

Chapter 2

Bacterial Endophytes of Plants: Diversity, Invasion Mechanisms and Effects on the Host

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Abstract Plant inner tissues are colonized by bacterial organisms known as endophytes. The relatively recent application of culture independent and molecular high throughput techniques allowed the description of a large diversity of endophytic bacterial taxa. These microorganisms can be found in any plant organ, including fruits and legume nodules. Some endophytic bacteria benefit the host by several mechanisms, and their application to economically important crops represents an interesting alternative to the use of agrochemicals. However, more studies are required to clearly assess their effects on the hosts (especially in co-inoculation with other beneficial bacteria) and the molecular events that lead to the interaction between plants and endophytic microorganisms. In this chapter, we focus on bacterial endophytes from legumes and non-legumes plants, analyzing their diversity and effects on the hosts. We also discuss the endophytic colonization of legume nodules, with emphasis on the endophytic bacterial diversity, the mechanisms involved in the nodule invasion and their effects on the hosts.

Keywords Endophytes · Biocontrol · Symbiosis · Rhizobia · Legumes

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2.1 Introduction

Plants are known by their ability to interact with a large number of diverse microorganisms. In fact, it is thought that this ability constitutes one of the main innovations that allowed the algal ancestor of plants to colonize land (Delaux et al. 2015). Microorganisms interacting with plants include prokaryotic and eukaryotic taxa and can colonize the surface or internal parts of the host. Those prokaryotic microorganisms that can be detected within the tissues of apparently healthy plant host are considered as endophytic bacteria (Schulz and Boyle 2006). Although this definition is arbitrarily limited to non-pathogenic bacteria, its functional nature is useful for the purpose of this chapter. Here, we will use the term “endophyte” to refer to those bacteria detected by molecular methods or isolated from inside tissues that cause no visible harm to the plant. Indeed, some endophytes are able to benefit the host in several ways such as conferring biotic and abiotic stresses resistance and tolerance, enhancing nutrient availability, degrading toxic substances, and producing phytohormones (Wilson 1995; Hardoim et al. 2008; Doty 2011; Gaiero et al. 2013; Kandel et al. 2015).

Years ago, analysis of endophytic microorganism diversity relied on the identification of those that can be recovered in rich culture media from surface sterilized plant organs. However, culture-dependent methods confer selective advantage to some bacteria and do not allow a complete overview of the endophytic population (Bhattacharjee et al. 2008). Recently, the use of molecular approaches (including high throughput techniques) allowed the description of a larger diversity of plant endophytes.

2.2 Rhizobial and Non-Rhizobial Endophytes of Non-Legume Plants

Endophytic bacteria have been recovered from a wide array of plant species, suggesting a ubiquitous presence in nearly all higher plants (Luo et al. 2012). The structure of these communities depends on soil biotic and abiotic factors affecting bacterial survival, host factors that allow colonization and microbial determinants that shape the ability of the endophytes to survive and compete within the plant hosts (Gaiero et al. 2013). Microorganisms can reach the plants through a variety of sources, such as soil (Hallmann et al. 1997), water from precipitation or irrigation, the fall of atmospheric dust or wind (Agrios 1997; Morris et al. 2010; Savage et al. 2012), animals that can carry microorganisms (Villate et al. 2012), seeds, seedlings, plants from distant areas (Agrios 1997; Dobbelaere et al. 2001; Alabouvette et al. 2006; Truyens et al. 2014), and plant remnants (litter, crop residues) (Leplat et al. 2013). Moreover, seed endophytes can be vertically transmitted from generation to generation in plants that are propagated vegetatively (Moënné-Loccoz et al. 2015).

