgarelli et al. (2012) illustrated the context-specific interaction of microbes and plants. Moreover, a particular plant can interact with microbiota based on the soil type.

15.3 Soil Type and Its Microbiome

15.3.1 Grassland Soils

Grasslands are a type of geographical area where grass species dominate other groups of plants. They are also characterized by short growth and non-woody plant species (Gibson 2009). Microbes in soil are considered as crucial decomposers of these ecosystems, and they have a role in nutrient cycling (Freschet et al. 2013). Arbuscular mycorrhizal fungi are extensively found in these grasslands, and they develop mutual plant-rhizome interactions with plants (Brundrett 2002). However, additional interactions comprising ecto- and ericoid mycorrhizal fungi are not frequent in these ecosystems since these interactions are particular to arboraceous and ericaceous plant species (Brundrett 2002). Arbuscular mycorrhizal fungi benefit the host plant in many ways such as in the absorption of limiting nutrient (Smith and Read 2008), decomposition of organic matter (Hodge et al. 2001), embellishing the capacity to take in moisture (Ruiz-Lozano 2003), decreasing the occurrence of pathogenic diseases to plants (Newsham et al. 1995) and defence in opposition to herbivory (Johnson and Gilbert 2015).

Another group of microorganisms that dwell in grasslands are soil protists that belong to Eukarya and are single-celled organisms (Fiore-Donno et al. 2019). These organisms aid in developing community structure of flora and other groups of microbiotas like bacteria, fungi and algae (Trap et al. 2016; Geisen et al. 2018). Soil protist diversification is correlated with functional group diversity of the vegetation present in grasslands (Ledeganck et al. 2003). In contrast to other microorganisms, insights on protists are very constrained.

15.3.2 Forest Soil

If we calculate the overall forest area of earth, it will be around four billion hectares. Furthermore, the tropical rainforest (lungs of the planet) is a critical carbon sink on the globe. The whole forest architecture is influenced by climate and soil conditions. Zhou et al. (2016) demonstrated the bacterial and fungal varieties expand together with temperature gradient. When the temperature of the forest is very less, it freezes microbial functions, decay of carbon-containing compounds and nutrient cycling and further leads to carbon aggregation and nitrogen deficiency. However, increased temperature can lead to overall reversal of the above-mentioned processes (Reinsch

et al. 2017; Malhi et al. 1999). In forest ecosystems massive intricacies and heterogeneity of soil microbiota occur even regionally. This phenomenon may be due to huge trees that develop exclusive microhabitats and allows the survival of diverse microorganisms (Štursová and Baldrian 2011). Rhizome of trees tends to form mutual interactions with ectomycorrhizal fungi. Eighty percent of the fungal genera in forest belong to these ectomycorrhizal fungi, and this accounts for one-third of the overall microbiota of forest (Högberg and Högberg 2002). These ecosystems mostly carry elevated fungal/bacterial ratios. Moreover, bacterial varieties are lesser than grasslands or agricultural soils (Roesch et al. 2007; Delgado-Baquerizo and Eldridge 2019). Weathering of rocks and subsequent mineral deposition in soil is carried out by most of the microbes in the soil (Richardson and Simpson 2011). Fungal genera extensively accomplish this task. Nevertheless, only certain groups of forest bacteria have the ability to take part in this (Uroz et al. 2009; Adamo and Violante 2000).

15.3.3 Desert Soils

The initial groups that evade deserts are extremophiles which include bacteria and archaea (Mapelli et al. 2012) accounting to their ability to thrive in extreme conditions (Colica et al. 2014). During secondary succession other microbial groups such as fungi, lichens and mosses may resume their growth and development in deserts, and they constitute biological soil crusts (BSC) (Li et al. 2018). BSC aids desert ecosystems to attain soil stability and regulate carbon and nitrogen cycles and survival of vascular plants (Li et al. 2018). The endurance of desert fauna is also associated with bacterial diversity in soil where they grow (Shelef et al. 2013). Jorquera et al. (2012) demonstrated that fauna structure affects the constitution of microbial populations and positively regulates their growth and development.

15.3.4 Peatland Soils

The peatlands are remarkably marked by their aggregation of dead remnants on the surface. The water-clogged condition of land surface accounts to this, and overall degradation of litter is prevented (Joosten and Clarke 2002). Peatlands stock carbon and are involved in its cycling and also cause release of methane (Page et al. 2011). The crucial factors that affect the microbes which perform these functions are depth, redox condition and carbon quality (Morales et al. 2006). With the difference in fauna of each peatland, its microbial functions and degradation of organic matter differ (Fisk et al. 2003). Alteration of the fungal population structure of various peatlands depends on the litter type of that particular peatland (Andersen et al. 2013). Trinder et al. (2008) demonstrated that litter type is crucial than groundwater levels for underlying fungal population in peatlands.

15.3.5 Tundra Soil

These ecosystems are massively amalgamated with constantly changing hydrothermal features. Rhizome of these ecosystems plays a critical role in biogeochemical cycling. Evaluation of the microbiome of these regions aids in understanding environmental alterations and finding remedial ways to cope with it (Lydolph et al. 2005; Li et al. 2011). Microbiota is less prominent in the Arctic, Antarctic, Qinghai-Tibet Plateau and Siberian permafrost. Soil temperature is a crucial parameter in these ecosystems which influence soil respiration, degradation of litter, nitrogen metabolism, fauna characters and nutrient absorption (Callesen et al. 2003). However certain ecosystems are also influenced by total carbon (Ganzert et al. 2011). Carbon content can regulate the carbon metabolism and hence influence the soil microbiota (Jangid et al. 2008). Barns et al. (1999) showed that other factors like soil type and pH can also regulate microbial community alterations in this ecosystem (Barns et al. 1999). Organic acids in the soil mostly account for the pH in tundra region (Hobbie and Gough 2004).

15.4 Factors Influencing the Soil Microbiome

The growth and development of microorganisms on soil are highly restricted. Reasons may be abiotic stress, intra- and interspecific competition arising between different organisms and non-uniform dissemination of nutrients (Kuzyakov and Blagodatskaya 2015). It is not possible to point out a peculiar biotic or abiotic factor that contributes extensively to the constitution of soil microbiota. Jones et al. (2009) pointed out that even the carbon compounds that ooze out from rhizomes can influence microbiota. Furthermore, the exact quantity of carbon that reaches this way cannot be estimated. However, investigation of soils having a wide magnitude of pH values (from pH 4 to pH >8) indicated that pH is the suitable parameter for revealing the bacterial and archaeal population structure (Griffiths et al. 2011; Lauber et al. 2009). But a limited range of pH values sometimes will not influence the soil microbiome. Moreover, there is no possibility that every member of the microbiome will react to altered pH. Multiple determinants may affect the makeup of microbiomes in soil. But the factor which contributed extensively to alteration in soil microbiome is not yet confirmed. Along with pH, other crucial factors include nitrogen and phosphorus content of soil (Cederlund et al. 2014), organic carbon composition (Sul et al. 2013), temperature (Oliverio et al. 2017), soil oxygen and redox status (Pett-Ridge and Firestone 2005) and soil moisture (Maestre et al. 2015). The above-mentioned factors are not mutually exclusive, but they are correlated to each other in one or another way.

15.5 Plant-Soil Microbiome Interactions

Soil microbiota has a distinct and pivotal role in the soil ecosystem; it improves plant health, quality and fertility that contribute heavily to the quality and yield of agricultural products. Microbiota often encounters complex organic, dead-decaying matter having high energy, the nutritive value required for cell maintenance and stable growth (Dubey et al. 2020). Recent advancements in the food and agricultural industry predominantly depend on the usage of chemical-based fertilizers and pesticides which manipulate the quality of nutritional requirements, crop productivity and health status. However, the continuous exposure of these chemical-based constituents contributes to the accumulation of toxic compounds, which ultimately impacts human health. Hence, it is essential to come up with the alternatives to chemical pesticides and fertilizers for the overall wellbeing of agricultural products. The plant rhizosphere is a crucial ecological niche with an enormous diversity of microorganisms (Vishwakarma et al. 2020). The rhizospheric microbiome possesses the properties like plant growth promotion, disease suppression, removal of toxic materials and assimilation macro- and micronutrients in plants. Enabling such a beneficial microbiome for crop productivity represents an effective way out of modulating the overall crop yield and plant quality employing bio-formulations (Prasad et al. 2021). The microbiomes that colonize the plants can be categorized into two different categories, i.e. epiphytes, which are present onto the surface and endophytes, which are found within the plant tissues, phyllosphere and rhizosphere, close to the roots. The rhizosphere is considered to play the most dynamic and remarkable impact on the plant nutritional status and overall wellbeing (Thapa and Prasanna 2018). It mainly comprises of soil and roots along with root hairs that establish their interactions by communicating with a variety of microbes in the rhizosphere space, thereby significantly affecting the plant growth, and offer resistance against numerous environmental stresses (Vacheron et al. 2013). The whole system comprises plant roots interacting with the rhizomicrobiome facilitating the plant-root microbiome. The intertwining characteristics of host-microbial interactions open up the possibility of numerous signalling events, namely, plant root–root and root–microbe interactions. However, root–nematode communications may also serve as a determining mode to understand the overall behaviour of plants for such factors.

The recent technological advancements concerning genome and proteome-based analysis identify the mutual association between microbiome and plants, with emphasis on the mechanisms for improved production. Various compartments of plants harbour distinct endophyte signatures, which solely depend on the source allocation of a plant. Upon evaluating the structure of phyllosphere, it was observed that *Curtobacterium*, *Bacillus*, *Pseudomonas*, *Methylobacterium*, *Frigoribacterium*, *Sphingomonas*, *Pantoea*, *Acinetobacter*, *Citrobacter*, *Enterobacter* and *Erwinia* are the predominant genera, whereas endophytes are occupied by a collection of *Burkholderia*, *Staphylococcus*, *Ralstonia*, *Mesorhizobium*, *Pseudomonas*, *Propionibacterium* and *Bacillus* (Rana et al. 2020). The dynamic nature of microbiome communities that are associated with the plant roots preferably

undergoes horizontal transfer, enriched from the soil-rich communities populated by *Bacteroidetes*, *Acidobacteria*, *Proteobacteria*, *Actinobacteria* and *Planctomycetes* (Ecker et al. 2019). On the contrary, the transfer of bacteria could also occur by modulating an essential source of dividing microbes from the plant roots up to its development. However, the narrow layers of soil microenvironment in the near vicinity of the root system are thought to be a highly active area for microbial movement, making it one of the most intricate environments.

15.6 Soil Enzymes and the Soil Microbiome

Microbes mainly degrade organic matter for their nutritional requirement and as their energy source. As a result, the organic matter is oxidized, and an electron is released and is used as an energy source to drive the microbial metabolic pathways. Biogeochemical cycles and biodegradation processes are interlinked. Different bacterial genes are responsible for mitigating the process of biodegradation. How a genetically modified organism can perform biodegradation process has become an interesting area of research. Degradation of an organic compound with the help of microbial enzymes in the process is widely known as biodegradation (Karigar and Rao 2011). Several classes of enzymes are present in the environment which help degrade the organic matter. Various organic compounds, viz., carbon, nitrogen, sulphur, phosphorus, chlorine and many other minerals, help the microorganism fulfil their nutritional requirements (Verma et al. 2014). A different type of organic matter disperses throughout the earth and in various habitats like marine, freshwater and terrestrial as well. Both prokaryotic and eukaryotic microbes can help degrade organic matter. Enormous organic matter found in nature includes the plant residues, animal wastes, microbial biomass, organic compost, detritus, pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Das and Chandran 2011).

Microbial enzymes are often called extracellular enzymes. Bacteria secrete different organic material-degrading enzymes such as polysaccharide-degrading enzymes, pesticide-degrading enzymes, PAH-degrading enzymes and so on. Biodegradation is helpful in several aspects such as bioremediation, biogeochemical cycling, etc. (Ortiz-Hernández et al. 2013). The microbial plasma membrane is selectively permeable and cannot allow large polymeric substances into the cell. So, microbes first degrade large polymeric organic substances to small nutrients that can be taken up by the microorganisms (Costa et al. 2018). Some of the most striking examples of microbial extracellular enzymes are amylases, cellulases, chitinases, hemicellulases, hyaluronidases, ligninases, lipases, pectinases, proteases and so on. Sulphate (SO_4^{2-}) is used as an electron acceptor for organic matter oxidation in marine water under anaerobic conditions. Sulphate-reducing bacteria (SRB) play a key role in degrading organic complex (Jørgensen et al. 2019). Sulphate-reducing bacteria have been divided into six major groups, *Desulfotomaculum*, *Desulfobulbus*, *Desulfobacterium*, *Desulfobacter*, *Desulfococcus-Desulfonema-Desulfosarcina* and *Desulfovibrio-Desulfomicrobium*.

