Vadakattu V. S. R. Gupta
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Rhizosphere Biology: Interactions Between Microbes and Plants

Springer

Rhizosphere Biology

Series Editor

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Editors

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Preface

Rhizosphere plant–microbe interactions are diverse, spatially and temporally dynamic, influenced by plant, soil biophysical environment, and are critical to plant health and crop productivity. It is well established that bacteria and fungi around a plant's root in the rhizosphere, the miniature ecosystem around the root, can influence both the root's form and its physiology. The rhizosphere concept, coined in 1904 by Lorenz Hiltner about the roles of soil microorganisms in plant nutrition and health, has initiated a century-long research and discussion about organismic interactions between plants and microbes. More than 50 years ago, Dr Ralph Foster's (CSIRO, Adelaide) electron micrographs revealed the intricate structure of rhizosphere, rhizoplane, and endosphere environments and the interplay between plant root, microflora, and protozoa. Rhizosphere microbiota provides a valuable potential resource of plant probiotic and growth-promoting functions capable of conjugating crop productivity within sustainable agricultural systems. It is, therefore, important to understand the dynamics of rhizosphere interactions in order to develop practical strategies that would help improving yield and maintain ecosystem health.

During the last decade, there has been a renewed interest in exploring the dynamics of the rhizosphere, using *omics* tools, for its composition and organismal interactions occurring in the complex spatial structuring at the root–soil interface and their key drivers during the crop growth. Recent research has shown structural and functional diversification of root-associated microbial communities of crop varieties, wild and domesticated accessions of barley, maize, canola, peas, and various *Arabidopsis* accessions, etc. Some of these findings also identified bacterial taxa which were positively correlated with crop performance or yield. This led to an intense effort to identify the plant-based traits that modulate the genetic structure and diversity, gene expression, and functional profile from the outer realms of rhizosphere to inside the root. This new understanding has highlighted an attractive avenue that would help to better harness beneficial outcomes from plant–microbiome interactions.

This book is a compilation of the latest knowledge on plant and microbial aspects of rhizosphere biology covering different ecological, molecular, and biochemical characteristics of rhizosphere and endosphere interactions. It contains 15 chapters, each prepared by authors who are internationally recognized for their knowledge and

expertise in a particular area of rhizosphere interactions. Additionally, it covers the cross-talk between plants and microbes including quorum-sensing signal molecules, plant interactions with abiotic factors, and potential ways rhizosphere microbial composition and functionality could be manipulated for enhanced and efficient benefits. The different chapters cover key areas such as (1) factors driving rhizosphere biology and interactions, (2) diversity of phenotypic and functional groups, (3) functional significances of rhizosphere interactions, and (4) how best to manipulate rhizosphere interactions. A special feature of the papers is that they highlight the benefits of using the latest omics (metagenomics, transcriptomics, and proteomics) and isotopic tools in dissecting the plethora of mechanisms, genes, and metabolites involved in the multitrophic interactions.

Endophytic bacteria, those that colonize the internal tissue of the plant showing no external sign of infection or negative effect on their host, have been found in all plants and form a range of relationships including symbiotic, mutualistic, commensalistic, and trophobiotic interactions. This type of plant microbiome is now considered as the second genome of the host plant and concepts such as "holobiont" comprising the plant with its endophytic microbiome as an extended phenotype and a unified system. In the chapter on bacterial endophytes, Tosi et al. (Chap. 1) present a comprehensive summary of the latest knowledge about the diversity and functions of bacterial (including actinobacteria) endophytes, their influence on plant fitness, and the potential to manipulate their functions in agroecosystems. The presence of a taxonomic overlap between endophytic and rhizospheric communities and clear community shifts between these compartments confirms the idea that rhizosphere is a key habitat regulating endophytic communities. Although soil type can have a major influence, strong and significant host genotype effects on the diversity resulting in distinct taxonomic composition of endophytic bacteria have been shown with a variety of plants. However, the observation of differential abundance, core microbiomes, new knowledge on the heritability of the specific taxa and their links to plant genotype through genome-wide association studies is needed in order to develop designer plant–microbiome combinations that maximize beneficial functions. The involvement of root exudates as carbon and nutrient sources in modifying the rhizosphere microbial communities, as proposed by Albert Rovira more than 50 years ago, is now extended to include signal molecules and root architecture influencing the microbiome composition. In Chap. 9, Haichar and Achouak describe how newly introduced and modified genes in plants influence the quality and quantity of root exudates and in turn rhizosphere microbiome. It is increasingly becoming clear that the ability of soil microbes to colonize a particular plant species is fuelled and modulated by the release of signals by either or both partners and are only recognized by the right partner. Taking the knowledge from legume–rhizobia and plant–mycorrhizal symbioses, Antar et al. summarize (Chap. 13) the latest knowledge about the signals involved in other beneficial plant–microbe interactions and microbe–microbe signal interactions. In spite of recent findings about the intricacies of rhizosphere interactions, the exact molecular mechanisms governing the complex root–soil–microbe interactions remain largely unknown. Balasubramanian et al. present (Chap. 14) a review of

what is known about the strategies for manipulating the rhizosphere region with a focus on engineering the root H+ efflux and organic anions, secondary metabolite composition of root exudates, alterations in root biomass accumulation, and below-ground carbon allocations for improved plant performance.

The carbon inputs by plant roots not only provide the primary source of organic C into the soil modifying rhizosphere microbiome, but the chemical composition of exudates also strongly influences the metabolic potential of rhizosphere-enriched microbes along with mediating nutrient fluxes in the rhizosphere. In Chap. 2, Pett-Ridge et al. provide evidence showing the downstream effects of rhizosphere dynamics on the colonization of nearby soil minerals, degradation of prior season's root litter, and the balance of stabilized versus lost soil carbon. Furthermore, this study provides an excellent example illustrating the benefits of using the latest genomic and isotopic techniques to unravel the mechanisms of C flow between growing plant roots, soil microbial communities, and the surrounding mineral matrix. The beneficial effects of these rhizosphere interactions on nutrient fluxes and availabilities could also improve plant nutrition through increased nutrient use efficiency and, as discussed by Paterson and Mwafulirwa (Chap. 3), provide a realistic means of improving plant health and productivity while potentially also mitigating environmental impacts. Also, recent findings about the diazotrophic communities in the rhizosphere and endosphere in terms of their diversity and functional capacity have rejuvenated the old idea of harnessing the biological N fixation in nonleguminous crops through manipulation of this specific functional group; the chapter by Roley (Chap. 4) presents the latest knowledge on this topic.