Application of new tools such as next generation sequencing technologies to study the plants endophytic community has shown that its composition is highly underestimated. Hardoim et al. (2015) constructed and analyzed a database of all currently 16S rDNA sequences assigned to endophytes, including cultured and uncultured microorganisms, and found that, although the sequences belong to 23 different bacterial Phyla, 4 of them (Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes) encompass for 96% of the total number of endophytic prokaryotic sequences. Among them, Proteobacteria includes more than 50% of the sequences in the database. Within this phylum, isolates from the Gammaproteobacteria subclass are the most commonly found as endophytes, including genera such as *Pseudomonas*, *Enterobacter*, *Pantoea*, *Stenotrophomonas*, *Acinetobacter*, and *Serratia*. On the other hand, genera *Streptomyces*, *Microbacterium*, *Mycobacterium*, *Arthrobacter* (within Actinobacteria) as well as *Bacillus*, *Paenibacillus*, and *Staphylococcus* (Firmicutes) are also well represented among the endophytic microorganisms (Hardoim et al. 2015). As species from all these genera are common in soils, it has been suggested that the endophytic microbial community constitutes a subpopulation of the rhizospheric bacteria (Germida et al. 1998; Marquez-Santacruz et al. 2010; Santoyo et al. 2016). However, how the plants manage to select a certain group of endophytes is still not fully understood.

Rhizobia are a diverse group of soil bacteria known for their ability to establish a symbiotic interaction with legumes. They induce in their plant host the development of nodules that house these nitrogen fixing microorganisms. Interestingly, rhizobia have also been found colonizing non-legume plants tissues, but, with the exception of *Parasponia*, induction of nodule formation has never been reported (Yanni et al. 1997, 2001; Prayitno et al. 1999; Biswas et al. 2000a, b; Chaintreuil et al. 2000; Gutierrez-Zamora and Martinez-Romero 2001, Hilali et al. 2001; Peng et al. 2002; Lupwayi et al. 2004). As several studies indicated that endophytic rhizobia promote non-legume plants growth, their application as biofertilizers may represent a useful strategy in sustainable agriculture.

2.2.1 How Endophytes Gain Access to Plant Tissues?

Bacterial endophytes invade and colonize internal plant tissues, using organic plant metabolites for growth and survival, and avoiding host defense responses. The main site for endophytes entry into plants tissues is the root zone (Compant et al. 2005; Meneses et al. 2011; Gaiero et al. 2013), but they can also invade aerial tissues (Chi et al. 2005). Bacteria endophytes can entry through plant's flowers and therefore, they may be found in fruits. Another mode of invasion of the host plant is through infection of seeds, assuring their presence in new plants.

Chemotactic signals play a very important role in the first step of endophytes root surface colonization. Moreover, they can enhance their competitive performance and regulate the expression of genes involved in plant tissue invasion (Bais et al. 2006; Rosenblueth and Martinez-Romero 2006; Compant et al. 2010;

Carvalho et al. 2016). Meanwhile, the host plant recognizes and selects the beneficial bacteria to associate with and as a consequence, root endophytic bacteria communities may differ from bacteria communities in the rhizosphere. Therefore, microbe–microbe and microbe–plant signaling are involved in the plant tissue colonization process. Host plant–potential endophytic bacteria cross talk begins with signaling molecules released by plant roots. Chemical signals and nutrients excreted by the roots modulate and determine the abundance and diversity of bacteria that colonize the root (Bais et al. 2004). For example, flavonoids and some phytohormones were also found to improve *Serratia* spp. rice seedlings endophytic colonization (Balachandar et al. 2006). It has been observed that *Arabidopsis thaliana* selectively recruits the biocontrol agent *Bacillus subtilis* FB17 by secretion of malic acid to prevent pathogenic attack (Rudrappa et al. 2008). Rice and sugarcane plants modify their chemical signals when they interact with beneficial bacteria or pathogenic bacteria (Gaiero et al. 2013).

In addition to plant exudates, the quorum sensing system (QS) of potential endophytes has a main role in plant tissue colonization, since it regulates the expression of bacterial genes involved in this process. The most common QS signals found in Gram-negative bacteria are *N*-acyl homoserine lactones (AHLs) while in Gram-positive bacteria are peptides (Kleerebezem et al. 1997; Gaiero et al. 2013). It is known that plants can positively or negatively affect AHL-dependent QS responses.