Carbohydrate is a major component of organic matter and one of the major carbon and energy sources (Daly et al. 2000). To understand the biodegradation process, one must know about the microorganism that carries out the process. Microorganisms release several enzymes for this process (growth and co-metabolism). Organic matter is solely used as a carbon and energy source to support microbial growth. This process is crucial because it promotes complete mineralization or degradation. Different types of microbes need some growth substrate along with organic matter for supporting their growth; this is called co-metabolism. The process of biodegradation is diverse, but the end product is generally CO_2. It is carried out both aerobically and anaerobically. Moreover, different types of factors control the biodegradation process like temperature, pH, availability of nitrogen and phosphorus sources and genetic potential. The use of genetically modified organisms in biodegradation opens a new era in the field of bioremediation. The most striking feature of soil microbiota is to degrade hydrocarbons. Soil microflora contains an enormous amount of diverse microflora that includes bacteria, fungi, protozoa, algae and actinomycetes, possessing a capacity to degrade hydrocarbons (Sachidanand et al. 2019). The factors affecting microbial degradation are depicted in Fig. 15.1.

15.7 Degradation of Organic Matter by Microbial Enzymes

Plant root exudates may directly participate in the process of organic degradation by secreting several enzymes such as laccases, nitroreductases, phosphatases, dehalogenases, cytochrome P450 monooxygenases and peroxidases (Muratova et al. 2009). However, the research in microbial degradation of organic matter is limited and is evolving with time. It has been observed that in a phenanthrene-contaminated environment, the activity of oxidoreductases (e.g. peroxidises, tyrosinases, laccases) in sorghum (*Sorghum bicolor* (L.) Moench) root exudates increased as phenanthrene concentration increased in the soil (Hoang et al. 2020). A few investigations made on the degradation of petroleum hydrocarbons have shown that the plant enzymes were mainly involved in the oxidation of PAHs, and the initial attack on the contaminant was predominantly performed by soil microbial enzymes (Ghosal et al. 2016).

Plant roots can act upon the soil contaminants, but sometimes they are susceptible to contaminant-mediated stresses (Lareen et al. 2016). Vesicular-arbuscular mycorrhiza (VAM) fungi are ubiquitous and present as symbionts in plant roots (Bhattacharjee and Sharma 2011). The coexistence of these VAM fungi has prime importance when plants are under environmental stress such as contamination of petroleum or other PAHs. It has been found that the enzyme concentrations increase with increasing crude oil concentrations (Alarcón et al. 2019). Studies have also reported that some microbial enzymes take part in the degradation of crude oil and are able to withstand 20% (w/w) crude oil in the culture medium, and the tolerance could be explained by their ability to degrade and utilize the hydrocarbons as an energy source (Xu et al. 2018). Moreover, VAM may also cause induction of root

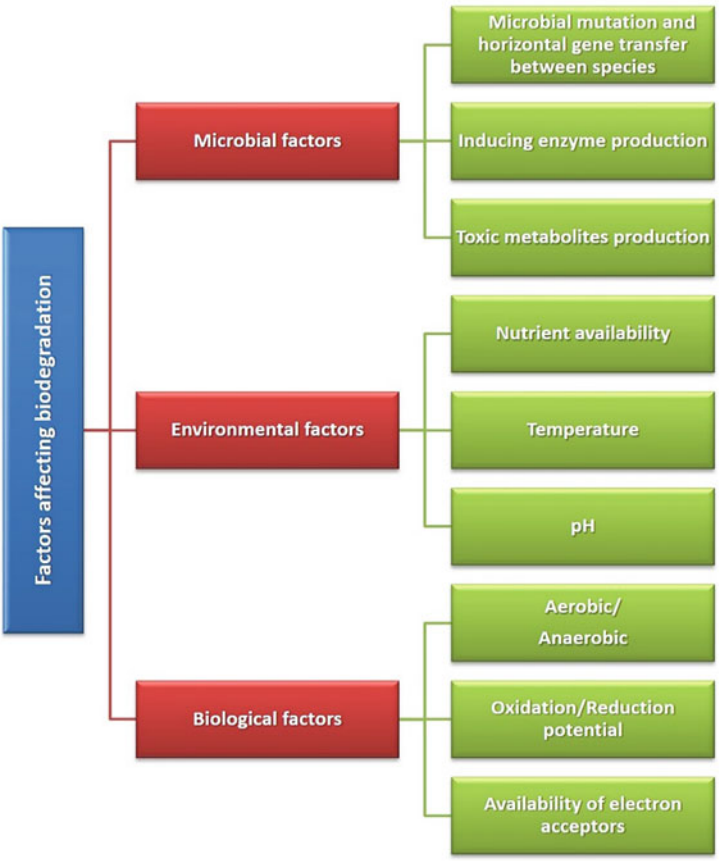

Fig. 15.1 Factors mediating biodegradation. (Adapted and modified from Boopathy 2000)

peroxidase production by their host plants, which are often involved in hydrocarbon metabolization (War et al. 2012). For example, wheat rhizosphere mycorrhized by *Rhizophagus irregularis* showed remarkably higher peroxidase activity than compared to the control (non-mycorrhized) (Pérez-de-Luque et al. 2017). A higher percentage of PAH and alkane degradation were also observed in *R. irregularis*-inoculated wheat in comparison to non-inoculated wheat (Ingrid et al. 2016). A detailed account of microbial enzymes and their role in the degradation of organic compounds have been summarized in Table 15.1.

Table 15.1 Secreted microbial enzymes and their role in the degradation of organic compounds (Adapted and modified from Hoang et al. 2021)

Enzymes	Catalytic functions	Microorganisms
Dehalogenase	It hydrolyses the chlorine (Cl) and fluorine (F) from the halogenated aliphatic hydrocarbon moieties (e.g. trichloroethylene) and aromatic hydrocarbons (e.g. DDT, PCBs, etc.)	Bacteria: *Xanthobacter autotrophicus*, *Sphingobium chlorophenolicum*
Dioxygenase	Degrades a variety of organic compound	Bacteria: *Pseudomonas* sp., *Mycobacterium* sp.
Peroxidase	Degrades various aromatic compounds; reductive dehalogenation of aliphatic hydrocarbon	Fungi: *Phanerochaete chrysosporium*, *Phanerochaete laevis*
Nitrilase	Digests the cyanide groups from aromatic and aliphatic nitriles	Fungi: *Aspergillus niger*
Nitroreductase	Reduces nitrogen group on aromatic compound bearing nitrogen	Bacteria: *Comamonas* sp., *Pseudomonas putida*
Phosphatase	Remove phosphate groups from organophosphates	Mostly soil bacterium and *Aspergillus* sp. fungi

15.8 Importance of Soil Microbiome in Bioremediation

Bioremediation is a microorganism-mediated process that degrades and detoxifies environmental pollutants in a sustainable and environmentally favourable manner. Soil microbiomes have clearly emerged as a significant component of bioremediation, as they are more stable and efficient than pure cultures, and have been identified as one of the scientific frontiers in the domains of soil environmental science and technology (Ying and Wei 2019).

Persistent organic pollutants (POPs) are extremely lipophilic in nature, and their biomagnification in the food chain has negative consequences for the ecosystem and human health (Chakraborty and Das 2016). Diverse soil microorganisms have shown the ability to flourish on harmful organic substances in soil, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and plastics, among others (Teng et al. 2015). Microbes use gene mutation, rearrangement and differential regulation to help them survive in unfavourable conditions such as contaminated environments (Thomas and Nielsen 2005). Several microbes including those belonging to bacterial genera such as *Pseudomonas*, *Burkholderia*, *Stenotrophomonas*, *Corynebacterium*, *Staphylococcus*, *Micrococcus*, *Mycobacterium*, *Rhodococcus*, *Sphingobium*, *Bacillus*, *Aeromicrobium*, *Brevibacterium*, *Desulfotomaculum*, *Desulfovibrio*, *Dietzia*, *Escherichia*, *Gordonia*, *Methanosaeta*, *Moraxella*, *Pandoraea* and *Pelatomaculum* have been found to show chemoorganotrophy for degradation of POPs (Chowdhury et al. 2008; De Roy et al.

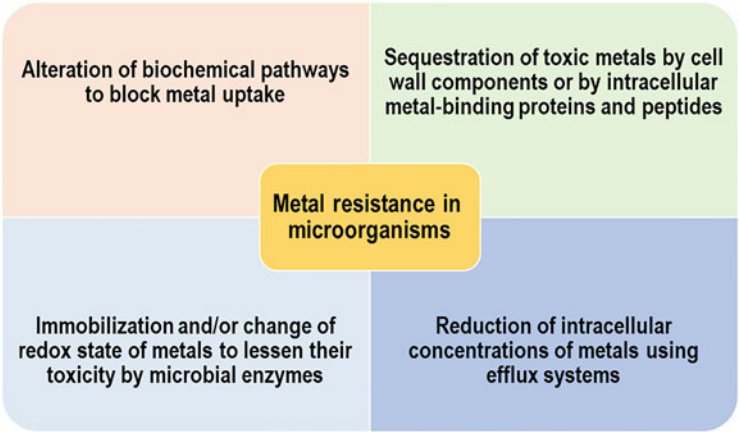

Fig. 15.2 Factors responsible for metal resistance in microorganisms

2014; Chakraborty and Das 2016). Similarly, fungal genera such as *Amorphotheca*, *Neosartorya*, *Talaromyces*, *Graphium* and *Irpex* have been identified as promising POC degraders (Gupta et al. 2016; Lenoir et al. 2016).

Heavy metal remediation may be hindered by their low bioavailability due to insolubility and soil-bound characteristics (Teng et al. 2015). Bioremediation using indigenous microorganisms to treat heavy metal-polluted soils by converting toxic heavy metals into non-hazardous forms is a cost-effective method (Gupta et al. 2016). Some soil microorganisms' metabolism could enhance metal bioavailability in soil by altering soil pH, resulting in the production of chelators (i.e. siderophores) and organic acids that can improve metal complexation and mobility (Schalk et al. 2011). Microbial volatilization is a preferred technique for metal bioremoval of metals like selenium and mercury, which can be accelerated by a variety of soil bacteria (Zhang et al. 2012). Factors responsible for metal resistance in microorganisms are shown in Fig. 15.2.

Microbes can assist phytoremediation by immobilizing or activating heavy metals (Ojuederie and Babalola 2017). Nitrogen fixation, phosphate solubilization, phytohormone synthesis, siderophore release and synthesis of indole acetic acid, 1-aminocyclopropane-1-carboxylic acid deaminase and volatile chemicals are all methods used by plant growth-promoting microbes in soil to stimulate plant growth (de Bashan et al. 2012; Hao et al. 2014). Furthermore, some findings suggest that consortia of bacterial strains exhibit better bioremediation of heavy metals than a single strain. For example, Kang et al. (2016) evaluated the bioremediation of combined lead, cadmium and copper-contaminated soils using a bacterial consortium composed of *Viridibacillus arenosi B-21*, *Sporosarcina soli B-22*, *Enterobacter cloacae KJ-46* and *Enterobacter cloacae KJ-47*. When compared to single strains, the bacterial consortium demonstrated higher heavy metal resistance and remediation efficacy. Polycyclic aromatic hydrocarbons (PAHs) are common organic pollutants that, due to their hydrophobic character, persist in the

environment (Lamichhane et al. 2016). In the bioremediation of PAH-contaminated soils, soil microbiomes are commonly used. Wang et al. (2016) enriched a unique aerobic microbial community for complete phenanthrene degradation from a petrochemical-contaminated soil.

Crude oil is a complex mixture of hydrocarbons and other chemical compounds, as well as some organometallic components, especially vanadium and nickel (Varjani and Upasani 2017). A wide range of soil microorganisms have the potential to decompose oil sludge (Shankar et al. 2014). The use of consortia of microorganisms in bioremediation of oil spills in soil demands further investigation. Mariano et al. (2008) investigated the biodegradation of commercial and weathered diesel oils, concluding that consortia had greater biodegradation capability than pure cultures and that individual isolates might not help in degradation. Cerqueira et al. (2011) used a heterogeneous bacterial consortium and five pure petroleum-degrading isolates to examine the biodegradation potential of aliphatic and aromatic hydrocarbons in petrochemical oily sludge. The results of the heterogeneous bacterial consortium exhibited excellent outcomes. In a similar manner, Shankar et al. (2014) identified 32 oil-degrading isolates that were positive and created a consortium to degrade the mixture of petrol, diesel and engine oil. They confirmed that consortia of microorganisms are superior bioremediation agents for oil-contaminated soil than individual microorganisms. Haque et al. (2021) in their study concluded that biosurfactant produced by *Pseudomonas aeruginosa* ENO14 has potential to be used as a crude oil bioremediation agent.

