Sustainable Development and Biodiversity 26

Dinesh Kumar Maheshwari
Shrivardhan Dheeman *Editors*

# Endophytes: Mineral Nutrient Management, Volume 3

Springer

# Sustainable Development and Biodiversity

## Volume 26

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# Endophytes: Mineral Nutrient Management, Volume 3

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# Preface

The present book entitled *Endophytes: Mineral Nutrient Management, Volume 3* is in continuation of two previous volumes published on endophytes, their biology, biodiversity, crop productivity, and their role in crop protection. Based on the idea that endophytic microbes are far stronger than the other bacteria in the rhizospheric microbial world, scientists are paying great attention to the understanding of the beneficial nature of endophytes in particular as they bear enormous potentialities for boosting the sustainable growth of crops and agroecosystems.

This book is a comprehensive collection of reviews on current science and evidence of biofertilizer ability of endophytes for nutrient management. Some chapter on the endophytic lifestyle in the plant tissues is an attraction of the book. There is an elaborated account of their special traits like competence, root colonization, and/or endophytic phytohormone secretion which act for plant protection and stress sequestration. Entirely, the synergism of practical and theoretical wisdom on endophytes makes this book alive. An elaborated content has also been available in the book on the science of enhancement of nutrient utilization efficiency.

Endophytes increase the availability of important nutrients to enrich soil fertility and fulfill the nutritional demand of plants. Further, plant-endophyte interaction evidences a mutual alleviation of biotic and abiotic stresses in diverse habitat and agroclimatic conditions. Genomic tools and techniques can further identify endophytes with the ability of mineral nutrient management which can be utilized in the production of microbial inoculants for future farming. The book presented under the series "Sustainable development and biodiversity" is entirely dedicated to various endophytic genera, able to mineralize micro and macronutrients in the soil and rhizosphere.

This book will not only benefit the scientific diaspora but also to the teachers, researchers, graduation and postgraduation students in various streams of life sciences such as Agriculture, Horticulture, Biotechnology, Microbiology, Phytopathology, Agronomy, and Environmental Sciences. We desire to pay our thanks to all the subject specialists and contributors, who lent their cooperation and patience in the completion of this book. Our research team members, who generously assisted in the compilation and completion of this task also deserve a big thanks. We extend our sincere thanks to Dr. Ineke and her colleagues for their valuable support in the completion of this project. Support from MHRD UGC—BSR is also duly acknowledged.

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Dinesh Kumar Maheshwari
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# Part I
# Endophytes in Agriculture

# Chapter 1
# Endophytes in Mineral Nutrient Management: Introduction

**Dinesh Kumar Maheshwari and Shrivardhan Dheeman**

**Abstract** The second green revolution can beat the challenge of food requirement, which will be a game-changer of this decade. It will boost the fertility of the soil, food security, and global crop production. The microbial world of endophytes having the ability of nutrient mineralization has been proved a boon to mitigate hunger of the global population. In this scenario, crop yields must be increased substantially to glorify the coming decades and mitigate global food demand using endophytes-based biofertilizers. Over the past few years, researchers are engaged to re-discover endophytes to help us to produce healthier crops with higher yields while reducing the need for fertilizer and other chemicals. This is a summary account of reviews of the subject experts from the entire globe bringing their idea(s), commentaries, and views on the current research on endophytes and the mechanistic role of endophytes to sustain agriculture production in major and micro mineral nutrient management precisely.

**Keywords** Endophytes · Mineral nutrients · Soil · Rhizosphere

## 1.1 Introduction

Research of plant growth-promoting bacteria (PGPR) and endophytes is entering in the third decade of the twenty-first century. Scientific chronicles are converting old policies of agriculture into golden policies for sustainable agriculture, to achieve a new revolution and combat against food security. Like plant growth-promoting bacteria, endophytes are also known for providing several direct and indirect benefits to their host friend. Hence, newer studies become important to understand and

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harness the beneficial influence of the past and future of mineral nutrient management and sustainable agriculture by endophytes. Nutrient management is an approach of sustaining mineral nutrients in plants and soil systems. Originally, this approach is a derivation of integrated nutrient management (INM) holding focus on nitrogen (N), phosphorus (P), and potassium (K) nutrients by blending agrochemicals with effective microorganisms (EM). In the insights of mineral nutrient management (MiNuM), endophytes have been used to increase the spectrum of soil fertility and mineralization or immobilization of various trace elements like N, P, K, Zn, Fe, Cu, Mg, and S.

The soil microorganisms can improve plant responses against biotic and abiotic stresses and aid them in health management are called 'beneficial bacteria'. Altogether the plant-microbe-soil creates a tripartite relationship in soil ecology, which is often studied under plant-microbe interaction. Plant-microbe interaction (PMI) is a complex relationship that exists above-ground and below-ground. The below-ground PMI is more complex than the above, because of consisting complex interface with soil. The soil affects these relationships via its physico-chemical properties in addition to abiotic and biotic factors.