Once the potential endophyte is attracted to the plant root, it has to attach to it. Type IV pili are essential for bacterial adherence and colonization of host cell surfaces (Carvalho et al. 2016). Moreover, a mutation in *Azoarcus* sp. pilin, a major component of Type IV pili, reduced its adhesion and colonization of rice roots (Dörr et al. 1998). In addition, Gram-negative bacteria surface components (exopolysaccharides (EPS) and lipopolysaccharides (LPS) are involved in the attachment and colonization. Moreover, plant–bacteria recognition may be modulated by bacterial effectors delivered into the plant cells by a type III protein secretion system (TTSS) (Carvalho et al. 2016).

After the potential endophyte bacteria are attracted to the root and attached to its surface, they multiply and reach a population density that enables them to form biofilms. Biofilm formation allows non-spore-forming soil bacteria to colonize their surrounding habitat. The major components of biofilms are water and bacterial cells. The next most important component is an EPS matrix, which provides a physical barrier against diffusion of defense substances from the host and protection against environmental stressing factors. Minor components include macromolecules such as proteins, DNA, and other products released by cells lysis (Rinaudi and Giordano 2010). Meneses et al. (2011) demonstrated that EPS biosynthesis is required for *Gluconacetobacter diazotrophicus* PAL5 biofilm formation and rice endophytic root colonization, since when they knocked out a gene involved in EPS biosynthesis, mutant bacteria were defective in biofilm formation, root surface attachment, and endophytic colonization.

Bacterial signals recognition by plants is mainly mediated by the plant receptors-like kinases (RLK), such as leucine-rich repeat–receptor-like kinases

(LRR–RLKs), wall-associated kinases (WAK), lectin receptor-like kinases (LecRLKs), Lys-motif receptors (LysM), among others; and by plant small RNAs (sRNA) as miRNA, and small interfering RNA (siRNA) (Carvalho et al. 2016).

After the initial colonization, some endophytes enter roots and gain access to the interior tissues, migrating endophytically upward into the leaf or stem bases. They may pass through root tips (root tip pathway) or through the middle lamella of the epidermal layer (Compant et al. 2005). Three modes of nitrogen fixing organism entry into roots have been described: (a) through wounds particularly where lateral or adventitious roots protrude, (b) through root hairs, (c) between undamaged epidermal cells (Cocking 2003). It has been proposed that cellulolytic and pectinolytic enzymes produced by endophytes are involved in the infection process (Hallmann et al. 1997). The mechanism is known as “crack entry” allows some endophytes to passively gain entry the interior part of plant using epidermal junctions between root hair and adjacent epidermal cells, or disrupted endodermal cell layers resulting from the emergence of developing lateral roots. This mode of entry (often combined with active penetration) has been suggested for different bacterial species such as *Burkholderia* (Compant et al. 2005; Govindarajan et al. 2006), *Bacillus* (Ji et al. 2008), and *Herbaspirillum* (James et al. 2002) among others. It is interesting that this entry route is an ancient strategy also used by rhizobia in the interaction with some legumes to establish a symbiotic relationship (Fabra et al. 2010; Huang et al. 2011). Instead of that, *Pseudomonas* spp. use root hairs as the main entrance for endophytic colonization of olive roots, regardless they have been previously colonized, but well-known root hair morphological changes induced by rhizobia in legumes were not observed (Prieto et al. 2011).

2.2.2 Plant Growth Promotion by Endophytes

Plant endophytes can promote plant growth by fixing atmospheric nitrogen, producing phytohormones, controlling phytopathogens, or by enhancing the uptake of minerals. In this sense, there are many studies demonstrating the beneficial effects of endophytes. For instance, the endophytic diazotrophic bacteria *Gluconacetobacter diazotrophicus* improves sugarcane growth (Cocking 2003). In this plant, as well as in other non-legumes plants, the role of endophytic diazotrophic bacteria in N nutrition has been demonstrated by quantifying ^{15}N (Chalk 2016).