15.9 Factors Affecting Bioremediation by Soil Microbiomes

Microorganisms are extremely sensitive to their growing environment and react rapidly to changes (Varjani 2017). Decades of research have demonstrated that soil parameters, such as pH, organic carbon concentration, salinity, texture and available nutrient concentrations, have a broad range (Fierer 2017). In most soil environments, however, microbial survival and growth are often severely limited. There can be persistent abiotic stressors (such as low water availability, limited availability of organic carbon sources, acidic conditions and a wide range of pollutants), intense competition with other soil microbial groups, frequent disturbances (such as drying-rewetting and freezing-thawing events and predation by earthworms and/or other fauna) and unequal distribution of different kinds of resources across space and time (D'Costa et al. 2006; Kuzyakov and Blagodatskaya 2015).

15.9.1 Effect of Conditions of Soil Environment on Bioremediation

The soil environment, which includes soil type, aeration status, temperature, bioavailability of nutrients, presence of other inhibitory pollutants or co-contaminants, soil moisture, water activity and microbial competition, all have a significant impact

on a remedial system's efficiency and effectiveness (Varjani and Upasani 2017). To improve remedial efficiency and assure field-scale success, these parameters must be properly optimized. In bioremediation, temperature is crucial (Varjani et al. 2014). It has an impact on both the physical and chemical states of pollutants, as well as the microbiomes (i.e. microbial growth rate, gas solubilities, soil matrix, microbe metabolism and the physical and chemical state of contaminants) (Chandra et al. 2013; Varjani and Upasani 2017).

Thamer et al. (2013) found that soil microbes biodegraded crude oil (80% rate in 27 days), which they attributed to environmental conditions, the development of emulsion materials, the presence of bacterial enzyme and the availability of appropriate temperature. They hypothesized that temperature and nitrogen demand were critical factors in increasing microbial effectiveness in degrading crude oil components. Varjani (2017) reviewed that petroleum hydrocarbons lack important nutrients for microbial development, such as nitrogen and phosphorus. Studies have found that the best C/N/P ratio for promoting microbial development is 100:10:1 (Zhao et al. 2011; Dias et al. 2012). Furthermore, researchers have frequently observed that soil pH is the strongest predictor of bacterial and archaeal community composition while studying a collection of soils with a wide range of pH values (from pH 4 to pH $>$ 8) (Fierer 2017). They predicted that soil pH would have a significant impact on the diversity and richness of soil microbiome, which affects soil functioning, including biodegradation of pollutants and biotransformation.

15.9.2 Effect of Bioavailability of Pollutants and Biosurfactants in Soil on Bioremediation Process

The amount of a substance that is physico-chemically available to microorganisms is known as bioavailability (Souza et al. 2014). The longevity of POPs in the environment is attributed to their low water solubility and ability to be incorporated into soil organics, limiting their availability to degrading microbes (Chakraborty and Das 2016). It has been found that the bioavailability of a compound in different contaminants might cause it to be degraded to varying degrees by the same organism or consortium, rather than its chemical structure (Varjani 2017). Bioavailability is influenced by soil physico-chemical properties (such as composition, texture, water content, pH, sorption, occlusion and ageing) and has a significant impact on the feasibility of risk-based remediation, the type of microbial transformations that occur and whether POCs will be used as a primary, secondary or co-metabolic substrate or energy source (Kuppusamy et al. 2017). Varjani and Upasani (2016) observed that soil microorganisms can produce a variety of products (such as gases, biosurfactants, biopolymers, solvents and acids) to aid in remediation. Biosurfactants are among the products that have received a lot of attention since they play a key role in increasing hydrocarbon pollutant bioavailability (Souza et al. 2014). As a result, using biosurfactants to increase the bioavailability of POPs, particularly PAHs, is a promising strategy (Gupta et al. 2016).

15.9.3 Role of Indigenous Microorganisms of Soil in Bioremediation Process

Exogenous microorganisms struggle to survive and flourish in soil environments due to abiotic stresses as well as indigenous bacteria (Varjani and Upasani 2017). A significant degree of competition between inoculated microbes and indigenous soil microbes has been indicated, as evidenced by the widespread prevalence of antibiotic-producing and antibiotic-resistant soil bacteria (D'Costa et al. 2006). Microbial consortia exhibited an advantage over single strains in that their great diversity of microbes could aid exogenous microorganisms in surviving in new environments (Großkopf and Soyer 2014).

15.10 Soil Microbiome and Climate Change

Climate change is threatening the very existence of almost all the structures thriving on the earth surface (both biotic and abiotic structures). Any ecosystem, either it is terrestrial, aquatic or even a human body (can be considered as ecosystem as they harbour various microbes), should be in stability via homeostasis to accomplish competence in the changing environmental condition (e.g. climate change).

Soil, being an abiotic assembly that hosts vast majority of living organisms on the planet, right from the plants to animals, is also influenced by the climate change. Climate change affects the soil environment in various ways which includes elevated levels of carbon dioxide (CO_2), temperature rise, permafrost thaw, drought, elevated levels of rainfall and floods, increased frequency of wildfire, soil warming and so on (Jansson and Hofmockel 2020). Influence of climate change on soil environment also affects the soil microbiome (Fig. 15.3). Soil microbiome is crucial in the operation of various biogeochemical cycles and nutrient processing. Soil microbiome serves as the deciding authority of plants' health, and also, they play a key role in the decomposition of dead and decaying materials dumped into the soil. In fact, apart from the physical and chemical processes, the formation of soil structures is majorly influenced by the soil microbiome.

15.11 Effect of Elevated CO_2 and Temperature on Soil Microbiome

Dunbar et al. (2012) have reported that the elevated levels of CO_2 in the soils can increase the abundance of *Acidobacteria* through a 10-year cross-biome study. Elevation of CO_2 in the soils can initially increase the plant productivity; however, its long-term elevation in soil can cause detrimental effects by rapid decomposition of soil organic matter by soil microbes, which can inversely affect the soil health (Drake et al. 2011; Van Groenigen et al. 2014). Environmental disturbances caused due to climate change are being mitigated by the soil microbes as they have certain limits of tolerance threshold (microbes are existing in the earth surface since early

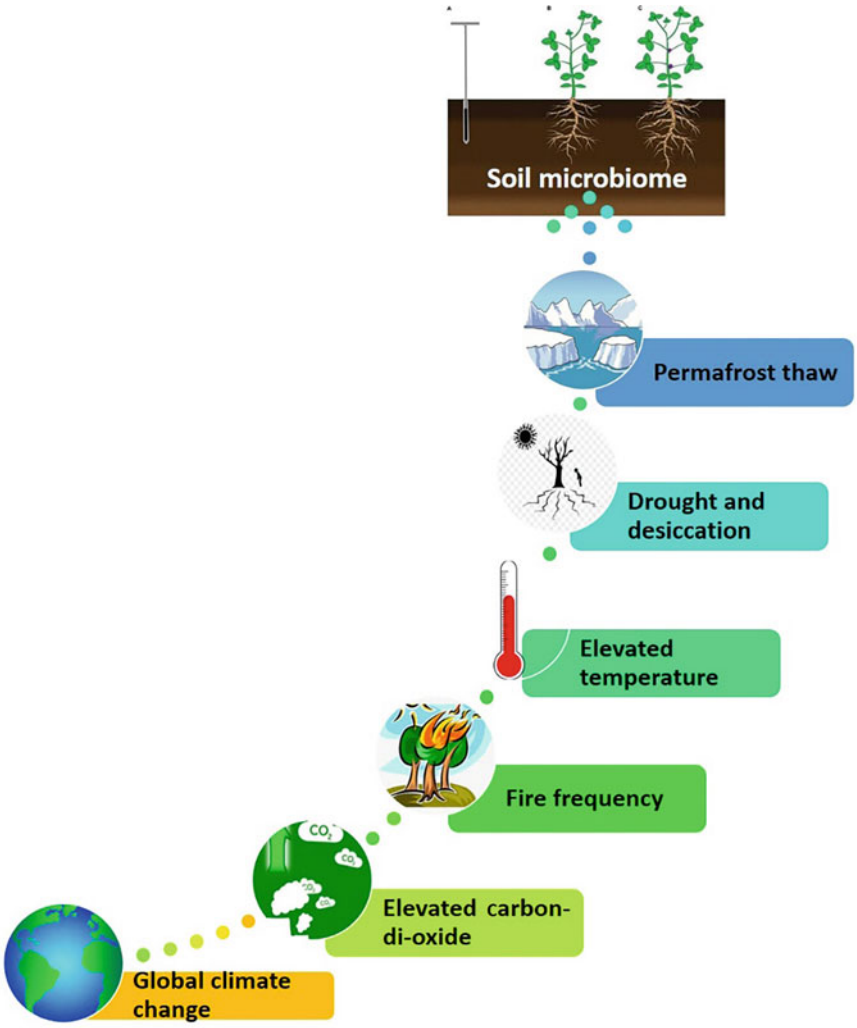

Fig. 15.3 Impact of climate change on various environmental factors that determines soil microbiome

Precambrian era mitigating various environmental perturbations). Microbes adapt certain cellular mechanisms to mitigate the environmental stresses. For instance, microbes thriving in high temperatures exert tolerance by changing the cell membrane lipid composition and also by producing heat-shock proteins (Jansson and Hofmockel 2020). Long-term soil warming study conducted by the Harvard Forest Ecological Research Station for a period of 26 years by increasing the temperature to 5 °C that has adversely affected the soil carbon degradation by increasing the microbial activity (i.e. degradation of recalcitrant soil carbon), which ultimately affects the sustainability of the soils.

15.12 Effect of Permafrost Thawing on Soil Microbiome

The serious consequence of global climate change is the thawing of permafrost. Permafrost thawing can lead to rise in the global sea level and also expose the dormant microbes in the permafrost soils into an active state (i.e. favourable condition for microbial growth). During permafrost thaw, frozen or dormant microbes become active by utilizing the available stored carbon from the arctic soils, thereby emitting greenhouse gases; in particular, methane can be more readily formed by microbes in the permafrost soils (Jansson and Hofmockel 2020). Moisture is one of the main factors that determines the microbial activity, and permafrost thaw gifts this advantage to the microbes that are ever-dormant in the freezing condition. Redox conditions in the permafrost soils encourage iron reduction by microbes, as evidenced by Alaskan permafrost (Hultman et al. 2015). Mostly, *Actinobacteria* are found to be increased during permafrost thaw; however, there is a difference in microbial community with respect to the location of the study (Taş et al. 2018; Müller et al. 2018). Genome-centric metagenomics exploiting the technology of metagenome-assembled genomes (MAGs) for studying the permafrost soil microbiome has revealed the presence of various functional genes responsible for cold-shock proteins, heat-shock proteins, cryoprotectants, DNA repair-related proteins, methanogenesis, plant polysaccharide degrading enzymes and so on (Woodcroft et al. 2018).

15.13 Effect of Drought on Soil Microbiome

Drought which directly implies the loss of moisture in the environment is negatively impacting the microbial growth. Drought leads to loss of microbial activity, thereby suppressing the soil respiration. Surface-dwelling photoautotrophs dominate the dryland systems as they possess adaptive mechanisms such as dormancy/reactivation, osmoregulation, extracellular polymer production, etc., to cope up with moisture and carbon limitations (Jansson and Hofmockel 2020). Mostly *Actinobacteria* and *Bacilli* are well adapted as they can become dormant (conserving their bioactivity) during desiccation.

15.14 Effect of Elevated Precipitation and Increased Fire Frequency on Soil Microbiome

Elevated rainfall (precipitation) and excessive flooding can cause depletion of nutritional resources for the soil microbiome, thereby decreasing the microbial diversity and functioning. On the other hand, excessive precipitation can fill the soil pores due to increase in moisture and results in an anaerobic environment. The anaerobic condition can facilitate the process of methanogenesis and denitrification, thereby releasing methane and N_2O to the atmosphere, adding up the burden in managing global warming (Jansson and Hofmockel 2020). Increasing wildfire

frequency can intensify the effect of CO_2 elevation. Wildfire can cause depletion of soil carbon. Fire directly attacks the microbial activity by protein denaturation; however, archaea can counteract heat as they possess heat-resistant cell walls. Hinojosa et al. (2019) reported the decrease in proportion of fungi, whereas the proportion of *Actinobacteria* is highly increased post fire in in a Mediterranean shrubland exposed experimentally to drought stress. Moreover, the fungal diversity was initially found to be increasing due to decrease in pH post fire, but later diversity started to decrease due to prolonged fire exposure. Hence, with focused insights into the combinatorial effect of elevated CO_2 with respect to changes in temperature, precipitation is essential to better understand the impact of soil microbes on the health status of the terrestrial ecosystem.