The rhizobia-legume and mycorrhizae–plant interactions are two well-established examples of plant–microbe symbiosis with extensive research and knowledge about the mechanistic aspects of the beneficial interactions. Recent research has shown that the interactions of "Arbuscular Mycorrhizal Fungi" (AMF), the obligate biotrophs, in the rhizosphere are not just restricted to host plant but involve bacteria in the rhizosphere and hyphosphere; Wang and Feng discussed (Chap. 11) new insights into interactions between AM fungi and other organisms in the rhizosphere.

Plant health status affected by the presence of pathogens and root disease incidence could be a driver for change in the root microbiome as discussed in the chapter by Barnett (Chap. 8) and it was proposed that microsite-based variation between healthy and diseased niches in the root system could ultimately lead to the development of disease suppressive microbiomes; however, the exact mechanisms for such community changes remain elusive at present. Alternatively, plant–microbe interactions in the rhizosphere can have a significant impact on plant health acting as the first line of defense in the rhizosphere. Therefore, identification of plant genetic traits involved in the recruitment of beneficial microorganisms, i.e. promoting pro-biotic microbial community, would help improve plant defenses against biotic stresses; the chapter by Chiaramonte et al. (Chap. 7) discusses strategies and potential to explore this option through plant breeding programs.

Plant root–biota associations in the rhizosphere involve complex networks and interactions between micro- and macroorganisms across multiple microsites and in intricate spatial structuring that can vary temporally during crop growth from

seedling to maturity. Recent evidence from genomic and transcriptomic studies clearly indicates that there is a strong association between rhizosphere development and taxonomic makeup including the succession in bacterial community in field environments. This has been demonstrated for several crops including cereals, canola, cotton, and other crops (Richardson et al., Chap. 5). The major predators of microorganisms such as protists, faunal nematodes, and microarthropods can modulate the composition of rhizosphere microbiome through preferential feeding, with functional consequences in plant performance by affecting nutrient cycling, pathogen density, etc. (Geisen and Quest, Chap. 12). As rhizosphere food webs are not universally identical, the functional importance of microbial–faunal interactions is modulated by the soil habitat structure and management.

The concept of succession in rhizosphere microbiome dynamics also leads to the idea of "legacy" in that the rhizosphere of crop leaves a footprint in the soil systems affecting the following crops. Since rhizosphere interactions involve modifications in soil physicochemical and biological components, the legacy effects should incorporate physical, chemical, and biological effects that potentially endure beyond the root that created it. In the chapter by Oliver et al. (Chap. 6), they suggest that constraints of destructive sampling can be overcome from the recent advances in micro X-ray computed tomography, but it still requires other complementary techniques to determine the extent of the rhizosphere legacy.

It was considered that the most effective form of manipulation of rhizosphere and endosphere microbiomes is through the use of beneficial microorganisms, "bio-inoculants" either singly or as consortia for biocontrol to reduce or eliminate plant disease effects or effects of abiotic stresses such as from drought/water-stress or salinity effects. Through the use of latest *omics* tools (metagenomic, transcriptomics, and metabolomics), it is now possible to describe in-depth the networks of members of rhizosphere microbiomes including the effects of introducing inoculants as well as identifying mechanisms to manipulate and engineer microbiomes (Franco, Chap. 15). For example, recent evidence suggests that rhizobacteria with the capacity to produce ACC deaminase can initiate a cascade of changes in plant physiological and biochemical responses resulting in increased tolerance to abiotic stresses in a broad range of plant species (Sharma et al., Chap. 10).

As a result of fast-changing global climate scenario with predictions for reduced rainfall and increased effects of other abiotic stresses across many agricultural regions worldwide, exploitation of such beneficial plant–microbe interactions to alleviate abiotic stress effects in crops should be one of the key approaches to promote resilience and improve global food production. A majority of the recent research on the makeup and dynamics of rhizosphere microbiome until now has concentrated on taxonomic/phylogenetic makeup of the microbiome mainly about who is present and variations with plant type, management, and soil environment. In view of the extensive diversity and the dynamic spatial and temporal structure of the microbiome, interpretation and extrapolation of variations in phylogenetic makeup in terms of their functional potential and resilience have been found to be not straightforward. Hence future research on plant-trait based microbiome interactions requires investigations targeting specific functional groups associated with key

plant traits to help with the development of management interventions that can improve productivity in agricultural systems. Through a combination of genomic, transcriptomic, and isotopic tools, it should be possible to directly follow the dynamics of specific microbial functional group and link it with associated functional fluxes. Such research would facilitate the identification of key drivers from plant, microbial, and process perspectives, thereby assisting in the development of new designer plant holobionts that utilize native soil microbiome through next-generation crop breeding, "syncoms" or synthetic communities and management practices for sustainable and resilient food production systems.

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Anil K. Sharma is a Professor at the Department of Biological Sciences, CBSH G.B. Pant University of Agriculture & Technology, Pantnagar. He was a Visiting Scientist at the University of Basel, Switzerland from July 2003 to November, 2003, and at the University of Helsinki, Finland in 2013. He completed his postdoctoral studies at GSU, Louisiana, USA, and he has extensive research and teaching experience. He is a reviewer for DBT, DST, and MOEF projects and for journals such as the Biocontrol Journal, International Journal of Agriculture, and Microbiology. He holds three patents in the field of plant biology and microbiology and has received a number of prestigious grants. His laboratory is involved in various international collaborations, and he has published more than 84 research articles, 32 review articles, and two books with renowned publishers. He has presented his research on several internationally acclaimed platforms.