In *Zea mays*, the endophyte *Azospirillum lipoferum* alleviates drought stress symptoms through production of abscisic acid and gibberellins (Cohen et al. 2009). In *Solanum tuberosum* and *Vitis vinifera*, the endophyte *Burkholderia* sp. promotes plant growth by reducing the level of the inhibitory hormone ethylene through production of high levels of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Fommel et al. 1991; Barka et al. 2000). Citrus plants were protected against the pathogen *Xylella fastidiosa* by the endophyte *Curtobacterium flaccumfaciens* (Araujo et al. 2002). The inoculation of *Bacillus* sp in *Arachis hypogaea* plants induced the systemic resistance against *Sclerotium rolfsii* (Tonelli et al. 2011).

Some studies indicated that co-inoculation of endophytes with different ecological niches is a promising alternative to individual PGPR inoculation. For example, *Avicennia germinans*, *Laguncularia racemosa*, and *Rhizophora mangle* plants co-inoculated with the phosphate solubilizing *Bacillus licheniformis* and the nitrogen-fixing *Phyllobacterium* sp. showed better nitrogen and phosphorous assimilation than plants inoculated individually with the endophytic bacteria (Rojas et al. 2001). It is important to highlight that not always the co-inoculation of beneficial endophytes results in an improved plant growth effect compared to individual inoculation. Bent and Chanway (1998) showed that the plant-growth-promoting ability of some rhizobacteria in *Pinus contorta* can be significantly reduced in the presence of another rhizobacterium, even when individually both strains can benefit plant growth.

2.3 Non-Rhizobial Endophytic Bacteria Within Legume Nodules

Although the interior of any plant organ can be colonized, a particular endophytic colonization takes place in legume root nodules. We refer to nodule endophytic bacteria as the occupants of the nodules unable to induce their formation, therefore excluding compatible rhizobia. At first, nodule endophytic bacteria were considered artifacts derived from a deficient surface disinfection of the root nodules. Later, it was found that they were capable to effectively colonize the interior of nodules induced by compatible rhizobial strains (Bai et al. 2002; Ibáñez et al. 2009). Currently, endophytic colonization of legume nodules is a promising field for identifying bacterial strains with new PGP activities or for optimizing plant growth promoting rhizobacteria (PGPR) inoculation. In fact, these endophytes share the nodule resources with rhizobia and, at least in theory, can positively or negatively affect biological nitrogen fixation. Moreover, nodules offer a controlled and rich in carbon source environment where endophytic bacteria can multiply. Afterward, releasing of bacteria with PGP properties from senescent nodules could represent a new source of inoculum to the soil.

2.3.1 Diversity of Endophytic Bacteria Found Inside Nodules and Their Hosts

As research expands to include new geographic regions or other legume clades, more and more endophytic bacterial groups are described inside nodules. To date, a wide range of bacteria was described as nodule endophytes. They comprise Gram-negative or Gram-positive bacteria included within Phyla phylogenetically diverse such as Proteobacteria, Firmicutes, Actinobacteria (reviewed in Peix et al. 2012, 2015; Velázquez et al. 2013) and the Cytophaga-Flavobacterium-Bacteroides (CFB) group

(De Meyer et al. 2015). Within Proteobacteria, endophytes were found mostly in alpha (Zakhia et al. 2006; Muresu et al. 2008; Deng et al. 2011), beta (Valverde et al. 2003; Li et al. 2008; Hoque et al. 2011), and gamma (Zakhia et al. 2006; Li et al. 2008; Ibañez et al. 2009; Deng et al. 2011; Hoque et al. 2011) subclasses. In Firmicutes, genera *Bacillus* and *Paenibacillus* encompass the majority of non-nodulating rhizobial endophytes (Zakhia et al. 2006; Li et al. 2008; Deng et al. 2011). Within Actinobacteria, bacteria belonging to the genera *Microbacterium*, *Mycobacterium*, *Agromyces*, *Ornithinococcus*, *Nocardia*, *Streptomyces*, and *Micromonospora* were described as nodule endophytes (Zakhia et al. 2006; Trujillo et al. 2010; Deng et al. 2011). Considering all these reports, bacteria from *Agrobacterium*, followed by *Bacillus* and *Pseudomonas* are the most frequently genera obtained from inside nodules of a vast diversity of legumes.