Few PGPR develops an intimate relationship with plants and becomes colonized inside tissues without any visible symptoms that are usually termed as endophytes. Endophytes are also found in the seeds of few plants, thus, these are termed as 'seed endophytes'. Basically, in the below-ground PMI, they are known for their versatility and helping plant via several mechanisms such as nitrogen fixation, phosphate solubilization, potassium (K) and zinc (Zn) solubilization, siderophores production, phytohormone production, volatile production of hydrogen cyanic (HCN) acid, 1-aminocyclopropane, 1-carboxylic acid (ACC) deaminase production, biocontrol of fungal phytopathogens, induced systemic resistance (ISR) and systemic acquired resistance (SAR). Endophytes are important to consider in the usage of nutrient mineralization due to their significant traits (Maheshwari and Dheeman 2019).

The heterogeneous community of endophytes includes root-nodulating Rhizobia to facultative endophytes such as *Bacillus*, *Pseudomonas*, *Azotobacter*, etc. The abundant existence of *Bacillus* in soil may be attributed due to spore formation, resistance to high temperature, and cold shock resistance (Pandey et al. 2018). Endophytes can solubilize phosphorus (P) and potassium (K) along with the ability to mineralize Zinc (Zn) and oxidize sulfur (S). On the other hand, few endophytes involve in N-fixation (mineralization of N to fix in the form of Ammonia). This way, endophytes have emerged as a versatile candidate. They endure in harsh environments with their feasible strategies and deals with the limitation of agricultural production caused by soil factors. The exact mechanism by which endophytes improve plant health remains largely speculative; however, possible explanation includes mineral nutrient management (include acquisition of nutrients as direct involvement in plant growth promotion largely reviewed in this book by eminent scholars). Endophyte to serve as biofertilizer or Phyto-stimulator helps in maintaining the soil. These include the acquisition of nutrients as a direct involvement in plant growth promotion; however, other mechanisms support indirectly toward plant growth and sustainable agriculture.

The beneficial microorganisms have been established for efficient mineral solubilization, mobilization, and acquisition of soil nutrients. Being major tissue colonizer of plant's interior and to improve plant growth by enhancing nutrient use efficiency (NUE) with phosphorus (P) from the organic stock to available form via nutrient mineralization. Biological control against few soil-borne diseases and improved water uptake in drought conditions via the maintenance of ACC-deaminase activity and ethylene regulation influence growth promotion (Aeron et al. 2019). A revised scheme of the utilization of endophyte as bioformulation is depicted in Fig. 1.1.

The use of fertilizers, including mineral fertilizers and organic manures, to enhance soil fertility and crop productivity (Etesami and Maheshwari 2018) has often influenced the complex system of the biogeochemical cycles. The synthetic fertilizer and chemicals caused leaching and nutrients run-off, especially N and P, leading to environmental degradation. Low fertilizer use efficiency and continuous long-term use are important causes of this aggravated problem. Thus, it necessitates the involvement of endophytes to acquire suitable strategies for the plant's mineral nutrient management.

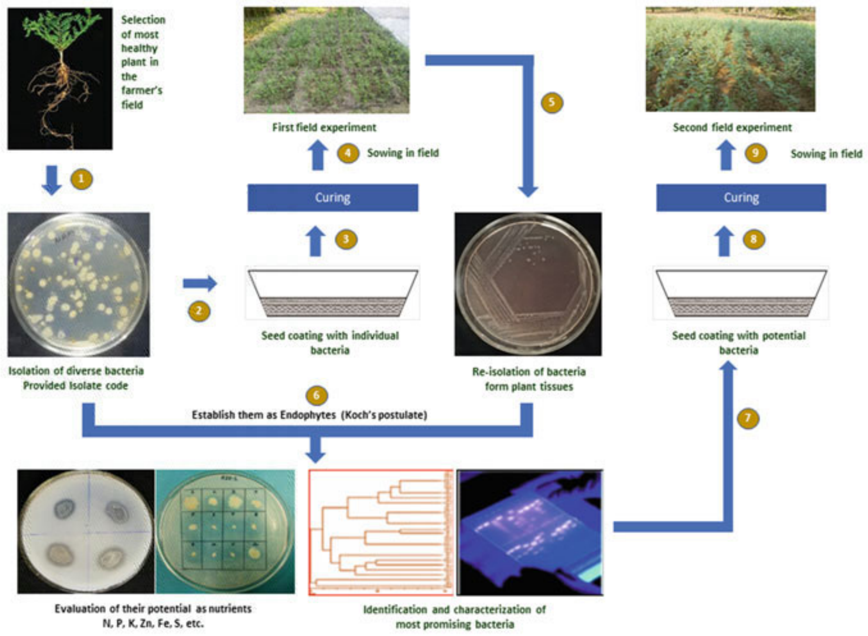

**Fig. 1.1** A revised scheme of isolation and characterization of endophytes for plant growth promotion, molecular identification, and use as bioinoculant for raising agricultural crops

## 1.2 Frontiers of Endophyte

The frontiers of endophyte research have been determined with unique mixes of varied contributions of the chapters and unification of the most influential researches, and historical advances. It finds more about how endophyte owns their frontiers and go beyond.