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Bacterial Endophytes: Diversity, Functional Importance, and Potential for Manipulation

1

Micaela Tosi, Jonathan Gaiero, Nicola Linton, Tolulope Mafa-Attoye, Anibal Castillo, and Kari Dunfield

Abstract

Even though beneficial plant–microbe relationships have been studied for over one century, the recognition of a complex microbiome inhabiting the plant is relatively recent and reveals new opportunities for manipulating plant growth and health. Endophytes, commonly defined as non-pathogenic microorganisms inhabiting the plant interior, constitute an important component of the plant microbiome. Specifically, bacterial endophytes gained research interest only in the past decades, due to their role in plant-growth promotion and their potential use in agriculture. New research is continuously published in this topic, with increasing sophistication provided by new technologies such as omics. For this reason, this chapter aimed to summarize current knowledge on bacterial endophytes focusing on three major aspects: (1) current knowledge on their bacterial endophytic diversity and regulation by plant and soil factors, (2) functional aspects of bacterial endophytes and available tools to study them, and (3) role of bacterial endophytes on plant fitness and potential manipulation tools in agroecosystems. To fit the scope of this book, which is the rhizosphere, the chapter focused on soil-borne facultative endophytes, even though we acknowledge the relevance of obligate vertically transmitted endophytes.

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1.1   Bacterial Endophytes: Definition, Classification, and Scope of this Chapter

Research on bacterial endophytes began growing exponentially in the late twentieth century, mostly fueled by an interest on their role in plant-growth promotion and potential application in agriculture (Turner et al. 1993; Hallmann et al. 1997; Sturz et al. 2000). The term endophytic was applied to microorganisms that could colonize internal tissues of a plant locally or systemically, residing there latently or actively for at least part of their lifetime without causing damage (i.e., commensal or mutualistic relationships) (Wilson 1995; Hallmann et al. 1997). Later findings reported microbial species with both a pathogenic and a beneficial life form in the plant (Kogel et al. 2006), making this definition controversial and giving birth to a new notion where these categories are extremes in an operational continuum instead of two defined groups (Schulz and Boyle 2005; Partida-Martínez and Heil 2011). To what extent this versatility is widespread is still uncertain, therefore, here we exclusively refer to endophytes as those non-pathogenic microorganisms inhabiting the plant interior. Isolating organisms from the plant interior from those inhabiting plant surfaces, such as the rhizoplane or the phyllosphere, is a challenging step. Typically, plant tissues are surface-sterilized before isolation or nucleic acid extraction but, to date, no standardized methods have been defined for different plant species or tissue types, despite the fact that the chosen methodology can strongly affect the results (Reinhold-Hurek and Hurek 2011) (See Box 1.1).

Some beneficial plant–microbe relationships have been studied for more than a century, as is the case for mycorrhizal fungi (Frank 1885), rhizobia–legumes (Beijerinck 1888), and *Frankia*–actinorhizal plants (Bottomley 1911). However, these studies were focused on just those few known associations, and they were mostly approached as one-to-one interactions between a microorganism and a host plant. It was in the last decades that most endophyte research switched to a community approach, acknowledging the different microorganisms that co-exist, as well as the interactions that occur between them and with the host plant (Andreote et al. 2009; Bulgarelli et al. 2013; Gaiero et al. 2013). New technologies like DNA profiling and sequencing became key in unraveling the complexity of endophytic microbial communities and, nowadays, the increasing number of beneficial traits found suggests that a microbe-free plant would hardly survive under natural conditions (Partida-Martínez and Heil 2011). Endophytic communities are such an essential piece of plant fitness that some authors are considering them the second genome of a plant host, where plant and microbiome work as a meta-organism (Lakshmanan et al. 2014). Similar concepts are now widespread in the literature, like "extended phenotype" (Partida-Martínez and Heil 2011) or "holobiont," comprising the host organism with its symbiotic microbiome (Vandenkoornhuyse et al. 2015). This change of perspective is in synchrony with animal health, where researchers are also recognizing the host and its microbiome as a unified system (Ramírez-Puebla et al. 2013).

Bacterial endophytes are usually classified based on their life strategies: while some of them are considered obligate, which means they need the host plant to fulfill

their life cycle, others are known as facultative for having a free-living phase in the soil (Hardoim et al. 2008, 2015). These authors also introduced the categories of opportunistic endophytes, as those who thrive as epiphytic but sporadically enter the plant, and passenger or passive endophytes, who enter and inhabit the plant without actively seeking to colonize it (Hardoim et al. 2015). Different life strategies are usually associated with different degrees of dependency with the host plant. Facultative endophytes must colonize the plant from the rhizosphere, for which they will probably go through a mutual signaling phase to then enter through root cracks or wounds, germinating radicles, emerging root hairs, among others (Reinhold-Hurek and Hurek 2011; Santoyo et al. 2016). Obligate endophytes, on the other hand, are thought to be transmitted vertically, via seed (Hardoim et al. 2008; Truyens et al. 2015). Bacterial endophytes can inhabit multiple tissues (e.g., roots, stems, leaves, flowers, fruits, seeds, vascular tissues), their communities fluctuate with plant development, and they carry out many functions that could potentially regulate growth and development of the host plant during its whole lifecycle. These effects could begin as early as seed establishment, as was shown by their indispensable role in primary colonization and rock-dwelling by some cacti species (Puente et al. 2009). Continually, more and more complex interactions are being unmasked, from non-rhizobia bacteria inhabiting legume nodules with the potential for horizontal gene transfer (HGT) (Li et al. 2008) to endophytic fungi hosting symbiotic bacteria (Lackner et al. 2009; Desirò et al. 2015). There is much we do not know about their assembly rules, distribution in the bulk soil-rhizosphere-endosphere continuum, or effects on plant fitness, but past research on widely studied endosymbionts and phytopathogens can act as a useful reference for future studies (Sasse et al. 2018).