There are several ways by which soil microbes can mitigate the consequence of climate change, namely, sequestration of carbon by necromass (dead microbial biomass) formation, water retainment by production of extracellular polymeric substances (EPS) to mitigate drought stress, PGPR (plant growth-promoting rhizobacteria) inoculation to promote plant growth by producing phytohormones to overcome the stressful conditions, mycological association of plants with beneficial arbuscular mycorrhizal fungi to reduce water stress by producing aquaporins and so on (Jansson and Hofmockel 2020).

15.15 Resistance and Resilience of Soil Microbiome

Disturbance can be defined as the process by which the stability of an environment is negatively affected by various stress factors, viz., natural or anthropogenic stresses (Shade et al. 2012). Based on the time span, environmental disturbances are grouped into two types, namely, pulse disturbance and press disturbance. In pulse disturbance, the time span is short and discrete, whereas press disturbance lasts constantly for a longer period. Soil environment experiences both pulse and press disturbances globally, either naturally or due to anthropogenic actions. For instance, Intergovernmental Panel on Climate Change (IPCC 2007) has predicted the CO_2 elevation and ocean acidification as press disturbance and extremities in weather conditions (elevated rainfall or temperature) and fire frequency as pulse disturbance. Either it is pulse or press disturbance, environment tends to maintain its homeostasis to certain extent by responding to the disturbance through the process of stability (Rykiel 1985). While studying the environmental perturbations, it is essential to understand three key terms, namely, resistance, resilience and functional redundancy (Fig. 15.4).

15.15.1 Soil Resistance

The process by which the microbe, macro-organism (plant or animal) or an environment retains its potential without being affected (intact) by the prevailing stressful condition is termed as resistance. For example, drought-resistant microbes produce

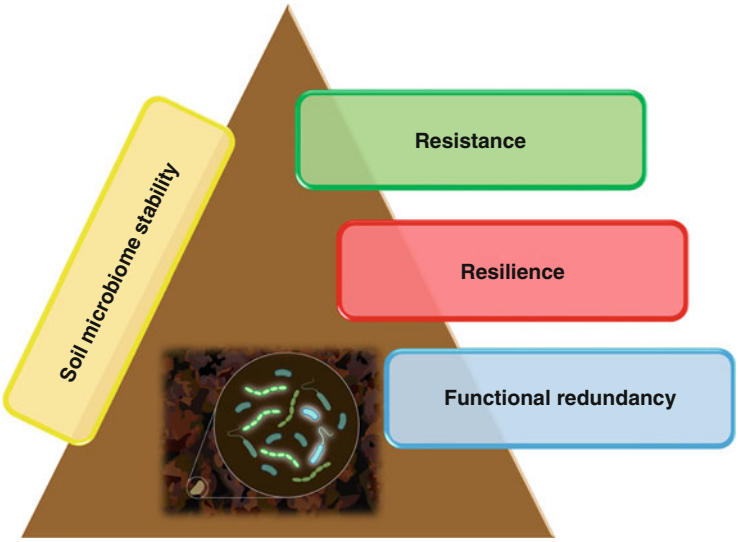

Fig. 15.4 Soil microbiome stability determining factors—resistance, resilience and functional redundancy

EPS to manage desiccation caused due to drought stress (Jansson and Hofmockel 2020). Resistance against environmental disturbance is mainly influenced by the ability of the microbial cell to tolerate the perturbation by acquiring various tolerance mechanisms such as physiological plasticity (ability of the cell to adapt to different environmental conditions in a short period of time), dormancy and so on. Microbial entities in the soil, forming a resistance community that maintains the soil in the undisturbed state (insensitive to environmental disturbances) despite pulse or press disturbance, and the soil in which this phenomenon occurs exerts soil resistance.

15.15.2 Soil Resilience

The process by which the cells or any ecosystem that is recurring back to its original state by overcoming the environmental disturbance is called as resilience (Pimm 1984). Soil resilience studies are gaining focus in the recent years which employs the experimentally induced pulse as well as pressing disturbances. Hartmann et al. (2014) have reported that the soil compaction experimentation by using harvesting machine (cultural practices) showed decrease in soil permeability and water infiltration. In terms of microbial diversity, resilience is limited and there is a significant increase in abundance of sulphur reducers, and fungi is said to be the most affected species (especially ectomycorrhizal fungi; Longepierre et al. (2021). Application of vinasse (an organic fertilizer obtained from sugarcane industry as a by-product in the production of ethanol) as fertilizer into the soil has shown good resilience property of the soil microbiome. In addition to these stresses, metal dumping (Azarbad et al.

2016) and agro-land deposition of organic and inorganic contaminants (Jiao et al. 2019) also showed notable soil resilience.

Resilience is mainly dependent on the R-K strategist microbes. R strategists show rapid growth rate with less resource exploitation, and they are highly resilient but shows less resistance. K strategists in the contrary exhibit comparatively slower growth rate with higher resource utilization, and K-strategists exhibit lesser resilience but higher resistance (Shade et al. 2012). Functional redundancy is the ability of microbes to retain the community functions despite changes in the microbial diversity (Allison and Martiny 2008).

15.16 Metagenomic Tools to Study Soil Microbiome

Improvements in metagenomic analysis have revolutionized the study of microbiomes in various ecosystems of our planet. Metagenomics comprises of various sequencing platforms which can analyse short (Illumina) or long (PacBio and Nanopore platforms) reads. The process of metagenomic analysis involves the following steps: environmental DNA extraction, library preparation, sequencing, quality check, assembly, annotation, taxonomic profiling, binning, statistical analysis, gene prediction, etc. (Fig. 15.5). There are various web-based databases and locally installed software and programs available to analyse the sequenced data (Taş et al. 2021). Metagenomic analysis of soil microbiome involves several bioinformatic tools for its analysis.

15.16.1 Quality Tools

The raw data obtained after high-throughput sequencing is subjected to various quality checks to diminish the sequencing bias by removing primer and adapter sequences, reducing low-quality base calls and contaminating sequence reads. The sequence read quality tools includes the following: (1) FastQC which runs some realistic quality control statistical data, (2) MultiQC tool is the collection of data outcomes from multiple samples and forms as a single report, (3) FastQ Screen

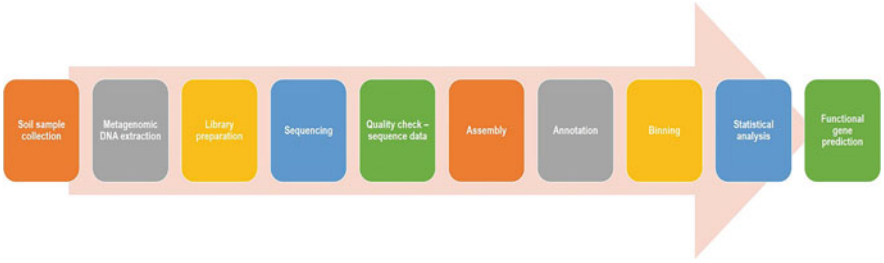

Fig. 15.5 Processes involved in metagenomic analysis of soil

works by aligning the query sequence data against a reference data set, (4) BBDuk tool removes the contaminating sequences with respect to k-mer-based analysis and (5) Khmer tool normalizes the sequences by trimming unwanted sequences by using k-mer analysis (Taş et al. 2021).

15.16.2 Assembly Tools

The high-quality sequence reads obtained after quality check were assembled using the following tools (Taş et al. 2021): (1) CLC Assembler creates assembly based on the de-Bruijn graph, (2) Meta-IDBA aims to contain high- as well as low-abundant genomes through k-mer size-based iteration, (3) MetaVelvet-SL modification of Velvet assembler integrating a support vector machine to improve the performance, (4) megahit exploits the k-mer as well as de-Bruijn strategies to obtain computationally cost-effective analysis tool and (5) MetaSPAdes is the modification of SPAdes for high-throughput sequence assembly. There are several assembly quality check tools such as QUAST (which evaluates genome or gene-centric metagenome assemblies by calculating contig length, N50, GC content), dnAQET (runs by Java programming to determine the contigs or scaffolds with respect to reference data and GenomeQC (which integrates multiple quality parameters to illustrate both gene assembly and annotation).

15.16.3 Annotation Tools

There are several web-based annotation tools available which include EBI metagenomics, MG-RAST, KBase and IMG/M A platforms. Several tools require local installation which include MetAMOS, MOCAT2 and Anvi'o (integrated analysis of genomics, metagenomics, metatranscriptomics, pangenomics, metapangenomics, phylogenomics, microbial population genetics; Taş et al. 2021).

15.16.4 Binning and Metagenome-Assembled Genome Refinement Tools

Binning is the process of grouping contigs with reference to genomes. Metagenome-assembled genome (MAG) refinement is the combinatorial strategy which exploits the metagenomic, metatranscriptomic and metametabolomic data of a particular environment to assemble the genome of a specific organism.

MetaBAT2 clustering is based on binning of contigs by determining pairwise distance. Maxbin2 uses an expectation-maximization algorithm for contig clustering. CONCOCT is a blinded contig binning method utilizing the nucleotide composition, k-mer content and coverage data. GroopM is an automated binning strategy that exploits differential data coverage to acquire high-quality bins. DAS Tool cumulates the reports of various binning algorithms to obtain defined results by

calculating improved and unclassified bins. CheckM is the common tool used to quality check the MAGs (Taş et al. 2021).

15.16.5 Statistical Tools

Metagenomic data analysed with statistical tools are considered to be highly reliable data. The tools include the following: (1) Metastats is the specific statistical method developed particularly to handle two different population data instantaneously (Paulson et al. 2011), (2) LefSe employs the method of genetic biomarker (Segata et al. 2011), (3) ShotgunFunctionalizeR compares the functional roles of genetically identified reads and (4) SourceTracker employs Bayesian method for estimation of the portion of a novel community from a group of source environments (Knights et al. 2011).

15.16.6 Gene Prediction Tool

FragGeneScan is the most predominantly used tools for gene prediction from short reads which combines the sequence error model with that of codon usage statistics to predict gene function. Glimmer-MG utilizes the interpolated Markov models (IMMs) to detect the coding regions. Prodigal helps in prediction of genes in the prokaryotic reference-based genomes as well as metagenome analysis. MetaGeneMark investigates the metagenomic analysis-based protein coding genes. Prokka is the hub for assessing a series of external databases to annotate genomes of bacteria, fungi and viral genomes (Taş et al. 2021).

15.17 Conclusion

Soil microbiomes are extremely diverse, and their constituent communities differ substantially in both form and function between habitats. It has an impact on the substrate and surroundings while also being impacted by it, resulting in a complex whole that is more than the sum of its parts. Many microbiome constituents are indigenous to their specific location, making soils a great reservoir of biodiversity. Soil microbiomes are a promising soil remediation approach. We need to figure out how to manipulate and manage soil microbiomes to improve remediation efficiency while also increasing soil fertility. Advances in community characterization and analysis will continue to shed light on the complexity of this diverse and heterogeneous environment, allowing for a better understanding of how the soil microbiome changes as the climate changes.

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Rhizobacteriome: Plant Growth-Promoting Traits and Its Functional Mechanism in Plant Growth, Development, and Defenses

16

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Abstract

The rhizomicrobiome comprises a wide variety of microorganisms that are essential for microbial colonization and root development in a wide variety of plants. A plant's growth, development, and defense mechanisms would be impossible without the rhizomicrobiome's microbes. In order to develop and operate properly, roots are essential to plants because they give structural support and aid in the intake of water and nutrients. This rhizobacteriome, a diverse bacterial population with particular roles that affect plant health, may be found in plant root exudates due to the complex variety of elements present. There are

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several metabolites produced by the plant-growth-promoting rhizobacteria (PGPR) in the rhizosphere near the plant roots that stimulate the plant's development. Many PGPRs have the ability to solubilize phosphate, fix N2, produce biosynthesis of hydrolytic enzymes (hydrolase), produce phytohormones (phytoestrogens), produce siderophores (antibiotics), and more. Climate change, population growth, and the use of herbicides and insecticides have all had a significant influence on crop productivity in recent decades. Studies show that PGPR can boost plant growth and yield in a variety of species. As a result, PGPR dynamic microorganisms can be used as biofertilizers or biopesticides in agricultural techniques, which is critical to alleviating the urgent call for sustainable production. Rhizobacteriome, in particular PGPR found in the rhizosphere, and their many strategies for enhancing plant production are summarized in this chapter.