### *1.2.1 Avenues in Pharmaceuticals*

Metabolism is a universal phenomenon of all living creatures and science evidenced the beneficial and harmful gears of metabolites. Most in cases secondary metabolites are considered toxic, and due to antibiosis mechanisms, some sort of bacterial metabolites is considered beneficial for therapeutic purposes. Chapter 6 encased with direct shreds of evidence to understand the usage of secondary metabolites from endophytic fungi. It is focused on biological activity correlated to chemical diversity and exclusive use in agriculture, medicine, and industry.

### *1.2.2 Fungal Endophytes*

In the micro-niche of mycorrhizal spore, actinobacterial endophyte creates a scope of future research. As a plant growth promoter, they bear vast applications in agriculture. Actinobacteria lives in association with arbuscular mycorrhizal (AM) spores under abiotic and biotic stresses and, therefore, can alleviate climatic adversity in grain crops like rice; fairly reviewed in Chapter 10.

A few endophytic fungi living asymptomatically within the plants can confer improvement in plant nutrient uptake, as a need of a sustainable farming and soil-recharge with biogeochemical cycle. Fungi boost nutrient uptake via several mechanisms understood in Chapter 3. This countered on how fungi hold multiple benefits to plants under stressed conditions and mineral nutrient uptake.

### *1.2.3 Spore Bearing Endophyte Enhancing Plant Nutrient Uptake*

Abiotic factors are determinantal for plant growth but, a few endophytes like spore-forming bacilli are resistant to the climatic adversity and contributing to the agricultural productivity. These microorganisms can enhance crop growth with great potential while having an endophytic life. Especially in tropical ecosystems, they colonize the internal tissues of plants and bear an ecological advantage explored by their genomics. Thus, opportunities to commercialize these beneficial bacteria, create a deep-down sense to investigating more about their roles and mechanisms has been covered in Chapter 7.

### *1.2.4 Endophyte for Crop Protection*

Plant-microbe interactions have many faces of benefit and deleterious effects. In this trend, endophytes diminish disease development by several mechanisms but, precisely by involving antagonistic and parasitic interaction with pathogens. ISR and SAR contributed by endophytes using a sum of different strategies. Hence, endophytes are now utilizing as a source of biological control. Endophyte in biological control and an image that they're intertwined in the network for mineral nutrient uptake/management.

### *1.2.5 Phosphorus Management by Endophytes*

Phosphorus (P) is the second most essential mineral nutrient after nitrogen for plant growth and development. Phosphate solubilizers have been characterized as an important candidate for soil microbes. Like PGPR, endophytic microorganisms associated with different plants release organic acids and solubilize phosphate complexes into ortho-phosphate for easy uptake by plants. Genetic manipulation in the strains of phosphate solubilizing (PS) bacteria can increase the capacity and efficacy of PS biofertilizer for sustainable agriculture, significantly covered in Chapter 3.

Along with several mechanisms of PGPR, phosphate solubilization facilitates the conversion of insoluble P and intertwines in the biogeochemical cycle. Chapter 9 reviews the urgency of industrial agriculture to move for modern agricultural biotechnology and exploiting microbial inoculants, which can enhance plant growth and thereby reduce the use of agrochemicals. In this way, giant information on endophyte involved in P solubilization becomes crucial to be collected. This chapter focused on endophytic phosphate solubilization and its role in mineral management.

### *1.2.6 Endophytes: Ecological Advances*

The realization that the plant microbiome can improve the management of plant health, soil fertility, and crop productivity is one of the most fascinating scientific discoveries in the world. Endophytic bacteria are unique plant microbiome that establishes them within their tissues. Chapter 12 is enhancing wisdom on putative functions of endophyte for plant mineral nutrients acquisition and is advantageous to provide better opportunities and viable strategies for sustainable agriculture.

### *1.2.7 PGPB: Nutrient Use Efficiency*

Plant growth-promoting bacteria (PGPB) has a potential tool for sustainable agriculture utilizing for a long time in plant growth and development. Also, these PGPB increase plant nutrient uptake capacity and nutrient use efficiency. Chapter 13 illustrates several studies focusing on PGPRs in nutrient use efficiency of various crops and covered their long-term application to reduce the use, cope with the negative effects of chemical fertilizer thereby.

### *1.2.8 Iron Management: Endophytes in Siderophore Production*

Iron is an essential nutrient for plant growth and soil salinity is a leading cause for iron limitation. Plants and microbes overcome this iron limitation by producing iron-chelating agents known as Siderophores. Effect of Iron on siderophore production, pH levels, antagonism, and root colonization was identified. Chapter 5 is providing a case study for salinity stress by managing iron via siderophore by Pseudomonads being the most dominant bacteria in the soil ecosystem after bacilli. This further strengthens how siderophore is important for soil nutrient management and sustainable farming.