According to the review by Partida-Martínez and Heil (2011), a key question is *how endophytes affect plant physiology, ecology, and, in the long term, evolution*. If endophytes can shape the plant's response to multiple biotic and abiotic factors, and if they can be transmitted from generation to generation, they will most probably affect population dynamics and ecological interactions such as competition, herbivory, or pollination (Friesen et al. 2011). In fact, Friesen et al. (2011) stated that the large populations and short generation times of microorganisms would allow trait mediation to evolve on an ecological time scale. Although the effects of bacterial endophytes at the plant community and ecosystem level are still understudied, with most studies carried out on single plants or monocultures, there is increasing interest in their impact beyond the individual plant level (van der Heijden et al. 2016). For instance, synergistic effects on plant biomass and diversity could be expected from the interaction between different groups of symbionts, likely with outcomes affecting ecosystem functioning (van der Heijden et al. 2008, 2016). Most certainly, these complex communities inhabiting plants have major implications in agroecosystems, as they are widespread in grain, pasture, horticultural, floral, and forestry crops (Baldan et al. 2014). It is also possible that plant breeding has inadvertently modified the microbiome of wild ancestors, which was probably more adapted to marginal soils (Wissuwa et al. 2009; Bulgarelli et al. 2013). But bacterial endophytes are not only important for their impact on plant and ecosystem functioning; they also

constitute a source of novel metabolites to be applied in medicine or industry (Strobel 2003; Smith et al. 2008). A promising aspect for bioengineering is in their capacity to synthesize, at a higher rate, biologically active substances analogous to those synthesized by the host plant (Gunatilaka 2006; Wang and Dai 2011).

Box 1.1 Methodological Constraints in DNA Analyses of Bacterial Endophytes

Despite great improvements in the last decades, the study of bacterial endophytes still faces many methodological challenges and inconsistencies that need to be solved to validate and consolidate different findings. Many reviews are discussing the potential and the flaws of studying and manipulating bacterial endophytes, but we still lack standardized methodologies to unify these research efforts. During our literature review, we could identify different areas in need of a critical methodological revision, testing, and development of a standardized protocol. The work by Richter-Heitmann et al. (2016) constitutes a useful approach to understanding the effectivity and risks attached to different endosphere isolation methods, while it evidences the variability resulting from root morphology traits.

Surface-sterilization. Sodium hypochlorite (NaClO) is the most commonly used agent. Usually, a pre- or post-NaClO treatment with ethanol (70 or 95%) is done to improve penetration and sterilization, although it was suggested it could lead to contraction of the plant tissues (Sieber 2002). For optimal results, concentration, exposure time, and agitation should be tested for different plant species and growth stages (Hallmann et al. 2006). Underexposure to NaClO leads to contamination with surface-dwelling microbes and amplifiable DNA (Reinhold-Hurek and Hurek 2011), but overexposure can damage endophytes (Lundberg et al. 2012). Since residual NaClO can cause DNA mutations and artifacts, we must rinse several times with sterile water, and some authors also included a sodium thiosulfate rinse (Rosenblueth and Martínez-Romero 2004; Pereira et al. 2011). Alternative sterilizing agents, like propylene oxide vapor, hydrogen peroxide, or formaldehyde, are less commonly used (Hallmann et al. 2006; Nassar et al. 2005).

Prior to chemical treatment, shaking with sterile glass beads (McClung et al. 1983; Reinhold et al. 1986; Sessitsch et al. 2012) or sonication can be used to physically remove microbes attached to the plant surface. Sonication has been used both complementary to surface disinfection (Conn and Franco 2004) or as the main removal procedure (Lundberg et al. 2012; Bulgarelli et al. 2012). Microscopy studies have shown that physical removal was less efficient to remove rhizoplane microorganisms than NaClO (Richter-Heitmann et al. 2016; Reinhold-Hurek et al. 2015), while sonication in particular could cause root tissue damage (Richter-Heitmann et al. 2016).

(continued)

Box 1.1 (continued)

Sterilization control. Usually an aliquot of the last water rinse (or an imprint of the sterilized tissue) is incubated in a general culture medium (e.g., TSA or LB), either liquid or solid. Microbial growth (turbidity or colony growth) indicates incomplete removal of surface bacteria (Hallmann et al. 2006). Culture-dependent techniques might not be suitable if the study has a culture-independent approach, and alternative methods should be examined, like microscopy (Turner et al. 2013), PCR (Wemheuer et al. 2017), or sequencing DNA from the last water rinse, similarly to the "kitome" analysis carried out to check for contamination from DNA extraction kits (Kim et al. 2017; Salter et al. 2014).

DNA extraction. Generally carried out directly from plant tissues, whole or ground in liquid nitrogen. On pre-extracted endophytic bacteria, it may overcome some issues of target specificity due to the large amount of plant material (Sessitsch et al. 2012). Extraction methods differ in cell lysis efficiency and removal of PCR inhibitors, biasing downstream analyses, and diversity estimations. Hence, when comparing endophytic and soil microbial communities, there is a compromise between optimizing the procedure for each sample type or treating all samples equally, for example, by using only soil DNA extraction kits (Bulgarelli et al. 2012). Finally, there may exist a trade-off between increased reproducibility and total yield of endophytic diversity, as found when comparing commercial kits and SDS- or CTAB-based DNA extraction tests (Maropola et al. 2015).