Keywords

Microbiome · Plant–microbe interactions · Rhizobacteriome · Biocontrol · PGPR · Rhizobacteria

16.1 Introduction

To decompose organic matter, cycle nutrients, and grow crops in a sustainable manner, the soil microbiome is essential (Chandler et al. 2008; Ahemad et al. 2009). Researchers have found a substantially larger concentration of bacteria in the rhizosphere (the area around the roots; the rhizomicrobiome) than in free soil. In the rhizosphere, 1011 cells per gram of soil contain more than 30,000 different bacterial species, according to Berendsen et al. (2012). Figure 16.1 shows that the roots of plants release a wide range of nutrients, including sugars; vitamins; amino acids; enzymes; nucleosides; organic acids; inorganic ions; organic compounds; and gaseous molecules (Fig. 16.1) (Dakora and Phillips 2002). In addition, the microbiome in the rhizosphere is fed by these tiny root exudate chemicals (Babalola 2010; Carvalhais et al. 2015). Rhizobacteria, or the rhizobacteriome, is the community of rhizobacteria living in the rhizosphere, where they are known for their role in promoting plant growth and rhizoremediation, which is why they are referred to as plant growth-promoting bacteria or PGPB (Olanrewaju et al. 2017) or plant growth-promoting rhizobacteria (PGPR). Rhizobacteria that promote plant health are also known as PGPR or nodule-promoting rhizobacteria (NPR).

Plants and PGPR can interact in a variety of ways, including an endophytic, epiphytic, or symbiotic connection with the roots and the surrounding soil (Souza et al. 2015). When the PGPR colonizes root cells, it generates an environment that is ideal for its activity. The rhizobacteriome uses the same processes to promote plant development as the endophytic bacteria (Santoyo et al. 2016). Non-symbiotic PGPR

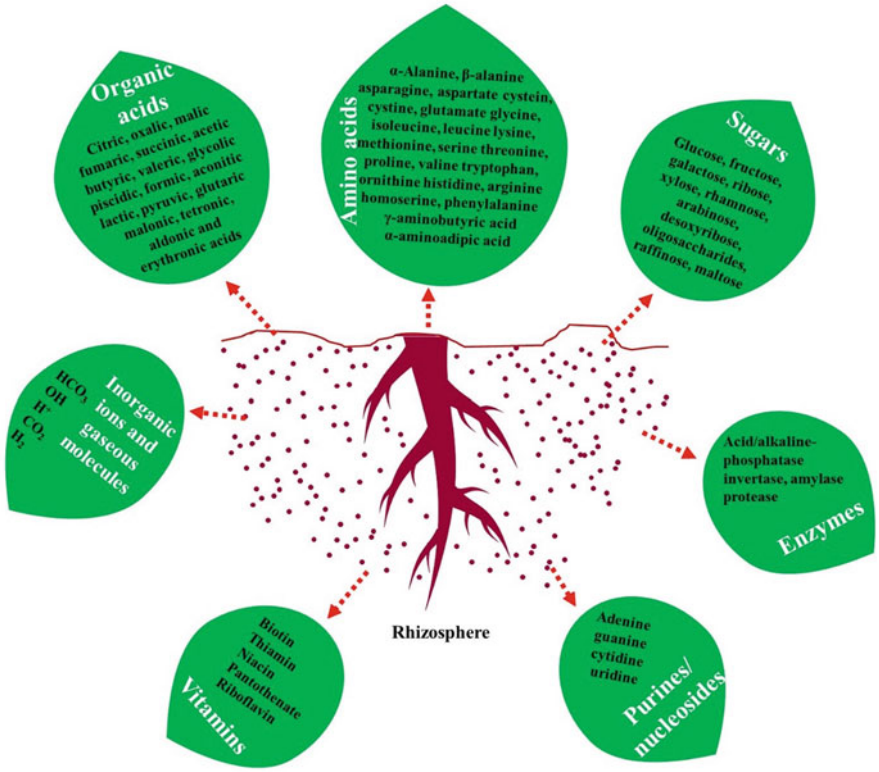

Fig. 16.1 Secretion of different organic compounds in root exudates of different plant species

such as Azotobacter, Klebsiella, Pseudomonas, Bacillus, Azospirillum, and Azomonas have also been frequently employed as biocontrol agents for improving plant development (Ahemad and Kibret 2014). Biocontrol agents and biofertilizers based on many PGPR are now commercially available (Reed and Glick 2013; Calvo et al. 2014; Olanrewaju et al. 2017). PGPR has been widely documented for promoting and managing plant development, but its whole mechanism has yet to be established. In addition, there is a lack of consistent outcomes in laboratory, greenhouse, and field research when it comes to the application of PGPR for sustainable plant development (Gouda et al. 2018). The most recent research on PGPR and its processes for promoting plant development is presented in this chapter.

16.2 Rhizosphere: A Warehouse of Rich Microbiomes

Known as the "warehouse" of soil microorganisms, the rhizosphere is a dynamic area of soil that surrounds the root zone and is immediately controlled by root exudations (Walker et al. 2003). In addition, the term "rhizobacteria" refers to a group of bacteria capable of colonizing the root system of a plant or animal (Kloepper et al. 1991). In addition to supporting the plant's structure, the root system is a chemical factory that creates a wide range of substances (Walker et al. 2003). The rhizomicrobiome comprises an immense number of different heterotrophic microbial communities in soil is attracted to plant roots by these tiny chemicals (Ahemad and Kibret 2014). A soil environment's chemical composition is influenced by the physical qualities of the compounds, the kind of plant species, and the sorts of microbes present (Kang et al. 2010). Microbes in the rhizosphere transform tiny carbon and nitrogen compounds released by plants into microbe-oriented chemicals that plants may use to grow and develop (Kang et al. 2010). Root exudation, according to Marschner (1995), transfers 5–21% of the carbon ingested by photosynthesis to the rhizosphere environment. Therefore, the rhizosphere can be recognized by plant roots and its connected root hairs and root secretions (Dessaux et al. 2009). Three different parts such as the rhizosphere soil, the rhizoplane, and the root only are recognized in the rhizosphere, of which, the microbial function affected by substances secreted in the soil of the rhizosphere is regulated by roots. Besides, the root surface known as rhizoplane is where the soil particles firmly attach for microbial colonization (Barea et al. 2005), whereas numerous microorganisms that can colonize plant inner tissues are known as endophytes (Santoyo et al. 2016). The rhizosphere environment has a 1000× higher concentration of bacteria than free soil (Gouda et al. 2018). Soil microbiome must be competent with rhizomicrobiome for nutrients produced by root to sustain their healthy root environment. Plant and the roots interactions are crucial to obtaining macronutrients and micronutrients from the soil. These interactions are also advantageous to the plants and the soil microbiome.

16.3 Different Types of PGPR

Various bacterial species are found in the rhizobacteriome, which is beneficial to plants. There are varying degrees of closeness and proximity between plant roots and the rhizosphere PGPR. PGPR can be classified as extracellular (ePGPR), which occurs outside of cells, or intracellular (iPGPR), which occurs within cells, based on their position in the rhizosphere (Viveros et al. 2010). Rhizobacteria occurring on the rhizoplane or in the root cortex intracellular spaces can be categorized as ePGPR. Several bacterial genera are classified as ePGPR such as *bacillus, Pseudomonas, Micrococcous, Serratia, Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium,* and *Cellulomonas flavigena*. PGPR which occurs inside specialized nodular structures of root cells is classified as iPGPR. These bacteria usually produce specific structures such as nodules and reside inside them. Bacteria that belong to iPGPR consist of

rhizobium, Allorhizobium, Sinorhizobium, Bradyrhizobium, Mesorhizobium, Azorhizobium, and *Frankia*. Growth facilitated by nitrogen fixation in higher plants is one of the positive influences of endophytic iPGPR (Bhattacharyya and Jha 2012). In addition, the actinomycetes group of bacteria is generally recognized for the production of antibiotics and other pharmaceutically important molecules (Genilloud 2017). However, actinobacteria genera *Streptomyces, Micromonospora, Streptosporangium,* and *Thermobifida* have widely been reported for their immense potential to control various plant pathogens therefore prospects to use of effective biocontrol agents (Bhattacharyya and Jha 2012). Recently, Vurukonda et al. (2018) reviewed and summarized the perspective of actinomycetes especially *Streptomyces* species-derived bioactive compounds and commercial products that are being used as biofertilizers, and biopesticides, or consortiums of plant beneficial microbes to suppress plant pathogens. Several studies have reported and experimentally demonstrated the ability of actinomycetes in plant growth promotion, hence focusing on actinomycetes as a biocontrol agent will enhance agricultural production and ensure food security (Vurukonda et al. 2018).

16.4 Functional Mechanisms of PGPR in Plant Growth Enhancement

No single mechanism has been influenced to induce plant growth as the rhizobacteriome produces abroad range of substances that influence plant growth-promoting (PGP) attributes and it varies among the types of bacterial species and host plants (Table 16.1). Besides, different pH, soil temperature, and environmental conditions are also critical for PGPR activity toward plant growth promotion (Olanrewaju et al. 2017). In general, PGPR enhances plant growth by influencing it directly and indirectly (Glick 1995). Production of various plant hormones (auxin, cytokinin, and gibberellin), enzymes (ACC deaminase), and other traits such as siderophore production, nitrogen fixation, and phosphorous solubilization are recognized as direct mechanisms as they directly influence the plant growth (Olanrewaju et al. 2017). Indirect mechanisms are identified as bacterial attributes that suppress the growth of different phytopathogenic fungi and bacteria. The bacterial attributes implied in indirect mechanisms comprise the production of antibiotics, siderophores,1-aminocyclopropane-1-carboxylate deaminase, lytic enzymes and hydrogen cyanide, competition, quorum quenching, and induced systemic resistance (Olanrewaju et al. 2017). None of the individual strains of PGPR is capable of producing all the bacterial traits involved in both direct and indirect mechanisms. However, a few or some traits will take part in prompting plant development(Saharan and Nehra 2011; Reed and Glick 2013).

Table 16.1 Rhizobacteriome and their plant growth-promoting (PGP) traits in plant growth and productivity (Ahemad and Kibret 2014; Gouda et al. 2018)

PGPR strain	Host plant	PGP traits and effects in plants	Reference
Pseudomonas cepacia	*Phaseolus vulgaris*	Inhibit *Sclerotium rolfsii*	Montano et al. (2014)
	Gossypium hirsutum	Prevent *Rhizoctonia solani*	Montano et al. (2014)
	Cucumis sativus	Inhibit *Pythium ultimum*	Montano et al. (2014)
Pseudomonas fluorescens	*Medicago sativa*	Increase metabolism, sequester cadmium from solution and biodegradation	Ramadan et al. (2016)
	Triticum aestivum	Inhibit *Fusarium culmorum*	Santoro et al. (2016)
	Phaseolus vulgaris	Prevent halo blight	Ramadan et al. (2016)
	Gossypium hirsutum	Inhibit damping off of cotton	Ramadan et al. (2016) and Santoro et al. (2016)
	Maize	Increase plant height, seed weight	Gholami et al. (2009)
	Maize	Promote plant growth, facilitate soil metal mobilization, enhance Cr and Pb uptake	Braud et al. (2009)
	Soybean	Increase plant growth	Gupta et al. (2005)
	Peanut	Enhance pod and haulm yield and nodule dry weight over the control	Dey et al. (2004)
	Alfalfa	Improve Cu and Fe translocation from root to shoot	Carrillo-Castaneda et al. (2003)
	Pea	Production of ACC-deaminase	Zahir et al. (2008)
Pseudomonas putida	*Cynara scolymus*	Nitrogen fixation, increase plant yield	Jahanian et al. (2012)
	Arabidopsis thaliana	Enhance utilization of plant secondary metabolites	Ahemad and Khan (2012b)
	Maize	Increase plant height, seed weight, shoot dry weight, leaf area, and number of seeds	Gholami et al. (2009)
	Lectuca sativa L.	Significant increase in shoot and root length	Rekha et al. (2007)
	Mung bean	Stimulate the plant growth, reduce Pb and Cd uptake	Tripathi et al. (2005)

(continued)

Table 16.1 (continued)