In the plant-soil interaction uptake of nutrients specifically, phosphorus, nitrogen, iron, and potassium is facilitated by plant growth-promoting rhizobacteria. Recently, endophyte was identified to produce a variety of siderophores such as pyoverdine, hydroxymate, ferrioxamines increase three times iron transportation efficiency to the plant for the development of root and shoot growth. Chapter 14 understands the applications of siderophore in bioremediation, weathering of soil-mineral particles, and plant growth as a major account.

### *1.2.9 Endophyte: Biotechnology and Bioinformatics*

Endophytes have emerged as an important tool for plant growth promotion and crop productivity enhancement. Their application is extending the frontiers of medicine industry and environmental remediation. Chapter 8 is an important part of elaborating major findings of endophytes and their applications in emerging ‘omic’ tools and to cast the light on biotechnological and bioinformatics aspects.

## 1.3 Conclusions

In current scenario, due to the abundant use of synthetic chemicals on crops, the sustainability of agriculture systems has distorted; the cost of cultivation has increased at a high rate; the income of farmers stagnated, and the provision of food security and safety has become a frightening challenge.

For these reasons, estimation and re-investigation of the endeavors of endophytes via mineral nutrient mineralization become important. The harmless inputs in safeguarding soil health and the quality of crop products are holding the inspiration to scientists to re-discover multi-faceted roles of endophytes in plant interaction and benefits to agriculture. The use of endophytes is a relevant strategy for the efficient and rational use of agricultural resources with minimal effects of adverse environmental impacts that may boost water resources, ecosystems, or the quality of human life.

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# Chapter 2
# Bioefficacy of Endophytes in the Control of Plant Diseases

**Fernando Matias Romero, Amira Susana Nieva, Oscar Adolfo Ruiz, Andrés Gárriz, and Franco Rubén Rossi**

**Abstract** Plants establish multiple kinds of interactions with microorganisms, which can be neutral, beneficial, or detrimental for the plant host. Interactions also occur between endophytic microorganisms that colonize inner parts of plants, beneficial in nature, and able to promote plant growth both directly or indirectly. Direct plant-growth promotion includes the production of phytohormones, nitrogen fixation, and an increase in nutrient availability. On the other hand, endophytes can promote plant growth indirectly by contributing some beneficial attributes to plant health. Direct interaction between pathogens and endophytes also induces systemic resistance in the host, which allows the plant to respond faster and/or more intensively upon pathogen infection. Usually, endophytes share more than one of these mechanisms so the outcome of the interaction is the sum of different strategies. In this chapter, review on bacterial and fungal endophytes as potential biological control agents and their mechanisms of action have been documented. Besides it analyzes the most recent information about the nutrient uptake/management, specifically iron and nitrogen nutrition, with the biological control exerted by beneficial microorganisms.

**Keywords** Endophytes · Biological control · Plant protection · Biological control agents

## 2.1 Introduction

Control of plant diseases that reduce crop yields is a pressing need in modern agriculture, as the demands of stable and healthy food supplies by a growing human population must be guaranteed (Emmert and Handelsman 1999; Maheshwari 2013; Maheshwari and Annapurna 2017). Although there are several strategies to control plant diseases, there is an increasing interest in finding new technologies that can

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diminish or replace the use of agrochemicals, which can result in negative consequences to human health and the environment. In this way, biological control has attracted the interest of researchers over the last few years as a non-polluting alternative. One of the many strategies is to adopt microbial inoculants since they have several benefits compared to traditional chemical pest management. Being effective in small quantities, because they can multiply themselves, and at the same time, the host and the native microbial community control their spread (Berg 2009). Another advantage of bioinoculants is that the development of resistance is limited because of the involvement of different control mechanisms simultaneously. Moreover, microbial inoculants can be used in conventional or integrated pest management (Berg 2009). Thus, it can be achieved by (i) creation of environmental conditions favorable for the action of controlling microorganisms already present in the crops, (ii) through the genetic improvement of the host's ability to interact with such microorganisms and (iii) by the genetic manipulation of the controlling microorganisms to give them advantageous characteristics, or the massive introduction of beneficial microorganisms into the host during the interaction process.

The first microorganisms receiving attention as potential bioinoculants were those inhabiting the host-rhizosphere because they were proven to have several traits regarding plant promotion and antagonistic activity against plant pathogens (Bhattacharyya and Jha 2012). However, the microorganisms able to colonize the inner cells and tissues of plant hosts also improve plant growth and health and seem to be excellent candidates as biological control agents (BCAs) as observed by several workers (Berg and Hallmann 2006; Kloepper and Ryu 2006; Maheshwari 2017). It is due to endophytic nature which is better protected from harsh environmental conditions (i.e. extreme temperatures and UV light) and is in closer contact with their host's cells and tissues than that of rhizosphere or phyllosphere microbes (Hallmann et al. 1997; Lindow and Brandl 2003).