Downstream target specificity. Ideally, PCR primers would cover all target taxa but, as we know, modifications and optimizations are continually made even for the highly conserved bacterial 16S rRNA gene (Caporaso et al. 2012). Another common issue is the co-amplification of non-target DNA (e.g., chloroplasts, mitochondria), since samples will have a high ratio of plant DNA relative to bacterial DNA. Several studies have compared the utility of many bacterial 16S primers for use in endosphere microbiome research (Beckers et al. 2016; Dorn-In et al. 2015; Thijs et al. 2017; Klindworth et al. 2013); 799F-1391R, 335F-769R, and 341F-785R have been suggested due to their high coverage of the domain Bacteria. Relic DNA is often discussed as a potential source of variation in soil microbial diversity analyses, since it may persist for months or years (Carini et al. 2017), but its impact is still controversial (Lennon et al. 2018) and, to our knowledge, it has not been explored in the endosphere. However, propidium monoazide may be used to remove contaminating DNA on the root surface, such as from dead microbial cells (Leff et al. 2017).

Understanding the limitations. Even though bacterial endophytes were initially defined as those who could be isolated from surface-sterilized plant tissues, complete removal of surface microbes is challenging. Ideally,

In this chapter, we will discuss current knowledge and prospects on the diversity and function of bacterial endophytes, their influence on plant fitness, and their potential to be manipulated in agroecosystems. The unique symbiotic relationship between legumes and nodulating N-fixing rhizobia, sometimes excluded from the group of endophytic bacteria (Partida-Martínez and Heil 2011), will be mentioned in some examples but detailed information can be found within the large body of published literature (e.g., Poole et al. 2018; Wang et al. 2018). Since this book is mostly dedicated to rhizospheric processes, we emphasize soil-borne facultative endophytes. Obligate endophytes may have a strong influence on plant functioning due to their dependency on their host for survival and reproduction, and that they represent an interesting tool for manipulation, since they are transferred between plant generations via seed (Sachs et al. 2004; Friesen et al. 2011). However, for their distinct behavior and the need to fit the scope of this book, we considered that they deserved a separate analysis. We will also purposely overlook bacteria inhabiting plant surfaces, like the phyllosphere and the rhizoplane, although their influence should not be neglected. Surface-inhabiting communities were shown to affect plant functioning (Oh et al. 2012; Vorholt 2012) and they might be intimately related to the endosphere (Hardoim et al. 2008).

1.2 Diversity of Bacterial Endophytes: What Do We Know?

Studies of bacterial endophytes began with simple systems of one-to-one microbe–host interactions, mostly using traditional techniques like isolation, culturing in synthetic media, and in vitro testing of morphological and physiological features. Many bacterial species have been isolated from different plant tissues and species, as reviewed previously (Hallmann et al. 1997; Sturz et al. 2000; Rosenblueth and Martínez-Romero 2006). Although culturing techniques provide some advantages, like working with isolated strains or working in a controlled environment, they are not amenable to studying the large diversity present in natural environments. In fact, many studies were able to capture more diversity with culture-independent than culture-dependent methods (Araújo et al. 2002; Conn and Franco 2004; Pereira et al. 2011; Qin et al. 2012). While for soils it is estimated that only about 0.001–1% of the microorganisms can be grown in synthetic media (Torsvik and Øvreås 2002), this percentage is unknown for endophytes. Yet, the review by Finkel et al. (2017) stated that the endophytic community encompasses a relatively higher percentage of culturable microorganisms. Supporting this idea, Le Cocq et al. (2017) established

that, compared to soil microorganisms, most endophytes are fast-growing, with a rapid response to nutrient and carbon substrate availability, thus amenable to culture-based work. So far, a complementary application of culture-dependent and -independent methods, together with an improvement of culturing techniques to capture a higher proportion of the actual diversity (Alain and Querellou 2009; Fierer 2017), seems to be a promising approach for exploring endophytic communities.

High-throughput culture-independent methods improved data acquisition, boosting our knowledge of endophytic communities and their ubiquity. First attempts to characterize endophytic bacterial diversity with culture-independent methods were carried out with profiling techniques such as terminal restriction fragment length polymorphism analysis (T-RFLP) and denaturing gradient gel electrophoresis (DGGE) (e.g., Conn and Franco 2004; Seghers et al. 2004; Andreote et al. 2009). More advanced technologies like next-generation sequencing (NGS) allowed more insight into the composition of endophytic communities. For instance, bacteria seem to be the dominant and most ubiquitous taxonomic group in the endosphere, followed by fungi and then, if present, archaea (Hardoim et al. 2015; Krishnaraj and Pasha 2017; Kroll et al. 2017). Studies show that only a few bacterial phyla are consistently dominant (Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes), with higher variability at lower taxonomic resolution levels (Bai et al. 2015; Santoyo et al. 2016; Kroll et al. 2017; Liu et al. 2017a). According to a curated database analyzed by Hardoim et al. (2015), these four phyla comprised ~96% of the total prokaryotic sequences consisting of 21 bacterial and 2 archaeal phyla. These authors reported that Proteobacteria was the most dominant group (54%, and Gammaproteobacteria being the most dominant class), followed by Actinobacteria (20%), Firmicutes (16%), and Bacteroidetes (6%). Bacterial phyla found to have a low abundance in the endosphere are Acidobacteria, Planctomycetes, Verrucomicrobia, and Gemmatimonadetes (Hardoim et al. 2015; Santoyo et al. 2016; Liu et al. 2017a). Several research papers whereby endophytic bacterial communities were analyzed using DNA sequencing studies are listed in the reviews by Bulgarelli et al. (2013) and Liu et al. (2017a). Archaeal phyla have also been detected in plant tissues (Euryarchaeota, Crenarchaeota, and Thaumarchaeota), although they are usually present in low abundance (e.g., <1% of prokaryote sequences) and diversity (Chelius and Triplett 2001; Sun et al. 2008; Sessitsch et al. 2012). However, in some cases archaea can thrive in the root interior, as was found for the genus *Methanobacterium* in rice crops (Edwards et al. 2015). It has been argued that the detection of archaea in root tissues could be an artifact from an incomplete surface-sterilization (Krishnaraj and Pasha 2017). Yet, this is less probable in archaeal endophytes found in aerial tissues such as coffee cherries (Oliveira et al. 2013) and olive leaves (Müller et al. 2015), in the latter case ranging from ~5 to 60% of total prokaryotic sequences, depending on the genotype and geographic origin. Further research is certainly needed to properly describe the distribution of archaea in the bulk soil-rhizosphere-endosphere continuum and within the plant, as well as their functional importance.

