American Journal of Botany

American Journal of Botany 100(9): 1738–1750. 2013.

SPECIAL INVITED PAPER—RHIZOSPHERE INTERACTIONS

Inside the root microbiome: Bacterial root endophytes and plant growth promotion[1]

Jonathan R. Gaiero, Crystal A. McCall, Karen A. Thompson, Nicola J. Day, Anna S. Best, and Kari E. Dunfield[2]

School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Bacterial root endophytes reside in a vast number of plant species as part of their root microbiome, with some being shown to positively influence plant growth. Endophyte community structure (species diversity: richness and relative abundances) within the plant is dynamic and is influenced by abiotic and biotic factors such as soil conditions, biogeography, plant species, microbe–microbe interactions and plant–microbe interactions, both at local and larger scales. Plant-growth-promoting bacterial endophytes (PGPBEs) have been identified, but the predictive success at positively influencing plant growth in field conditions has been limited. Concurrent to the development of modern molecular techniques, the goal of predicting an organism's ability to promote plant growth can perhaps be realized by more thorough examination of endophyte community dynamics. This paper reviews the drivers of endophyte community structure relating to plant growth promotion, the mechanisms of plant growth promotion, and the current and future use of molecular techniques to study these communities.

Key words: endosphere; microbial community; molecular methods; plant-growth-promoting bacteria; plant–microbe interactions; rhizosphere.

The interaction between plants and microorganisms in the soil is well recognized. Hiltner (1904; in Hartmann et al. [2008]) first observed that microorganisms were more abundant in the soil surrounding the plant roots than in soil remote from the root and called this area the rhizosphere. Plant roots exude many organic compounds that stimulate microbial growth and can have a major impact on the composition of the rhizosphere microbiome (Lemanceau et al., 1995; Grayston et al., 1998; Miethling et al., 2000). Recently, research focus has been redirected on the composition of the rhizosphere microbiome, examining the impact it can have on plant growth and health (Berg and Smalla, 2009; Mendes et al., 2011; Berendsen et al., 2012). The microbiome within plant roots can differ significantly from that within the rhizosphere, suggesting plants impact the microbial communities found inside their roots (Germida et al., 1998; Gottel et al., 2011). Microorganisms found within plant tissues, termed endophytes, are a subset of the root microbiome, which also includes the rhizosphere and rhizoplane microbiomes (Fig. 1). Extensive research has been done on the potential of root endophytes as plant inoculants for plant growth promotion (Thakore, 2006). However, our understanding of the drivers of endophyte communities is lacking and has hindered our ability to predict the success of endophytes to promote plant growth in the field. This review identifies three main mechanisms described in the literature that drive endophyte community structure: (1) soil factors that determine survival, (2) plant factors that determine colonization and compatibility, and (3) microbial factors that determine the ability of the endophyte to survive and compete within the root (Fig. 2). The study of endophyte communities is complicated in part due to their close relationship with plant roots. The advent of molecular techniques in microbial ecology has enabled more comprehensive studies of endophyte abundance, community composition, and function using genetic analysis. Application of molecular techniques will continue to enable extensive research on environmental factors that shape endophyte communities. Few studies have focused on endophyte community structure and how it relates to plant functions such as plant growth and health. In this review, we aim to summarize current knowledge regarding the drivers of the microbial diversity in the plant root and current methods of analyzing the community in the view of understanding the complex plant–endophyte relationship and promoting plant health.

BACTERIAL ENDOPHYTES

Endophytes are conventionally defined as bacteria or fungi that reside internally in plant tissues, can be isolated from the plant after surface disinfection, and cause no negative effects on plant growth (i.e., they are either beneficial or commensal) (Wilson, 1995; Hallmann et al., 1997; Reissinger et al., 2001; Coombs and Franco, 2003). Recent molecular advances require that this definition be adjusted since an abundance of unculturable endophytes have been sequenced, but not isolated (Hurek et al., 2002; Conn and Franco, 2004; Pereira et al., 2011). Furthermore, it appears that certain fungal endophytes can shift between parasitic and mutualistic life strategies, described as a balanced antagonism (Schulz et al., 2006). Therefore, a more

[1]Manuscript received 31 October 2012; revision accepted 1 April 2013.

This review was made possible by financial support to Graduate Students from the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). The authors thank Jeremy Greenwood Booth for his valuable technical assistance.