PGPR strain	Host plant	PGP traits and effects in plants	Reference
	Vigna radiata L.	Ethylene production	Mayak et al. (1999)
Pseudomonas aeruginosa	Maize	Improve plant growth, facilitate soil metal mobilization, enhance Cr and Pb uptake	Braud et al. (2009)
	Black gram	Decrease plant cadmium accumulation, widespread rooting, and improved plant growth	Ganesan (2008)
Pseudomonas aeruginosa	Mustard and pumpkin	Stimulate plant growth, reduce Cd uptake	Sinha and Mukherjee (2008)
	Solanum lycopersicum L., *Abelmoschus esculentus, Amaranthus* sp.	Increase dry biomass	Adesemoye et al. (2008)
	Cicer arietinum	Kindle potassium and phosphorus uptake	Ahemad and Kibret (2014)
	Vigna radiata	Inhibit root knot establishment	Ngumbi and Kloepper (2016)
Pseudomonas pieketti	Peanut	Enhance pod and haulm productivity and nodule dry weight	Dey et al. (2004)
Pseudomonas jessenii	Chickpea	Nitrogen fixing and P-solubilizing	Valverde et al. (2006)
Pseudomonas gladioli	*Gossypium hirsutum*	Resistance against *Helicoverpa armigera*	Ross et al. (1995)
Pseudomonas spp.	Soybean, mung bean, wheat	Promote growth of plants	Gupta et al. (2002)
	Brassica napus	Enhance plant growth and increase cadmium accumulation	Sheng and Xia (2006)
	Rice, maize	Biocontrol control activity	Lawongsa et al. (2008)
	Chickpea	Increase fresh and dry weight of plants at 2 mM nickel	Tank and Saraf (2009)
	Brassica juncea	Increase the biomass	Ma et al. (2009a)
	Soybean, wheat	Increase soil enzyme actions, total yield, and nutrient absorption	Sharma et al. (2011)
	Alyssum serpyllifolium, Brassica juncea	Enhance biomass under stress	Ma et al. (2011)

(continued)

Table 16.1 (continued)

PGPR strain	Host plant	PGP traits and effects in plants	Reference
	Greengram	Increase plant dry weight, nodule numbers, total chlorophyll content, leghaemoglobin, root N, shoot N, root P, shoot P, seed yield, and seed protein	Ahemad and Khan (2010a, 2011b, 2012a)
	Triticum aestivum	Prevent till disease	Richa et al. (2013)
	Dianthus caryophyllus	Inhibit *Fusarium* wilt	Rathore (2015)
Bacillus subtilis	*Brassica juncea*	Facilitate Ni accumulation	Zaidi et al. (2006)
	Hordeum vulgare	Prevent powdery mildew	Oyedele and Ogunbanwo (2014)
	Gossypium hirsutum	Inhibit from *Meloidogyne incognita* and *M. arenaria*	Oyedele and Ogunbanwo (2014)
Bacillus megaterium	*Camellia sinensis*	Phosphate solubilization	Stefanescu (2015)
Bacillus megaterium	*Brassica juncea*	Protect plant from metal toxicity, stimulate plant growth	Wu et al. (2006)
Bacillus licheniformis	*Piper nigrum*	Protection from *Myzus persicae*	Kumar et al. (2015)
Bacillus edaphicus	*Brassica juncea*	Stimulate plant growth, facilitate soil Pb mobilization, enhance Pb accumulation	Sheng et al. (2008)
Bacillus weihenstephanensis	*Helianthus annuus*	Increase biomass and the accumulation of Cu and Zn in the root and shoot systems	Rajkumar et al. (2008)
Bacillus amyloliquefaciens	*Solanum lycopersicum*	Prevent tomato molt virus	Oteino et al. (2015)
Bacillus circulans	*Vigna radiata*	Phosphate solubilization	Oteino et al. (2015)
Bacillus mucilaginosus	*Piper nigrum, Cucumis sativus*	Improve potassium intake capacity	Liu et al. (2012)
Bacillus spp.	Chickpea	Improve growth, nodule formation, chlorophyll, leghaemoglobin, seed production, and grain protein	Wani and Khan (2010)
	Rice	Increase the root and shoot growth	Beneduzi et al. (2008)
Azospirillium brasilence	*Festuca arundinacea*	High plant tolerance to polycyclic aromatic hydrocarbons	Orlandini et al. (2014)

(continued)

Table 16.1 (continued)

PGPR strain	Host plant	PGP traits and effects in plants	Reference
	Common bean	Root growth increase	Remans et al. (2008)
Azospirillum amazonense	Rice	Grain dry matter accumulation	Rodrigues et al. (2008)
Azotobacter chroococcum	*Brassica juncea, Triticuma estivum*	Stimulate plant growth and phosphate solubilization	Narozna et al. (2014) and Bhattacharyya and Jha (2012)
	Cotton	Increase seed productivity, plant height, and microbial population in soil	Anjum et al. (2007)
Azotobacter aceae	*Fagopyrum esculentum*	Nitrogen fixation	Bhattacharyya and Jha (2012)
Bradyrhizobium japonicum	*Glycine max*	Phosphate solubilization	Rathore (2015)
Bradyrhizobium sp.	*Vigna radiata*	Increase the nodule numbers, leghaemoglobin, seed harvest, and enhanced grain protein	Wani et al. (2007)
Bradyrhizobium sp.	*Lupinus luteus*	Increase both biomass, nitrogen content, and accumulation of metals	Dary et al. (2010)
Mesorhizobium sp.	Chickpea	Increase nodulation and leghemoglobin content, root N, shoot N, root P, shoot P, seed productivity, and seed protein	Ahemad and Khan (2009, 2010b, c)
Rhizobium leguminosarum	*Phaseolus vulgaris*	Phosphate solubilization	Ahemad and Kibret (2014)
Rhizobium sp.	Lentil	Increase nodule numbers and leghemoglobin content, root N, shoot N, root P, shoot P, seed harvest, and high seed protein	Ahemad and Khan (2010b, 2010c, 2011a)
Rhizobium phaseoli	*Vigna radiata* L.	Increase the plant height, number of nodules per plant, plant biomass, grain yield, and grain nitrogen concentration	Zahir et al. (2010)
Paenibacillus polymyxa	Pepper	Increase the biomass of plants and elicit induced systemic resistance against pathogen	Phi et al. (2010)
Rhodococcus sp., *Flavobacterium, Variovox paradoxus*	*Brassica juncea*	Stimulate root elongation	Belimov et al. (2005)

(continued)

Table 16.1 (continued)

PGPR strain	Host plant	PGP traits and effects in plants	Reference
Ochrobactrum intermedium	Sunflower	Increased plant growth and decreased Cr uptake	Faisal and Hasnain (2005)
Brevibacillus	*Trifolium repens*	Enhance plant growth and nutrition of plants	Vivas et al. (2006)
Sinorhizobium sp.	*Brassica juncea*	Increase the efficiency of lead phytoextraction	Di Gregorio et al. (2006)
Klebsiella pneumonia	*Triticum aestivum*	Increase the root length and shoot length	Sachdev et al. (2009)
Achromobacter xylosoxidans	*Brassica juncea*	Improve Cu uptake by plants and increased the root length, shoot length, fresh weight, and dry weight	Ma et al. (2009b)

16.5 Direct Mechanisms: Rhizobacteriome Traits Involved in PGP

16.5.1 Biosynthesis of Plant Hormones or Growth Regulators

A wide range of organic chemicals, including auxins, cytokines, gibberellins, and brassinosteroids, is generated by plants at low quantities to stimulate and control plant development (Damam et al. 2016). Lately developing roots and root hairs release a variety of plant growth regulators (PGRs) that favorably affect the microbes that live inside their environment (Sureshbabu et al. 2016). There are a surprising number of phytohormone-producing bacteria in the plant's rhizosphere. Most of the microorganisms that live in the rhizosphere are capable of producing auxins in the form of secondary compounds (Patten and Glick 1996). According to Souza et al. (2013), roughly 80% of the rhizobacteriome was sourced from rice that generated indole-containing chemicals. Biosynthesis of auxins, indole-3-acetic acid (IAA), Indole-3-acetamide, indole-3-pyruvate, and indole-3-acetaldehyde is carried out by a variety of microorganisms. In plant–microbe interactions, indolic chemicals play a critical function, varying from disease development to phytostimulation (Spaepen et al. 2007). Higher levels of indolic chemicals are produced by PGPR colonized by the rhizosphere than by free soil and endophytes (Khalid et al. 2004; Costa et al. 2014). L-tryptophan, the primary precursor for IAA biosynthesis, is found in root exudates and is essential for IAA production. Indole-3-pyruvic acid (IAA) was mostly produced by PGPR via the indole route. L-tryptophan is a key component of this alternate route. IAA is synthesized by plant pathogens through the indol-acetoamide pathway (Souza et al. 2015). It is possible for some species of *Erwinia* and *Agrobacterium* to manufacture IAA through the indole-3-pyruvic acid and indole-3-acetic aldehyde pathways. However, indolic chemicals are produced by pseudomonads and *Azospirillum* spp. via indole-3-acetic aldehyde (Glick 2012). The

indole-3-acetamide is synthesized by a variety of bacteria, including the pathogens *P. fluorescens,* P. putida, and *Agrobacterium tumefaciens*. To make IAA from tryptophan, the cyanobacterium *Synechocystis* creates indole-3-acetonitrile by turning tryptophan into indole (Ahemad and Kibret 2014). It is crucial for nodule development because IAA causes cell division, differentiation, and the creation of vascular bundles in plants. When it comes to nodule development in legumes, a high concentration of auxin is essential (Glick 2012; Spaepen et al. 2007). There have been reports of IAA synthesis in all *Rhizobium* species (Ahemad and Khan 2011a; Ahemad and Khan 2012a). *Bradyrhizobium japonicum* and Pseudomonas putida, among others, create IAA and use it in their metabolic processes, which may promote plant development and protect plants against infection (Jensen et al. 1995; Leveau and Lindow 2005).

There have been reports of the synthesis of cytokines and gibberellins by many soil bacteria, including PGPR (Tien et al. 1979; Williams and de Mallorca 1982; Taller and Wong 1989; Timmusk et al. 1999; Olanrewaju et al. 2017). Bacteria, algae, and higher plants are all known to contain cytokinins. It has been shown that many rhizobacteria, including those belonging to the *Pseudomonas* and *Rhizobium* families, as well as those belonging to the *Paenibacillus* and *Azotobacter* families, have been found to be capable of producing cytokinins in the cell-free media (Atzorn et al. 1988; Yahalom et al. 1990; Lorteau et al. 2001; Joo et al. 2005; Kang et al. 2009). However, nothing is known about the function of cytokinins generated by bacteria. This normally occurs in the root tips, where cytokinins are produced before being transported down the xylem to the plant's stems. Cell division, differentiation of xylem and chloroplasts, elongation of roots, seed germination, growth of flowers and fruits, signaling of nutrient mobilization, senescence process, and plant–pathogen interactions are all regulated by this protein (Sakakibara 2006; De Rybel et al. 2016). Most significant phytohormone and transducer of elicitor signals utilized to stimulate plant growth, such as seed germination and blooming and stem lengthening and fruit formation and increasing photosynthesis by decreasing chlorophyll breakdown are gibberellins (You et al. 2012; Zaidi et al. 2015; Khan et al. 2015). So far, bacteria have produced four gibberellins: GA1, GA3, GA4, and GA20 (Gupta et al. 2016). GA1 and GA4 have been shown to be the most active of these hormones, although nothing is known about their function or bioactivity (Nelson and Steber 2016). In order to produce gibberellin, several PGPR species have been identified, including Rhizobia sp. species, Azotobacter species, Bacillus species, Achroma sp. species, glucobacter diazotrophicus species, calcoaceticus species, herbaspirillum seropedicae species, and azospirillum species (Dodd et al. 2010; Deka et al. 2015).