A great diversity of microorganisms were reported to exist as endophytes in cultivation-based studies (Reinhold-Hurek and Hurek 2011; Suryanarayanan 2013). Among the bacterial endophytes, most isolates belong to the phylum Proteobacteria, even though Firmicutes, Actinobacteria, and Bacteroidetes were also represented (Rosenblueth and Martinez-Romero 2006). However, diversity and richness of endophytic communities are much greater than those reported in culture-dependent studies (Dissanayake et al. 2018). In this trend, the use of next-generation sequencing (NGS) techniques has helped to unravel the structure and composition of endophytic communities more truly (Bulgarelli et al. 2012; Hong et al. 2019; Romero et al. 2014). The rapid development and the relative low costs of NGS have contributed to study microbial communities associated to different plant genotypes and/or growth stages (Manter et al. 2010; Marques et al. 2014) and is helping to get new insights into dynamics of plant-endophyte-pathogen interactions (Ardanov et al. 2012; Bulgari et al. 2014; Tian et al. 2019). Moreover, these culture-independent technologies not only exhibit the composition of the endophytic communities but also, facilitate the study of the functions performed by communities in the system. However, the analysis of biological control (BC) related traits in endophyte microbial communities is still scarce. This information could lead to the development of improved and more efficient BCAs for sustainable agriculture.

In this chapter, we will review and summarize some of the works reporting the use of microbial endophytes as biological control agents for different plant diseases and their mechanisms of action. Finally, we will discuss the nutrient uptake/management during biological control mediated by beneficial microorganisms.

## 2.2 Bacterial Endophytes as BCAs

Endophytic microorganisms can display one or more mechanisms of action toward a specific pest or pathogen including direct interactions between endophytes, pathogens, and their hosts and this is one of the reasons why the use of microbial inoculants has notable advantages against chemical treatments (Fig. 2.1). The most commonly described mechanisms include inhibition of the pathogen by the

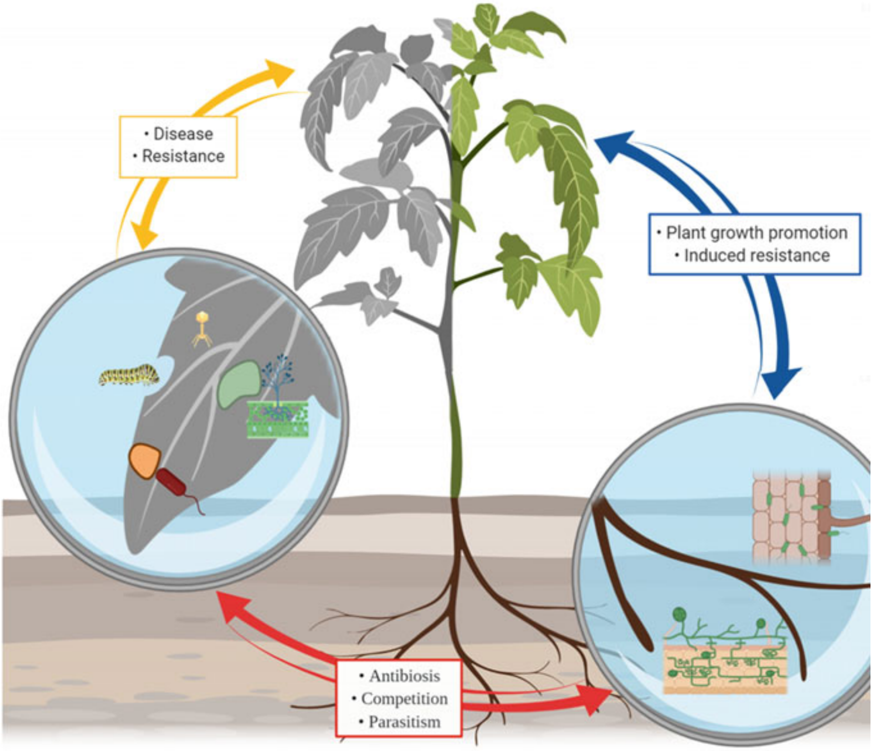

**Fig. 2.1** Modes of action in plant-microbe interactions promoting plant growth and health

production of antimicrobial compounds, competition for nutrients and space, induction of plant defense mechanisms, and parasitism. However, other modes of action remain to be explored in-depth, as the inactivation of pathogen germination factors or even degradation of virulence factors such as toxins produced by phytopathogens (Whipps 2001). Further, endophytes possess significant efficiency of protection since it is possible to prepare bioformulations with more than one microorganism for multiple trait benefits to combine desired traits and/or efficacy (Aeron et al. 2011; Baliyan et al. 2018; Pandey and Maheshwari 2007). For instance, Varo et al. (2016) tested several BCAs alone or in combinations, which showed great levels of protection against wilt in olives caused by *Verticillium dahlia*, reducing the incidence and mortality up to 90% (Varo et al. 2016).