In relation to the hosts, endophytic bacteria have been found to colonize nodules belonging to two of the three Fabaceae subfamilies (Papilionoideae and Mimosoideae) but, to our knowledge, there are no studies reporting nodule endophytic microorganisms on members of the basal Caesalpinioideae subfamily. This is probably related to the fact that nodulation is not so common within this basal legume group and also to the lack of deep studies on these plants. Expanding the studies of nodule endophytic bacteria to the nodulating members of this group of legumes will contribute to a better grasp of the bacterial diversity found within nodules.

Regarding the existence of specificity in the endophytic association, evidences suggest that there are no recognition mechanisms as strict as the ones involved in rhizobial symbiosis for endophytic colonization of the nodules. First, the great phylogenetic diversity of endophytic bacteria compared to the (relatively) narrow phylogenetic range of rhizobia. Second, some genera such as *Agrobacterium*, *Bacillus*, and *Pseudomonas* are able to colonize nodules of phylogenetically diverse legumes. Similarly, nodules from the same plant species can harbor a very diverse group of bacterial endophytes. For instance, bacteria from the phylogenetically distant genera *Bacillus*, *Agrobacterium*, and *Pantoea* were described as nodule endophytes of *Glycine max* (Velázquez et al. 2013). However, data seem to indicate that plants can select a specific subset of microorganisms to allow colonization of nodules. De Meyer et al. (2015) analyzed a large subset of nodule endophytic microorganisms from 30 species of indigenous legumes in Belgium and found that certain group of plants “prefers” some endophytes. Moreover, authors suggest a correlation between some rhizobial occupants of the nodules and certain groups of endophytic microorganisms. However, such concept is yet to be confirmed.

2.3.2 Mode of Entry of Bacterial Endophytes to Legume Root and Nodule Tissues

Bacterial genera most frequently isolated from inside nodules are also the most commonly found as root endophytes (including both legumes and non-legumes), suggesting that colonization of nodules does not rely on microbial specific traits

others than the ones required for root colonization. However, it is still not clear if there is any additional microbial trait particularly associated with nodule colonization, or a specific plant–microbe signaling for invasion of this specialized organ.

Sites for primary colonization and entry into the plant of non-symbiotic bacterial endophytes are undifferentiated tissues above the root tips and the points of emergence of lateral roots, as also described for rhizobia (Reinhold-Hurek and Hurek 1998). This first step in the tissue entry process of non-symbiotic endophytes also involves root adsorption and bacterial proliferation, forming biofilm structures at the surface of roots (Compant et al. 2010; Reinholdt-Hurek and Hurek 2011). Later, ways by which non-rhizobial endophytes can get access to the interior of legume roots have also been described in non-leguminous plants. In fact, they are able to use epidermal junction between root hair and adjacent epidermal cells, or disrupted endodermal cell layers resulting from the emergence of developing lateral roots, the mechanism known as “crack entry”. Root hairs also represent a site for endophytic bacteria entry. *Pseudomonas* spp. the main entrance for endophytic colonization of olive are root hairs, regardless they have been previously colonized, but well-known root hair curling and infection thread induced by rhizobia in legumes were not observed (Prieto et al. 2011). In *Vigna radiata*, the invasion of infection threads by *Pseudomonas* and *Klebsiella* strains led to nodule colonization when co-inoculated with host-nodulating *Ensifer adhaerens*. The presence of the three strains: *E. adhaerens*, *P. fluorescens*, and *K. pneumoniae*, within the same root hair was demonstrated, and the inability of *P. fluorescens* and *K. pneumoniae* to colonize the interior of root hairs was attributed to their inability to secrete cellulase and pectinase (Pandya et al. 2013). In *Lotus japonicus*, infection threads initiated by *Mesorhizobium loti*, symbiont of *Lotus*, can guide endophytic bacteria toward nodule primordia. Inside these cells, competent strains multiply and colonize the nodule together with the symbiotic partner (Zgadżaj et al. 2015) (Fig. 2.1).