16.5.2 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase Activity

Low amounts of ethylene, a vital gaseous phytohormone for plant growth, development, and senescence, are generated in the majority of higher plants. Plants are

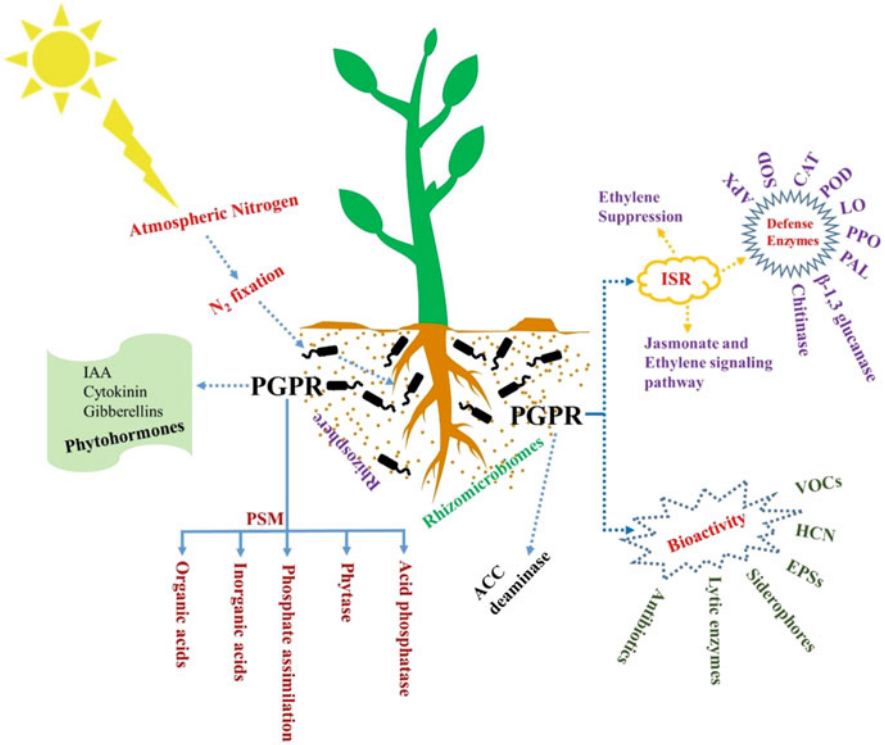

Fig. 16.2 Schematic representation of the effect of plant–microbiome interactions for plant growth promotion

protected from a variety of biotic and abiotic stressors as well (Abeles et al. 1992; Shaharoona et al. 2006; Saleem et al. 2007). When plants are exposed to environmental stresses such as drought, salt, and waterlogging and biotic stresses such as predator damage, pathogen infection, and organic and inorganic pollution, their roots and ultimately the entire plant suffer. Ethylene is a stress-responsible hormone (Barnawal et al. 2012; Ali et al. 2014). Ethylene concentrations in plants have also been studied extensively. Reduces plant ethylene levels by inhibiting the bacterial enzyme ACC deaminase that is found in soil (Glick 2005; Jalili et al. 2009; Farajzadeh et al. 2012). Plant 1-aminocyclopropane-1-carboxylate is converted to -ketobutyric acid and ammonia by bacterial ACC deaminase (Arshad et al. 2007; Saleem et al. 2007) which regulates ethylene production in higher plants (Fig. 16.2). As a result, microbial communities in the soil use ACC deaminase to hydrolyze the extra quantity of ACC released by plant roots, helping to keep the rhizosphere and the environment free of ACC (Glick et al. 2007). According to Yim et al. (2013), high levels of ACC may have an impact on plant development by triggering senescence, chlorosis, and leaf abscission in plants.

Soil microbiome activity, including PGPR, is evaluated by the amount of ACC deaminase produced in the soil (Glick 2005). There may be an overall reduction in the amount of ethylene manufactured by the plant if there is a large concentration of ACC deaminase-producing microorganisms on a wide variety of plant surfaces such as the rhizosphere, phyllosphere, and endophytes. Ethylene levels can be reduced by bacteria that produce tiny quantities of ACC deaminase, which bind to particular plants and are found in certain tissues (Glick 2005; Souza et al. 2015). The ACC deaminase is produced by a number of bacterial species from *Enterobacter, Acinetobacter* and *Bacillus, Pseudomonas, Azospirillum* and *Bradyrhizobium,* as well as *Ralstonia* and *Burkholderia* (Shaharoona et al. 2006; Bal et al. 2013; Ahemad and Kibret 2014; Souza et al. 2015). ACC 1-aminocyclopropane-1-carboxylate, which is the ethylene precursor, is successfully used by these bacterial species, which catabolize it into 2-oxobutanoate and ammonia (Arshad et al. 2007; Souza et al. 2015). In order to alleviate stress on plants and improve their health, using isolated PGPR containing active ACC deaminase from rhizosphere, phyllosphere, or endophytes might be a viable option (Glick 2010).

16.6 Biological Nitrogen Fixation

For plant development, nitrogen is one of the most important nutrients. There is a lot of nitrogen in the Earth's atmosphere, yet plants are unable to use it. Approximately two-thirds of the world's nitrogen is fixed by bacteria living symbiotically with plants and non-symbiotic microbes (the phytomicrobiome) (Shridhar 2012). Through the biological nitrogen fixation process, nitrogenase microorganisms transform atmospheric nitrogen into plant-usable compounds (Fig. 16.2). Symbiotic PGPR, like rhizobia with all legume plants and Frankia species with non-leguminous trees, is the most common nitrogen-fixing bacterium (Ahemad and Khan 2012a; Zahran 2001). Nitrogen-fixing symbiotic bacteria can improve soil quality and stimulate nodule development by inoculating the soil with them. Azoarcus, Burkholderia, and Frankia are some of the bacteria that can be found in soils that are infected with these microorganisms. *Rhizobium spp., Azorhizobium* spp., *Allorhizobium spp.,* and *Sinorhizobium* are some of the other bacteria that can be found in soil that are infected with these microorganisms (Babalola 2010; Perez-Montano et al. 2014; Ahemad and Kibret 2014; Turan et al. 2016). The quantity of nitrogen provided to the host plant by non-symbiotic nitrogen-fixing bacteria is minimal, therefore the two organisms must work together (Glick 2012). Free-living, associative, and endophytic bacteria are the most common types of non-symbiotic nitrogen fixers (Ahemad and Kibret 2014). *Anabaena, Nostoc, Azospirillum*, *Azotobacter, Gluconoacetobacter diazotrophicus*, and Azocarus are some examples of non-symbiotic nitrogen-fixing bacteria (Bhattacharyya and Jha 2012). Nitrogenase (nif) genes control biological nitrogen fixation in nitrogen-fixing bacteria, whether they are symbiotic or not. Iron protein, iron-molybdenum cofactor, electron donation, and other regulatory genes that are critical for enzyme production and control are triggered by gene nif in conjunction with other structural genes (Reed et al.

2011). The majority of nif genes in nitrogen-fixing bacteria are found in a cluster of more than 20–24 kb with seven operons expressing 20 different proteins (Glick 2012). A complicated regulatory mechanism has made genetic methods to improving nitrogen fixation confusing. Finding the right N2-fixing PGPR from the rhizosphere and using it to inoculate crops or apply it to crops and fields would unquestionably improve the soil's fertility, promote plant development, and reduce numerous phytopathogens.

16.7 Phosphate Solubilization

One of the most important nutrients for plants is phosphorous (P), which makes up less than 1% of the plant's dry weight. Plant development and production are negatively impacted by a lack of P in the soil. At the molecular level, phosphorous is involved in the production of macromolecular structures like those found in the phospholipid and nucleic acid cycles, as well as the energy transfer and signal transduction pathways (Khan et al. 2009; Richardson and Simpson 2011; Anand et al. 2016). Soil has a profound amount of P, about 400–1200 mg per kg of soil, of which approximately 99% of them are insoluble and precipitated as either an inorganic or an organic form. As an inorganic mineral known as apatite, it is difficult for plants to absorb the accessible forms of P in soils, such as inositol phosphate and phosphomonoester and phosphodiester (Glick 2012). Phosphate ions are highly reactive and undergo P fixation where they react with other minerals in the soil such as aluminum, iron oxides, and calcium resulting in insoluble compounds (Mahdi et al. 2012). P fixation predominates in acidic soils (reacting with oxides of iron, aluminum, and manganese) and in alkaline soils (reacting with calcium), resulting in unavailability to plants (Malhotra et al. 2018).

Plants may absorb both monobasic (H_2PO_4) and dibasic (HPO_4^{2-}) soluble forms of P. (Bhattacharyya and Jha 2012). As a result, the use of phosphatic fertilizers to soils lacking in P is a standard agricultural practice. Although plants may absorb a limited amount of phosphatic fertilizer supplied to soil, the remainder stays as insoluble complexes that cannot be absorbed by plants (Mckenzie and Roberts 1990). As a result, the cost of applying P fertilizers to the soil is high. In addition, it poses a danger to both human and environmental health. There is a pressing need to find a long-term solution for increasing agricultural yields on soils with low P. Soil microbes known as phosphate solubilizing bacteria may be found in this setting (Alori et al. 2017), which is one of the key properties of PGPR. It is possible to convert insoluble organic and inorganic P into more readily accessible forms of P that can be utilized by plants and are not damaging to the environment and are a viable alternative to chemical fertilizers (Khan et al. 2006). Phosphorus inorganic in soil can be dissolved in organic acids synthesized by soil microorganisms such as bacteria, fungus, and actinomycetes (Table 16.2) (Sharma et al. 2013). By producing organic acids, siderophores, and hydroxyl ions, bacteria have dissolved inorganic soil phosphates such as calcium, iron, and aluminum phosphates (Jones 1998; Chen et al. 2006; Rodriguez et al. 2006; Sharma et al. 2013). *Klebsiella, Enterobacter*, and

Table 16.2 Different groups of phosphate solubilizing microorganisms (PSM) and their organic acids production

Different PSM (Sharma et al. 2013)			
Bacteria	Fungi	Actinomycetes	Cyanobacteria
Alcaligenes sp., *Aerobactor aerogenes, Achromobacter* sp., *Actinomadura oligospora, Agrobacterium* sp., *Azospirillum brasilense, Bacillus* sp., *Bacillus circulans, B.cereus, B. fusiformis, B. pumils, B. megaterium, B. mycoides, B. polymyxa, B. coagulans, B. chitinolyticus, B. subtilis, Bradyrhizobium* sp., *Brevibacterium* sp., *Citrobacter* sp., *Pseudomonas* sp., *P. putida, P. striata, P. fluorescens, P. calcis, Flavobacterium* sp., *Nitrosomonas* sp., *Erwinia* sp., *Micrococcus* sp., *Escherichia intermedia, Enterobacter asburiae, Serratia phosphoticum, Nitrobacter* sp., *Thiobacillus ferroxidans, T. thioxidans, Rhizobium meliloti,* and *Xanthomonas* sp.	*Aspergillus awamori, A. niger, A. tereus, A. flavus, A. nidulans, A. foetidus, A. wentii. Fusarium oxysporum, Alternaria teneius, Achrothcium sp. Penicillium digitatum, P. lilacinium, P. balaji, P. funicolosum, Cephalosporium* sp., *Cladosprium* sp., *Curvularia lunata, Cunnighamella, Candida* sp., *Chaetomium globosum, Humicola inslens, Humicola lanuginosa, Helminthosporium* sp., *Paecilomyces fusisporous, Pythium* sp., *Phoma* sp., *Populospora mytilina, Myrothecium roridum, Morteirella* sp., *Micromonospora* sp., *Oideodendron* sp., *Rhizoctonia solani, Rhizopus* sp., *Mucor* sp., *Trichoderma viridae, Torula thermophila, Schwanniomyces occidentalis,* and *Sclerotium rolfsii*	*Actinomyces* and *Streptomyces*	*Anabena* sp., *Calothrix braunii, Nostoc* sp., and *Scytonema* sp.
Organic acids produced by PSM (Alori et al. 2017)			
α-Ketobutyric acid, tartaric acid, fumaric acid, glycoxalic acid, malic acid, citric acid, oxalic acid, acetic acid, isobutyric acid, isovaleric acid, itaconic acid, 2-ketogluconic acid, propionic acid, lactic acid, succinic acid, gluconic acid, aspartic acid, maleic acid, glutamic acid, glycolic acid, and malonic acid			

Pantoea rhizobacteria successfully dissolve calcium phosphate in soil more effectively than other forms of inorganic phosphorus (Chung et al. 2005). Solubilizing bacteria employ acids such as carboxylic and citric, as well as succinic and propionic to break down inorganic phosphates found in soil and slurry (Rodriguez and Fraga 1999; Souza et al. 2015). The capacity to dissolve tricalcium phosphate was found in 101 out of 336 rice plant-isolated bacteria. *Cronobacter, Cedecea,* and *Enterobacter* were some of the bacterial genera detected in this investigation. The bulk of bacteria

capable of dissolving phosphates are found in plant roots and rhizosphere soil (Ambrosini et al. 2012; Farina et al. 2012; Costa et al. 2013; Granada et al. 2013; Souza et al. 2014).

A number of PGPR genera including such *Pseudomonas, Bacillus, Enterobacter*, *Flavobacterium, Beijerinckia, Burkholderia, Arthrobacter, Azotobacter, Microbacterium, Erwinia, Rhizobium, Mesorhizobium, Serratia,* and *Rhodococcus* have been reported as potential phosphate solubilizing bacteria and have been used in sustainable agriculture as soil biocontrol agents to promote plant growth and productivity (Oteino et al. 2015). Some studies have confirmed that endophytic bacteria are also involved in phosphate solubilization. Parmar and Sindhu (2013) reported that *Mesorhizobium* spp. isolated from chickpea nodules showed significant phosphate solubilizing characters. An endophytic bacterium, *Pantoea dispersa* was isolated *Manihot esculenta* (cassava) roots, effectively solubilizes calcium phosphate, iron phosphate, and aluminum phosphate through the production of salicylate, benzene-acetic, and other organic acids (Chen et al. 2014). Therefore, the involvement of PGPR in soil and plants will certainly improve the P deficient soil and the release of readily available P for plant growth.