### *2.2.1 Antagonism*

Since endophytes share a similar niche as of many phytopathogens colonizing plant cells and tissues, with different degrees of association direct antagonism between them is a reliable screening technique to screen potential BCAs from a collection of endophytic isolates. Microbial balance in the tissue of plant, as a micro-niche microbial homeostasis is also important to notice, where microbe-microbe interactions interplay. The native microbe of endophytic nature sometimes competes with the other invading microbe under natural conditions. The in vitro proves of this phenomenon are constant and yet few.

Direct inhibition of pathogens is mainly mediated by the synthesis of antibiotics, volatile production of hydrogen cyanide (HCN), and antifungal metabolites (Raaijmakers et al. 2002, 2010). Antibiotics encompass a chemically heterogeneous group of organic, low-molecular-weight compounds. At low concentrations, these are deleterious to the growth or metabolic activities of other microorganisms, and most BCAs bacteria produce multiple antibiotics with different degrees of efficacy against pathogens, some of them with overlapping activity. Several compounds have been purified and identified from biocontrol bacteria. For example, pyrrolnitrin is produced by bacteria from the genus *Pseudomonas* and *Burkholderia*, which has been proven to be effective against a wide variety of plant pathogens such as *Rhizoctonia solani*, *Botrytis cinerea*, *V. dahliae*, and *Sclerotinia sclerotiorum* (Raaijmakers et al. 2002). Moreover, mutant strains unable to produce this compound lost the in vitro and in planta ability to control *R. solani* (Hill et al. 1994). In turn, 2,4-diacetylphloroglucinol (DAPG) is a phenolic antibiotic produced by BCAs from the genus *Pseudomonas* that exhibits antibacterial, antifungal, and anthelminthic activity (Haas and Defago 2005; Weller et al. 2007). DAPG not only was proven to inhibit the growth of pathogens directly but also, it has been demonstrated that *Arabidopsis thaliana* inoculated with *Pseudomonas* sp. mutant strains unable to produce DAPG are impaired in developing induced systemic resistance (ISR) against *Pseudomonas syringae* pv. *tomato* (Weller et al. 2012). Phenazines are also a class of well-studied natural antibiotics that are produced by diverse plant-associated bacteria, exhibiting unique redox properties and broad-spectrum antibiotic activity, and playing a wide variety of roles in nature (Mavrodi et al. 2006). Endophytes are essential to the production of several secondary metabolites in grasses, in the process of gummosis in trees, and the production of useful metabolites such as alkaloids, pestaloside, cryptocandin, enfumafungin, subglutinols, etc., for the host plant (Maheshwari and Annapurna 2017).

The transformation of *P. fluorescens* strain Q8r1-96 with the biosynthetic locus leading to the production of phenazine-1-carboxylic acid, a precursor of several phenazine compounds, conduces to an increase in the biocontrol efficacy against *Rhizoctonia* root rot in wheat. In this trend, a lower dose of the transformant antagonist is required to exert the similar level of control exerted by the parental strain (Huang et al. 2004). The presence of these traits in soils is thought to explain disease decline in suppressive soils, in which specific soil-borne plant pathogens cause only limited disease although the pathogen and susceptible host plants are both present. However, the quantification of these characteristics in different types of soils has no clear correlation between the presence of antibiotic synthetic genes and disease suppression (Garbeva et al. 2004; Imperiali et al. 2017). Further analysis is required to understand how these are expressed genetically and regulated in soil and/or inside plants.

Other metabolites with direct action against bacteria and fungi are lipopeptides such as iturin, surfactin, thanamycin, and fengycin (Ongena and Jacques 2008; Raaijmakers et al. 2010); and also the polyketide antibiotics bacillaene, difficidin, and macrolactin produced mainly, but not exclusively, by different strains of the genus *Bacillus*. The role of these compounds in biocontrol activity was also evidenced by the use of mutant strains defective in their production. For instance, the biocontrol activity of *B. subtilis* strain 6051 against *P. syringae* in *Arabidopsis* was impaired when a mutant strain unable to produce surfactin was used (Bais et al. 2004). Similarly, *Pseudomonas* strain SH-C52 reduces the incidence of stem rot disease of groundnut, whereas a thanamycin-deficient mutant strain was less effective (Le et al. 2012).