Nevertheless, how non-rhizobial rhizobacteria breach the rhizobial host specificity and enter root nodules remains unanswered. Even though symbiotic and non-symbiotic endophytes seem to use similar entry routes, to date, formation of nodules by endophytic bacteria other than rhizobia and *Frankia* has not been informed, with the exception of *Pseudomonas* spp. which induces nodules on *Robinia pseudoacacia* roots, probably after the acquisition in the soil of symbiotic genes from rhizobial species (Shiraishi et al. 2010).

Genetic diversity among nodule endophytes and their wide host-range suggest the absence of a sophisticated molecular recognition between the partners. However, it is becoming clear that plants are able to select their endophytic bacterial population by still not fully understood mechanisms. Possibly, the nodules endophytes use an ancestral form of colonization and accommodation, involving ancient traits. Studies focusing on the partners' genetic determinants allowing the endophytic colonization and accommodation inside the nodules could shed light on the evolution of the earlier steps of the beneficial interaction between plants and bacteria.

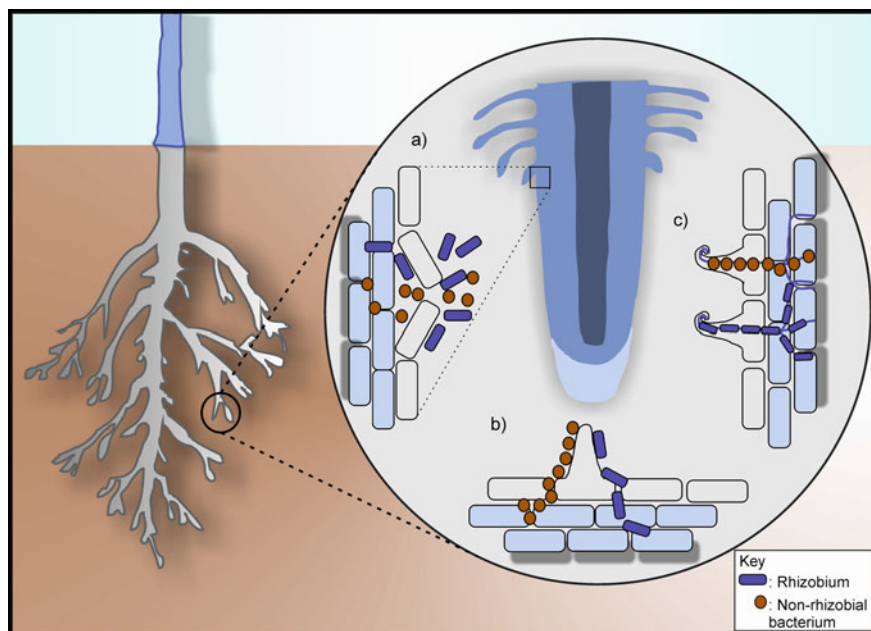


Fig. 2.1 Mode of entry to plant roots shared by rhizobial and non-rhizobial endophytes **a** through disrupted epidermal cell layers resulting from the emergence of developing lateral roots (“*crack entry*”), **b** root hairs colonization, without induction of morphological changes, and later invasion through intercellular spaces, **c** colonization of infection threads previously induced by rhizobial strains

2.3.3 How Plants May Benefit from Non-Symbiotic Nodule Endophytes?

Root nodule is an environmental niche induced by symbiotic bacteria. For a long time, it was believed that rhizobia or *Frankia* were the only nodule inhabitants in legumes and actinorhizal plants, respectively. Current data indicate that nodules may harbor a wide diversity of bacteria and that symbiotic and non-symbiotic endophytes coexist.