16.8 Siderophores Production

In addition to being a critical element for plants and bacteria, siderophores are tiny peptide molecules (400–1500 Da) (Goswami et al. 2016). The metabolic processes of carbon absorption, cellular respiration, chlorophyll production, and N_2 fixation all rely on the presence of iron (Fe) (Dixon and Kahn 2004). When it comes to soils that aren't easily digested by bacteria or plants, iron is the fourth most common element. As a result of its intractable nature, ferric ion (Fe+3) is only available in restricted quantities to living organisms for absorption and use (Ma 2005). Molecular siderophores with a high affinity for Fe+3 are produced by soil microbiomes, as are membrane receptors capable of binding the iron-siderophore complex. Microorganisms in iron-starved habitats are able to absorb iron by this mechanism (Neilands 1981; Hider and Kong 2010). More than 500 siderophore-compounds produced by microbes have been discovered thus far, with the chemical structures of 270 of these compounds being known (Hider and Kong 2010). The majority of siderophores are generated by plant-associated bacteria, according to several studies. Enterobacter and Burkholderia isolated from rice roots were shown to produce considerable amounts of siderophores, according to Souza et al. (2013, 2014). Costa et al. (2014) described that among the PGPR *Grimontella, Burkholderia,* and *Enterobacter,* species exhibit high siderophore production whereas species belonging to *Rhizobium, Klebsiella, Herbaspirillum, Stenotrophomonas,* and *Citrobacter* synthesis low amount of siderophores. PGPR produces siderophores to prevent the growth of phytopathogens. Due to its high iron affinity traits, siderophores strongly bind to a majority of the Fe^{3+} available in the rhizosphere soil, so either the host plant or rhizosphere bacterial communities (rhizobacteriome) acquire sufficient iron for their growth. In addition, this leads to depletion of iron in

the rhizosphere soil, hence it would be unable for the plant pathogens to proliferate in that environment. Therefore, the efficacy of biocontrol depends upon the magnitude of the PGPR siderophores and their affinity for iron (Kloepper et al. 1980).

16.9 Indirect Mechanisms: Rhizobacteriome Traits Involved in PGP

Rhizobacteriome especially PGPR acts as biocontrol agents and is the key player in the indirect mechanism of plant growth promotion (Glick 2012). PGPR produces repressive biomolecules against plant pathogens that naturally boost host plant resistance (Singh and Jha 2015). In addition, the involvement of PGPR in the indirect mechanism includes various cell wall degrading enzymes, a wide array of antibiotics against phytopathogens, synthesis of siderophores, volatile organic metabolites, exopolysaccharides, and induction of systematic resistance against various pathogens and pests (Nivya 2015; Gupta et al. 2014).

16.10 Antibiotics and Hydrolytic Enzymes

One of the primary mechanisms of PGPR is the production of a wide spectrum of antibiotics that inhibit or kill phytopathogens (Raaijmakers et al. 2002; Compant et al. 2005; Mazurier et al. 2009; Couillerot et al. 2009; Raaijmakers and Mazzola 2012). Several antibiotics synthesized by PGPR exhibit effective bioactivity and many of these potential strains have been commercialized. A disadvantage of frequent and increased application of antibiotic-producing bacteria as biocontrol agents in soil stimulates some plant pathogens to establish a resistance to particular antibiotics. Therefore, researchers reported that PGPR producing hydrogen cyanide (HCN) along with one or more antibiotics can be suggested as potential biocontrol agents to overcome the antibiotic resistance of phytopathogens since HCN synergistically acts with bacterially encoded antibiotics (Glick 2012). Several PGPRs are reported for producing a broad range of antibiotics, however, the genera *Bacillus* and *Pseudomonas* are the predominant antibiotic producers among the PGPR. Antifungal, antibacterial, antiparasitic, antihelminthic, antiviral, phytotoxic, antioxidant, cytotoxic, and antitumor agents are only some of the various metabolites they create (Olanrewaju et al. 2017). The bioactive compounds Tas A and sublancin, subtilosin, and bacilysin have been reported from *Bacillus* species while ecomycins and 2,4-diacetyl phloroglucinol have been isolated from ecomycins, pseudomonic acid and phenazine-1-carboxylic acid, pyoluteorin, and pyrrolnitrin as well as oomycin A, cepaciamide A, viscosinamide, butyrolactones, zwittermycin A, aerugine, azomycin, rhamnolipids, cepafungins, kanosamine, and karalicin were isolated from *Pseudomonas spp*. for controlling a broad range of plant-associated pathogens (Goswami et al. 2016; Olanrewaju et al. 2017).

PGPR secrete various types of hydrolytic enzymes such as proteases, chitinase, cellulase, β-1,3 glucanases, and lipases. These extracellular enzymes mainly break

down a part of the cell wall of diverse plant fungal pathogens (Husson et al. 2017). PGPR produce either one or many cell wall degrading enzymes for their biocontrol activity against different plant pathogenic fungi such as *Botrytis* spp., *Sclerotium* spp., *Fusarium* spp., *Phytophthora* spp., *Rhizoctonia* spp., and *Pythium* spp. (Singh et al. 1999; Frankowski et al. 2001; Kim et al. 2008).

16.11 Hydrogen Cyanide Production by PGPRS

Production of hydrogen cyanide or HCN in high amounts by PGPR accelerates the effectiveness of the biocontrol mechanism. Most of the PGPR can produce HCN. However, less amount of HCN produced by PGPR may not be potent in suppressing the growth of a majority of plant pathogens (Olanrewaju et al. 2017). Besides, it is frequently noted that the biocontrol mechanisms of PGPR are a synergistic action of producing HCN along with one or more antibiotics and hydrolytic enzymes (Ramette et al. 2006). HCN is toxic to many microbes including bacteria, fungi, and algae so PGPR survives by competing with its counterparts with this trait. Due to its host-specificity, PGPR may serve as a biological control agent by inoculating the plant with HCN (Zeller et al. 2007). Numerous PGPR taxa, including *Rhizobium, Pseudomonas, Alcaligenes, Bacillus*, and *Aeromonas*, have been shown to produce antifungal metabolites like HCN (Ahmad et al. 2008; Bhattacharyya and Jha 2012; Das et al. 2017; Zachow et al. 2017). By blocking the activity of cytochrome C oxidase and other metallo-enzymes in phytopathogens, HCN effectively halts respiration (Nandi et al. 2017). A variety of plant diseases and pests have been shown to be suppressed by HCN (Siddiqui et al. 2006; Kumar et al. 2015).

16.12 Production of Exopolysaccharides and Volatile Compounds

Exopolysaccharides (EPSs) are long chains of high molecular mass constituting monosaccharide residues and sugar derivatives produced by microorganisms and plants (Sanlibaba and Cakmak 2016). Biosynthesis of EPSs by rhizobacteriomes such as PGPR plays a critical role in enhancing plant development and production by retaining water, aggregating soil particles, and ensuring contact and interaction between rhizobacteria and plant roots (Pawar et al. 2016). They also aid the host plant to sustain under unfavorable conditions that alter the soil environment such as an increase in soil salinity, water deficit stress, and waterlogging conditions (Pawar et al. 2016). Several EPSs biosynthesized by PGPR such as *Rhizobium* spp., *Bacillus* sp., *Agrobacterium* sp., *Azotobacter* sp., *Enterobacter* sp., and *Xanthomonas* sp. (Mahmood et al. 2016), have been described for proliferating soil fertility and promoting crop yield.

Small volatile organic compounds (VOCs) produced by PGPR belonging to the *Bacillus* and *Pseudomonas, Serratia, Arthrobacter*, and *Stenotrophomonas* families have been shown to inhibit numerous plant pathogenic bacteria and fungi. In

addition, plants develop systemic resistance against phytopathogens as a result of the creation of VOCs (Raza et al. 2016a). Therefore, this coordinated activity, such as producing antimicrobial volatile metabolites that activate systemic resistance in plants, has a favorable influence on plant health indirectly (Raza et al. 2016b). VOCs 2,3-butanediol and acetoin generated by Bacillus spp. were found by Santoro et al. (2016). Furthermore, Sharifi and Ryu (2016) determined that PGPR-derived VOCs act as an elicitor to initiate plant-induced systemic resistance. VOCs such as dodecane, benzene(1-methylnonadecyl), benzene, methyl, tetradecane, 2,6,10-trimethyl, dotriacontane, 11-decyldocosane, cyclohexane, 2-(benzyloxy) ethanamine, morpholinocyclohexene, 1-(N-phenylcarbamyl)-2-decane, and 1-chlorooctadecane isolated from different PGPR strains participate in inducing systematic resistance in plants either direct or indirect mode to promote their growth, productivity, pathogen resistance and stress tolerance (Kanchiswamy et al. 2015; Gouda et al. 2018).

16.13 Induced Systemic Resistance

Interaction between plant roots and rhizomicrobiomes induces a systemic mechanism in plants which is evoked in response to environmental stimulus against various plant pathogenic organisms. This physiological condition of enriched defensive aptitude of the plant is known as induced systemic resistance (ISR) (Lugtenberg and Kamilova 2009; Prathap and Ranjitha 2015). ISR induces jasmonate and ethylene hormone signaling pathways to trigger the host plant's defense mechanism against different types of pathogens (Glick 2012). ISR stimulates plants to resist not only one type of pathogen but numerous diseases (Kamal et al. 2014). Several bacterial appendages, secretions, and metabolites such as flagella, lipopolysaccharides, lipopeptides, siderophores, homoserine lactones, cyclic 2,4-diacetylphloroglucinol, and VOCs like acetoin and 2,3-butanediol can induce the ISR in plants (Lugtenberg and Kamilova 2009). The signal for induced systemic defense mechanism in plants is stimulated through the vascular system during pathogen invasion. It leads to the activation of various defense and antioxidative enzymes such as chitinase, β-1,3 glucanase, phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), lipoxygenase (LO), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) including proteinase inhibitors to protect plant health (Gouda et al. 2018). Several researchers demonstrated the jasmonic acid/ethylene-dependent ISR activation by *Pseudomonas* spp. along with many other PGPR (Pieterse et al. 1998; Abo-Elyousr et al. 2009; Weller et al. 2012; Yi et al. 2013; Lucas et al. 2014). Application of PGPR to induce ISR in the plant could be an effective approach to sustainable agriculture and crop protection nevertheless, appropriate techniques and contemporary tools to carry plants from lab to field are still lacking.

16.14 Application of PGPR as Biocontrol Agents

Soil is a complex medium and an uncertain habitat hence targeting the desirable outcome from this medium is challenging. Although, PGPR can survive in any type of soil and its relative stress conditions, their role in plant growth and productivity differs in experiments conducted in a lab, greenhouse, and in-field conditions (Ahemad and Kibret 2014). Emerging climate change is also a concern on the efficacy of PGPR on crop production (Zaidi et al. 2009). The PGPR biocontrol traits that influence plant growth and productivity is not governed by an individual mechanism, rather they involve interaction among several mechanisms. The synergistic action of biological N_2 fixation, phosphate solubilization, phytohormones, enzymes, secondary metabolites, and other small molecules overall results in plant growth enhancement and biomass yield (Dakora and Phillips 2002; Ahemad and Kibret 2014). However, the application of PGPR under either natural or controlled soil environments effectively increased the productivity of many different crop plants (Ahemad and Kibret 2014). As people around the world embrace and prefer more organic foods, employing PGPR for controlling plant pathogens and promoting plant growth is advantageous. Several PGPR strains belonging to genera *Agrobacterium, Azospirillum, Azotobacter, Bacillus, Burkholderia, Delfitia, Paenobacillus, Pantoea, Pseudomonas, Serratia, Streptomyces,* and *Rhizobia* have already been commercialized for plant growth-promoting traits. Among them, *Streptomyces*, *Bacillus,* and *Pseudomonas* are more predominantly reported as potential biocontrol agents (Glick 2012; Ahemad and Kibret 2014; Vurukonda et al. 2018) (Table 16.1). PGPR can more exhaustively be commercialized nevertheless, some of the concerns need to be addressed (Glick 2012).

16.15 Conclusions and Future Perspectives

Researchers around the globe have executed intensive research for the last three decades on rhizobacteriome that how to plant growth-promoting bacteria (PGPR) influence plant health and the possibility of application of these microbes as biocontrol agents. It is undoubtedly revealed that employing PGPR as biopesticides, biofertilizers or biocontrol agents has shown remarkable improvement in plant growth, protection, and productivity. However, PGPR has synergistic direct and indirect actions on plant growth promotion. Besides, with more research on the application of PGPR, optimization of their growth and metabolites production and improvement of their adaptation to unpredictable soil environments would certainly increase its plant-growth-promoting and protecting traits. Further multidisciplinary research on PGPR for ecological and functional biological approaches is anticipated to substitute the chemical fertilizers, and pesticides and provide sustainable farming practices to decrease production costs in the future.

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