Even though there is taxonomic overlap between endophytic and rhizospheric communities (Santoyo et al. 2016), clear community shifts were reported between

these compartments. Studies comparing soil bacterial communities (bulk and/or rhizosphere) with endophytic bacterial communities have consistently shown lower diversity in the latter, independently of the plant species (Pereira et al. 2011; Gottel et al. 2011; Bulgarelli et al. 2012, 2015; Lundberg et al. 2012; Edwards et al. 2015; Saleem et al. 2016; Liu et al. 2017b). This was found at the phylum level but also at lower levels of taxonomic resolution; while in soils many species present low relative abundance or are rare (Fierer 2017), within plant tissues each phylum presents one or a few dominating classes or families (Bulgarelli et al. 2012; Lundberg et al. 2012). A reduced diversity was also found when comparing the root endosphere with the root surface (Bodenhausen et al. 2013). Besides being less diverse, the endophytic community shows a different community composition, with significant fluctuations in the relative abundance of certain taxa (Gottel et al. 2011). While some phyla are enriched with respect to their surrounding soils (e.g., Proteobacteria, Actinobacteria, and Bacteroidetes), others are reduced drastically, like Acidobacteria (Sessitsch et al. 2012; Bulgarelli et al. 2012; Lundberg et al. 2012; Edwards et al. 2015), which is one of the dominant phyla in soils (Crowther et al. 2014; Fierer 2017). The drivers of these changes in diversity and composition are not yet fully understood but some hypotheses are discussed in Sect. 5.3.2.

Results in terms of composition of the endophytic bacterial community are relatively consistent among studies, but we are still in the early phases of discovering the plant microbiome. Endophytic communities are dynamic in space and time, and we need to account for factors like plant genotype, tissue and growth stage, as well as environmental and edaphic conditions (discussed in Sect. 5.3). More studies comparing bulk, rhizosphere, and endosphere microbial communities are needed to identify hypothetical assembly mechanisms, as well as the relative importance of deterministic and stochastic events on endophyte recruitment (Haruna et al. 2018). Lemanceau et al. (2017) stated the need to identify which factors regulate the recruiting beneficial genes: augmenting populations carrying those genes, increasing the frequency of those genes, or increasing the number of populations carrying those genes via HGT (see also Niehus et al. 2015). Promising tools like NGS, bioinformatics, and network analyses are allowing to unmask relationships among different taxa in complex systems. New concepts will help simplify and understand assembly mechanisms, like "core microbiome," as a set of interacting microorganisms that optimize microbial functions (Lemanceau et al. 2017; Toju et al. 2018), and "keystone" or "hub" taxa, defining a group of microorganisms influential for both community structure and function, independently of their abundance (Agler et al. 2016; Banerjee et al. 2018).

1.3   Soil and Plant Shaping Bacterial Endophytes

The holobiont is shaped through the interaction between a host plant and colonizing microorganisms, under the influence of the prevailing environmental conditions. In this process, soils can act as a source of endophytic bacteria, which makes the rhizosphere a key habitat regulating endophytic colonization. This is supported not

only by taxonomic overlaps between rhizosphere and endosphere communities, as mentioned above, but also by several reports of soil type effects on endophytic bacteria inhabiting roots (Dalmastri et al. 1999; Deng et al. 2011; Prischl et al. 2012; Lundberg et al. 2012) or leaves (Knief et al. 2010). In fact, the rhizosphere has been considered one of the primary sources of endophytes, to the extent that bacterial diversity in the plant endosphere is sometimes considered a "subset" of rhizospheric diversity (Compant et al. 2010; Santoyo et al. 2016). The term "subset" can lead to confusion, however, since the relative abundances of different taxa are usually modified in the endosphere, as explained in the previous section. In spite of this, the contribution of soils to the endophytic community seems to be substantial and also dynamic, as it can modify the root endophytic community over time (Wagner et al. 2016).

The effect of plant species or plant genotype is generally smaller than the site effect (Dalmastri et al. 1999; Knief et al. 2010) or even negligible (Prischl et al. 2012). Yet, the relative importance of soil type or plant genotype on shaping the endophytic community is still a controversy (Berg and Smalla 2009; Kandel et al. 2017; Haruna et al. 2018), and the host genotype effect can even exceed the site effect (Li et al. 2012). For example, in a study on *Miscanthus × giganteus*, soil type (i.e., different sites) affected rhizospheric but not endophytic bacterial communities, suggesting a stronger effect of the host plant on the latter (Li et al. 2016). Host plants were also found to shape the structure and function of bacterial endophytes given the same environmental and edaphic conditions, and hence their effect should not be overlooked. Such host genotype effects were observed within the same species (e.g., Bulgarelli et al. 2012; Lundberg et al. 2012; Horton et al. 2014; Wagner et al. 2016), with wild relatives (Bulgarelli et al. 2015; Pérez-Jaramillo et al. 2016), and following genetic modification (e.g., Dalmastri et al. 1999; Dunfield and Germida 2004). These genotype-specific changes, together with the lower diversity and distinct taxonomic composition of endophytic bacteria compared to rhizosphere/bulk soil bacteria, evidence a clear process of selection/recruiting (Hardoim et al. 2008; Turner et al. 2013), where the plant acts as a "filter" or "gatekeeper" (Bulgarelli et al. 2013; Liu et al. 2017a). Further research is needed to unveil the relative weight of these two factors on the composition of endophytic communities. If soils are indeed the main source of bacterial endophytes, we must learn to what extent agricultural practices can determine the composition and function of the plant microbiome, and to what extent this can be controlled by plant-specific factors (see Sect. 5.5.3).