Recently, *Micromonospora saelicensis* was identified as the most frequently bacterial species isolated from nodules of both leguminous and actinorhizal plants (Valdés et al. 2005; Trujillo et al. 2006, 2007, 2010; Garcia et al. 2010; Carro et al. 2012, 2013). However, the ecological role of bacterial endophytes others than rhizobia and *Frankia* inside the roots nodules, as well as their interaction with these nitrogen fixing bacteria, is unknown. In *Lotus japonicus*, it has been reported that colonization of nodules by endophytic bacteria is a selective process, host controlled, and that bacterial EPS are required for chronic infection of nodules. Therefore, it seems that the legume host invaded by infection threads formation

controls not only the symbiont access into nodules but also the endophytes (Zgadzaj et al. 2015). However, no information is available in legumes infected intercellularly without infection threads.

Plants have evolved sophisticated mechanisms to control microbial presence and infection. Therefore, only particular microbes are able to colonize the internal tissues with minimal or no host damage. Intracellular accommodation and multiplication of compatible symbionts are allowed only inside nodules.

Considering that some legumes may control the endophytes entry to nodule, it is possible to speculate that those bacteria located inside nodules are beneficial. In fact, reports indicate improved plant health, nodulation, and yield when co-inoculated with nodule endophytes, compared to inoculation with rhizobia alone (Sturz et al. 1997; Bai et al. 2002, 2003; Rajendran et al. 2008). It has also been shown that *Micromonospora* inoculation enhances alfalfa aerial growth, and an increase of nitrogen uptake by the plant is a general phenomenon in this interaction (Martínez-Hidalgo et al. 2014). In the same sense, co-inoculation of peanut with the bradyrhizobial symbiont and endophytic gammaproteobacteria belonging to *Enterobacter* increased number of nodules (Ibáñez et al. 2009). Interestingly, these isolates were also capable to increase maize growth parameters when inoculated in a simulated peanut–maize rotation system (Ibáñez et al. 2014). In *Vigna radiata*, nodule endophytic bacteria belonging to genera *Klebsiella*, *Agrobacterium*, *Dyadobacter*, *Chitinophaga*, *Paenibacillus*, and *Bacillus* were beneficial for plant growth (Pandya et al. 2015). In *Melilotus dentatus*, it was demonstrated that an *Agrobacterium* strain originally isolated from nodules of *Onobrychis viciifolia* could co-inhabit root nodules with the symbiotic *Sinorhizobium meliloti* strain, without affecting the growth and nodulation of plants (Wang et al. 2006).

It is known that legumes can recognize rhizobia performances in the nodules and impose sanctions that affect the symbiont fitness (Kiers et al. 2003). Therefore, a positive (or at least non-detrimental) effect of the nodule endophytes on the plant host can also drive the ecological fitness of these endophytes. However, inoculation with nodule endophytic bacteria may have a negative effect on growth and yield parameters. In the common bean, the nodule endophytic *Agrobacterium* strains might reduce the nodulation of *Rhizobium gallicum* (Mrabet et al. 2006). This effect seems to be host-specific, since they did not affect nodulation of *Sinorhizobium meliloti* with alfalfa (Wang et al. 2006).

Our knowledge of the interaction among symbiotic, non-symbiotic bacteria coexisting in nodules, and host plant is still scarce, and more studies are necessary to understand fully not only the role of this ecological process but also the molecular interaction between plants and non-symbiotic nodule endophytes.

2.4 Conclusions

As knowledge on plant–microorganism interaction expands, researchers have begun to consider that plants host not only different endophytic communities but also can recruit a subset of microorganisms, presumably for specific functions. Even plant specialized organs such as nodules are now considered susceptible to be colonized by different bacterial species. Many studies suggest that plants and their microbiome are in constant communication through the exchange of signals. However, it is just beginning to understand mechanisms and functions of these interactions. Most functional studies have been performed using experimental strategies commonly applied to the study of plant–individual microorganism interactions. Therefore, additional research around these concepts may help to determine the interactive functionalities that occur between plants and their microbiome and would provide a mean to further increases plant growth promoting potential, reaching maximum crop yields.

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