Non-ribosomal peptides also contribute to the antagonism against bacteria and fungi (Abdalla and Matasyoh 2014). For instance, Tontou et al. (2015) demonstrated that an endophytic strain of *P. synxantha* isolated from *Actinidia chinense* showed antagonism against *P. syringae* pv. *actinidiae* (Psa) in vitro. To find out the molecular mechanisms involved in the antagonism, a mini transposon-mutant library was constructed and antagonism-deficient mutants were selected. Molecular characterization of these mutants showed that three genes could be involved in antagonistic activity, an acyl-homoserine lactone acylase gene, a glucose-6-phosphate dehydrogenase gene, and an mbtH-like gene. As these genes are directly or indirectly involved in the synthesis of non-ribosomal peptides, the authors claimed that these molecules are involved in the antagonistic ability of *P. synxantha* (Tontou et al. 2015). However, it is worthy to mention that these genes could also be affecting other antagonism-associated mechanisms. Thus, it has been shown that quorum sensing perturbation by the action of acyl-homoserine lactone degrading enzymes can interfere with interspecies competition (Amara et al. 2011; Kusari et al. 2014).

Even though the identity of the antimicrobial molecules has not been determined so far, several other studies proved that endophytic bacteria produce compounds with antimicrobial activity. These studies typically used cell-free supernatants from the cultures of these isolates and to inhibit the in vitro growth of phytopathogens. For instance, bacterial endophytes identified as *Pseudomonas*, *Bacillus*, and *Pantoea* isolated from field-grown tomato leaves showed antagonism against bacterial (*P. syringae*) and fungal (*Botrytis cinerea*) pathogens in vitro and planta (Romero et al. 2016). Moreover, their cell-free supernatants were able to inhibit the germination of conidia from *B. cinerea* for about 30–60% and also stopped the growth of *P. syringae* when added to growth media. Additional pieces of evidence of antibiotic production were demonstrated using a semi-purified ethyl acetate extract of the endophyte *B. velezensis* EB-39 against *Xanthomonas campestris* subsp. *citri* (Rabbee et al. 2019). Interestingly, this extract showed similar inhibitory activity to that observed in confrontation assays between EB-39 and *X. campestris*. Purification and identification of new compounds from new isolates will increase the possibility to develop new biocontrol strategies using whole microbes or their cell free metabolites alone.

The presence of genes involved in the biosynthesis of different antimicrobial compounds was also used as an indication of the ability to produce this kind of metabolites. An analysis performed on cultivable bacterial endophytes from mulberry cultivars having different resistance to sclerotiniosis showed that endophytic communities from resistant genotypes are more diverse than those from the sensible ones (Xu et al. 2019). In this work, dual-culture assays were performed with these endophytes against *S. sclerotiorum*, *B. cinerea,* and *Colletotrichum gloeosporioide* and most of the isolates that inhibit fungal growth were positive for the presence of genes involved in the biosynthesis of antimicrobial compounds, such as polyketides, non-ribosomal peptides, surfactin, iturin, and fengycin (Xu et al. 2019). Following this approach, Cui et al. (2019) isolated a *B. amyloliquefaciens* strain from Chinese cabbage with antagonistic activity against *Pectobacterium carotovorum* subsp. *carotovorum*, the causal agent of soft rot, possess genes involved in polyketides and dipeptide biosynthesis and showed a level of protection up to 75% in greenhouse experiments (Cui et al. 2019). It is worthy to mention that the mere presence of these biosynthetic genes is not sufficient to confirm the production of the antimicrobial molecules. For instance, Hazarika et al. (2019) demonstrated that a *B. subtilis* strain isolated from sugarcane as well as cell-free supernatants obtained from its culture showed antagonism against several pathogens. Moreover, it was positive for the presence of different genes involved in the synthesis of antimicrobial compounds, even though only one of them was detected in supernatants (surfactin) (Hazarika et al. 2019). This observation indicates that gene expression in combination with gene presence would be a more accurate indicator of antagonistic potential.

Volatile organic compounds (VOCs) are also responsible for the ability of certain isolates to inhibit the in vitro growth of different pathogens. For instance, an endophytic isolate from black pepper roots identified as *P. putida* inhibits the growth of several plant pathogens due to the production of volatile compounds as revealed by Gas Chromatography/Mass Spectrometry (GC/MS) (Sheoran et al. 2015). Moreover, the application of some of these chemically synthesized VOCs showed a high percentage of protection on black pepper shoots against *Phytophthora capsici*, especially when these compounds were applied at low concentrations (Agisha et al. 2019). The production of VOCs was also evidenced and identified in two isolates from cocoa that showed antagonism against the causal agent of a black pod, *Phytophthora palmivora,* both in vitro and in planta (Alsultan et al. 2019). The role of VOCs in plant protection is not only due to the direct effect on pathogen's growth as there is evidence that 2,3-butanediol and acetoin can induce systemic resistance to pathogens (Ryu et al. 2004).

### 2.2.2 Induction Disease Resistance in Plants

The recognition of microbial cell components and/or their metabolites can induce in the host's physiological state allowing them to respond faster and/or to a greater extent to future pathogenic attacks. This phenomenon is called induced systemic resistance (ISR) and shares characteristics with another type of systemic resistance triggered by a previous attack of the necrosis-producing pathogen (SAR, systemic acquired resistance). There are two possible molecular mechanisms activated during ISR. Thus, endophyte-inoculation can induce the expression of defense-related genes per se, or on the other hand, the presence of beneficial microorganisms primes plants for enhanced defense responses. In primed plants, defense responses are not activated directly but are potentiated upon pathogen attack, resulting in enhanced resistance (van Wees et al. 2008).