1.3.1   The Rhizosphere as a Source of Endophytic Bacteria

Soil abiotic properties can influence endophytes either via plant metabolism or soil microbial communities. The latter is related to the survival and proliferation of endophytes with a free-living phase in soil; by shaping the bulk soil microbiome, soil properties regulate who can eventually inhabit the rhizosphere and further colonize the plant. Since the rhizosphere is a highly competitive environment, those bacteria with traits like motility, chemotaxis, cell wall degradation, or

polysaccharide production will be favored (Santoyo et al. 2016). By triggering physiological responses in the plant, soil properties can influence the recruiting of endophytes, as well as the abundance or function of those already established (discussed in Sect. 5.3.2), although most studies do not separate these effects. A major constraint in studying soil properties is that they are usually interconnected and, thus, it is not always possible to isolate single effects. This is particularly challenging when edaphic changes are linked to other large-scale factors like climate and elevation, as when different sites are analyzed (Benson and Dawson 2007; Siles et al. 2016), and it might be the reason behind some contradictory results presented below. More studies relating soil properties with endophytic communities are needed, and when controlling co-varying factors is not possible, proper bio-geographical approaches might be suitable to unravel the main driving factors of microbiome composition (Deng et al. 2011). Below, we summarize current knowledge on the effect of different soil properties on bacterial endophytes.

Nutrient Availability It is one of the most studied soil properties in relationship with endophytes, mostly through the rhizobia–legume symbiosis and its negative response to nitrogen (N) availability (Arrese-Igor et al. 1997; Voisin et al. 2002; Carvalho et al. 2014). What was found for rhizobia, a symbiotic N-fixing (i.e., diazotrophic) bacteria, could be expected for other diazotrophic endophytes. Nitrogen concentration and form modified the plant defense response of sugarcane, leading to changes in colonization success of diazotrophic bacteria like *G. diazotrophicus* and *A. diazotrophicus* (Fuentes-Ramírez et al. 1999; de Oliveira et al. 2003). At the community level, N content can affect the structure of root diazotrophic bacteria (Tan et al. 2003; Rodríguez-Blanco et al. 2015), even if no effect is observed on total bacterial endophytes (Li et al. 2016). In another example with non-diazotrophic bacteria, high N favored colonization of tomato plants by *Enterobacter radicincitans* (Berger et al. 2013). In contrast, less attention has been put in other nutrients that could be affecting bacterial endophytes, like phosphorus (P), potassium (K), or sulfur (S), or in nutrient ratios that inform on soil stoichiometry (e.g., C:N, C:N:P). Robinson et al. (2016) found that both composition and abundance of wheat bacterial endophytes were affected by nutrient availability (N, P, K, and Mg), with low nutrient soils supporting higher abundance of endophytic bacteria. In an in vitro study, exudates from N-starved or P-starved maize impacted the transcriptome of the root endophyte *Bacillus amyloliquefaciens*, while small effects were observed from Fe and K starvation (Carvalhais et al. 2013). The authors found that, while N starvation seemed to negatively affect *B. amyloliquefaciens* growth, P starvation activated genes involved in chemotaxis and motility. Effects of soil nutrition can also be reflected in the long-term, for example, with low nutrient conditions favoring mutualistic relationships (Robinson et al. 2016).

Water Content It influences nutrient mobilization, oxygen diffusion and, thus, redox conditions, chemical reactions, and nutrient availability. Such effects on the edaphic environment could affect endophytes via plant physiology (Naylor and

Coleman-Derr 2018) or soil microbial communities, since they are sensitive to both drought (Schimel et al. 2007) and waterlogging (González Macé et al. 2016), as well as seasonal changes in water availability (Taketani et al. 2017). In rice, flooding caused a shift in endophytic diazotrophic bacteria (Ferrando and Fernández Scavino 2015) and seed-borne endophytic bacterial communities (Hardoim et al. 2012). Endophytic bacteria can also be affected by drought (see review by Naylor and Coleman-Derr 2018), as found in sorghum roots (Xu et al. 2018). Water availability could also lead to adapted endophytic communities; for example, Benson and Dawson (2007) found that intermediate soil water content was beneficial for *Frankia* sp. diversity compared to extreme water availability situations. Some endophytic species, however, seem to be more adapted to waterlogged conditions (Engelhard et al. 2000) or drought (Grönemeyer et al. 2012; Yandigeri et al. 2012).

pH The effects of pH on soil bacterial communities has been observed in many studies (Rousk et al. 2010; Bartram et al. 2014; Karimi et al. 2018), and thus, a repercussion on endophytes is also expected. pH was found to alter the composition of endophytic bacteria in wheat and soybean (Rascovan et al. 2016) and in rice seed-borne bacterial endophytes (Hardoim et al. 2012), but not in *Miscanthus* sp., even though rhizospheric bacteria were affected (Li et al. 2016). Studies carried out in legume-nodulating rhizobia found changes in their composition, either in soil or nodules, that could be attributed to direct or indirect effects of soil pH (Anyango et al. 1995; Bala et al. 2003; Han et al. 2009), although results were not always consistent (Diouf et al. 2007). Still, because of the unique nature of the legume–rhizobia symbiosis, its behavior may not be entirely relatable to other bacterial endophytes.

Texture This is one of the soil properties that can hardly be interpreted in isolation, since other variables are co-varying and could be at play (e.g., organic matter accumulation, water regime, and physical properties like aggregation and bulk density). Some studies, however, reported changes in the composition or diversity of soil microbial communities with soil texture (Sessitsch et al. 2001; Chau et al. 2011). When it comes to endophytic communities, the system becomes more complex as more influencing factors interact with soil texture, like water and air fluxes, or root penetration and architecture (Saleem et al. 2018). Comparing a fine-particle rich soil with a sand-rich soil, Bulgarelli et al. (2012) found that soil type not only influenced bacterial communities from bulk soil but also from the rhizosphere and root endosphere of *Arabidopsis thaliana*. Soil texture could also affect the proliferation and colonization success of inoculated endophytic bacteria (Afzal et al. 2011).