Both ISR and SAR contribute to resistance to a wide range of pathogens in systemic host´s tissues. However, the molecular mechanisms underlying both processes may differ. It was initially proposed that ISR is independent of salicylic acid (SA) signaling pathways but dependant of jasmonic acid (JA) and ethylene (ET), while SAR is dependant of SA and variable dependant of JA and ethylene (van Loon et al. 1998). However, a great number of systems studied afterward demonstrated that beneficial microorganisms induce resistance by activating both SA- and JA-signaling pathways (Mathys et al. 2012; Niu et al. 2011, 2012) or the SA-signaling pathway alone (Tjamos et al. 2005; van de Mortel et al. 2012). Thus, it is probable that nature and the molecular mechanisms underlying ISR depends on particular combinations of plant-beneficial microorganism-pathogen.

Regarding endophyte-ISR induction, it would be strictly necessary to test that endophyte and pathogen are physically separated in the plant to ensure that the mechanism involved in protection is ISR (Kloepper and Ryu 2006). This is difficult to perform with microbial endophytes that colonize the entire plant. In this section, we will discuss some examples of bacterial endophytes inducing defense responses in their host independently of their colonization pattern. Changes in gene expression due to endophyte inoculation can be both local (at the site of inoculation) and systematic (in inoculated and not inoculated tissues). A clear example of systematic responses is the interaction between olives and the endophytic bacterium *P. fluorescens* PICF7, which conduce to the overexpression of defense-related genes in both roots and leaves (Gómez-Lama Cabanás et al. 2014; Schilirò et al. 2012). Another example of induced resistance was described by Sahu et al. (2019), where three *Bacillus* strains from a collection of endophytes from tomato plants were selected due to their antagonism to *Sclerotium rolfsii*. These isolates reduced disease incidence up to 67% under greenhouse conditions (Sahu et al. 2019). Moreover, endophyte-inoculation reduced the reactive oxygen species (ROS) generated at the site of the *S. rolfsii* infection and induced the expression of pathogenesis-related (P R) genes. In this trend, one of the isolates was able to induce the expression of genes *PR1a*, *PR2b,* and *PR3* in absence of the pathogen, while others showed a mild induction of these genes that was enhanced upon infection with the pathogen. Further, these isolates were able to induce the activity of defense-related enzymes such as phenylalanine ammonia-lyase (PAL), peroxidase, polyphenol oxidase, and ascorbic acid oxidase (Sahu et al. 2019).

Importantly, besides their roles in the synthesis of bioactive molecules, these enzymes are involved in plant cell wall reinforcement. Thus, it is probable that their activities can help to prevent the infection by necrotrophic pathogens. This might explain also the mechanism of protection exerted by two *Stenotrophomonas* strains with the ability to colonize *Arabidopsis* leaves, which modified the expression of different enzymes involved in cell wall synthesis and reduce lesion sizes provoked by the necrotrophic pathogens *S. sclerotiorum* and *B. cinerea* (Marina et al. 2019). Accordingly, when cell wall extracts obtained from inoculated leaves were used as a substrate for pathogens growth on agar plates, there was a reduction in fungal colony radius compared to plates supplemented with cell wall extracts from mock-inoculated leaves. Besides, *Stenotrophomonas* inoculation induced callose deposition and expression of PR genes associated with the SA and JA signaling pathways. Similarly, endophytes isolated from *Solanum tuberosum* able to induce resistance to *Pectobacterium atrosepticum* in potato and are able to increase defense-related enzyme activities both before and after pathogen challenge. Moreover, these isolates primed the expression of PR genes involved in both, the SA and JA signaling pathways (Ardanov et al. 2011). The ability to induce the expression of genes involved in phytohormones signaling was also reported by an apoplast-colonizing endophyte from canola leaves (Romero et al. 2019). This isolate showed antagonism against different phytopathogens in vitro and planta. The mechanisms proposed to be involved include the production of antimicrobial compounds as well as the ability to induce defense mechanisms mediated by SA and JA in the host.

Transcriptional changes induced by beneficial microbes usually differ from the changes induced by pathogens, mainly in the intensity of induction (Romero et al. 2017). *Burkholderia phytofirmans* PsJN induced the expression of defense-related genes in grapevine cell suspension but to a lesser extent than non-host bacterium *P. syringae* pv. *pisi*. Both bacteria-induced medium alkalization, but the endophyte did not provoke ROS production or cell death, which are observed in pathogen-treated cells (Bordiec et al. 2011). This isolate also primed the expression of PR genes involved in the SA and JA signaling pathways upon pathogen infection in *Arabidopsis*, showing more sustained expression of *PDF1.2*, a JA dependent PR gene (Su et al. 2017).