Structure Less stable than texture, soil structure is susceptible to agricultural practices like tillage or cropping (Munkholm et al. 2013; Smith et al. 2016; Pires et al. 2017). Structure has a huge control over the soil environment, mainly by regulating the fluxes of air and water, root exploration, and the formation of multiple microhabitats through aggregation. Different microhabitats will host different

microorganisms and biochemical reactions (Mummey et al. 2006; Ruamps et al. 2011). Even so, to this day the effects of soil structure on microbial communities are considered uncertain (Santoyo et al. 2017), and even less is known about how it can alter the endophytic community. Li et al. (2016) found that bulk density, together with organic carbon, potassium, and phosphorus, explained variations in the rhizosphere and endophyte compartment of *Miscanthus* × *giganteus*.

Organic Matter Besides its role in aggregation and soil structure, soil organic matter regulates carbon, energy, and nutrient availability for microorganisms, therefore, affecting their functionality and eventually composition. It is also susceptible to disturbance and agricultural management, and it was associated with soil microbial community composition (Fierer 2017). The direct effect of organic matter content on endophytic bacteria has not been explored much in the literature, but it was one of the relevant factors shaping community structure in *Miscanthus* x *giganteus* rhizomes, as mentioned above (Li et al. 2016). Also, a study by Long et al. (2010) found that *Nicotiana attenuata* root bacteria were more diverse in organic than mineral soils.

Biotic Interactions In soils, especially in the substrate-rich rhizosphere, microbe–microbe interactions regulate community dynamics and plant colonization, although they have received less attention than abiotic factors (Agler et al. 2016). Microbe–microbe interactions include mutual signaling and coordinated invasion of the root, as well as competition, antibiosis, and predation (Gaiero et al. 2013). Using a systems biology approach on *A. thaliana* phyllosphere, Agler et al. (2016) exposed the importance of these interactions in community structure, and suggested that microbial "hubs" (i.e., groups of highly interconnected taxa) are the ones to respond sensibly to environmental factors, to then extend the effects to the rest of the community. Network analyses like this one can contribute to find co-occurrence patterns and taxonomic links, but mechanistic studies at the organism level are also needed to understand how these interactions operate and what triggers them. A significant amount of research was dedicated to quorum sensing and other microbe–microbe interactions, and their relevance for plant-associated microbes (Fray 2002; Faure et al. 2009). Quorum sensing is a bacterial density-dependent cell–cell signaling mechanism mediated by low molecular weight compounds (e.g., N-acyl homoserine lactones or AHLs), which coordinates processes such as plant–microbe communication, antibiosis, virulence, and siderophore production (Altaf et al. 2017). These processes are also relevant for soil inoculants, since introduced microorganisms will inevitably interact with the native microbiome before colonizing the plant.

1.3.2　The Plant as a Regulator of the Endophytic Community

Plants can regulate their microbiome either by altering the rhizospheric community, by recruiting and regulating the colonization process, or by affecting the persistence

or activity of established microorganisms. In this section we will focus on the colonization process and changes that occur within the plant. The effect of plants on potential endophytes inhabiting soils will not be reviewed here, but it is important to acknowledge the role of root exudates, litter and root morphology, which can act either directly (Dennis et al. 2010; Leloup et al. 2018; Saleem et al. 2018) or indirectly, by altering abiotic properties in the root surroundings (Angers and Caron 1998; Read et al. 2003). These three factors not only vary between plant species and genotypes but also fluctuate with plant growth stages and environmental factors (Ortíz-Castro et al. 2009).

The exact process of endophyte recruiting is still unknown, although root exudates are thought to play a key role (Badri and Vivanco 2008; van Dam and Bouwmeester 2016). Even though the role of many compounds in the root exudates has not been yet identified (Chagas et al. 2018), it is known that some can act as chemo-attractants and sources of carbon and energy, while others, like phenolic compounds, are involved in signaling or defense functions and can have a more selective effect on microbe recruitment (Badri et al. 2013). Bulgarelli et al. (2013) proposed a two-step model for endophyte recruitment, where the first step consists of shaping a rhizosphere microbiome by promoting organotrophic bacterial populations, via rhizodeposition of root exudates. Some plant cell wall features might also be determinant in this enrichment, as found when comparing bacterial colonization of sterile exudate-free wooden splinters with *Arabidopsis* roots (Bulgarelli et al. 2012; Hirsch and Mauchline 2012). A second selection step, more specific and dependent on the host plant genotype, determines the effective colonization by a chemical interaction between plant and microbes (Bulgarelli et al. 2013), involving signaling molecules such as flavonoids (Hassan and Mathesius 2012), glucosinolates (Bressan et al. 2009), salicylic acid (Lebeis et al. 2015), and malic acid (Rudrappa et al. 2008). Microbes also participate in this signaling; for example, secondary metabolites and other compounds help plants to screen beneficial and pathogenic microbes through microbe-associated molecular patterns (MAMPs or PAMPs) (Van Wees et al. 2008; Chagas et al. 2018). According to the review by Ortíz-Castro et al. (2009), elicitors or defense signaling molecules (e.g., salicylic acid, methyl jasmonate, and nitric oxide), many of them of microbial origin, can induce the plant to accumulate secondary metabolites associated with plant–microbe communication (e.g., glucosinolates, phytoalexins, and alkamides). Plants, at the same time, can interfere in microbe–microbe communication via anti-quorum sensing activity (Gao et al. 2003; Vasavi et al. 2016), a trait that has also been considered for application via genetic modification (see Sect. 5.5.2). The molecular mechanisms by which plants communicate with and select different bacterial endophytes over others remain largely unknown (Kandel et al. 2017). Detailed aspects of the chemistry of plant–microbe interactions can be found in the recent review by Chagas et al. (2018).

Once on the rhizoplane, soil-borne endophytes colonize or enter the cortical cell layer of the root by both active and passive mechanisms (Compant et al. 2010; Hardoim et al. 2015), the latter comprising the so-called passive endophytes or those incorporated through rhizophagy. Active colonizers will either explore potential