[2]Author for correspondence (e-mail: dunfield@uoguelph.ca), phone: +1-519-824-4120 ext. 58088; fax: +1-519-824-0756

doi:10.3732/ajb.1200572

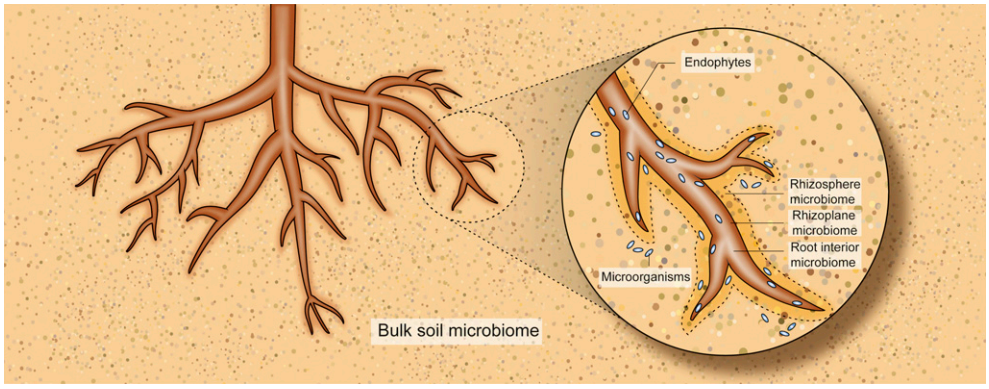

Fig. 1. Model of the root microbiome. The root microbiome consists of microorganisms in the rhizosphere microbiome, the area surrounding the plant root; the rhizoplane microbiome, the root–soil interface; and the internal root microbiome, the endophytes inside the root. The microbial community outside of the influence of plant roots is the bulk soil microbiome.

appropriate definition of endophytes is the set of microbial genomes located inside plant organs (Bulgarelli et al., 2013).

A vast number of wild and crop plant species have been shown to harbor endophytes (Mundt and Hinkle, 1976; Hallmann et al., 1997; Hallmann and Berg, 2006). Research on fungal endophytes, particularly mycorrhizal fungi, has been extensively reviewed (e.g., Carroll, 1988; Smith and Read, 2008). Similarly, the unique symbiotic relationship between nitrogen-fixing endophytes from the *Rhizobiaceae* family and their host plants has been the subject of a large amount of research and reviews (e.g., Long, 1996; Wang et al., 2012). The focus of this paper will specifically be root-associated bacterial endophytes with plant growth promoting life strategies.

***Distribution of endophytes*—**Endophytes can be classified into three main categories of plant-inhabiting life strategies

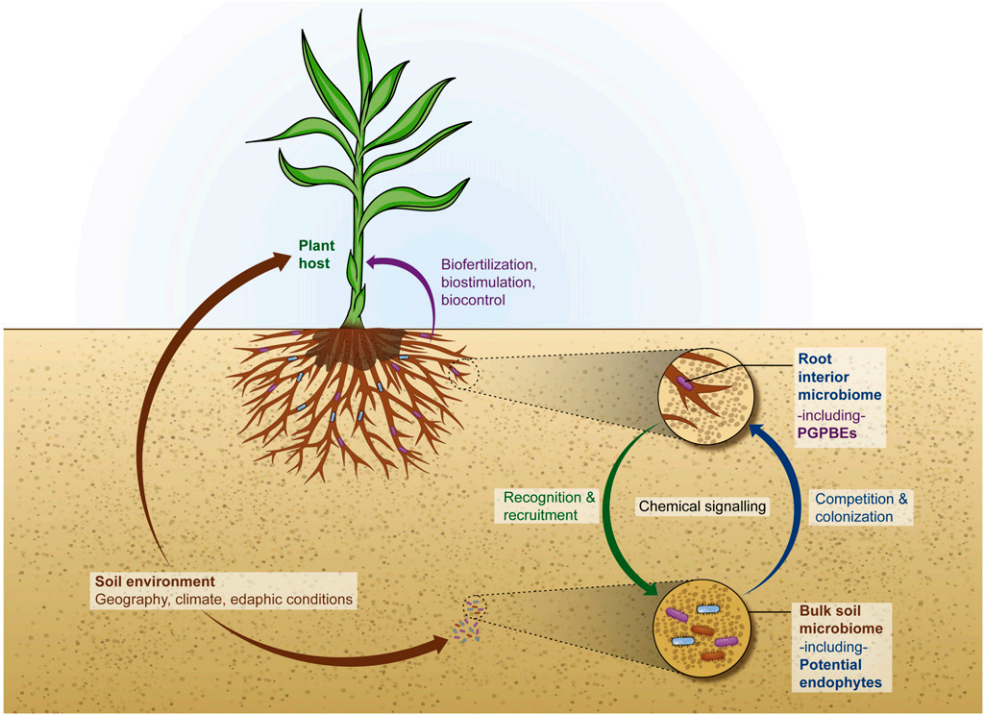

Fig. 2. Drivers of microbial community structure inside the root. Soil is able to influence the composition of the bulk soil microbiome through large-scale and local factors (brown arrows). Similarly, the soil environment also influences plant physiology (brown arrows), thus affecting biochemical interactions between plant roots and the soil microbiome. Plants select and recruit potential endophytes (green arrow) through root architecture differences and chemical signaling in root exudates. Potential endophytes cooperate and compete for invasion sites on the root (blue arrow). Once established inside the plant root, some endophytes can influence plant growth (purple arrow) through the release of phytohormones (phytostimulation), increasing the supply of nutrients (biofertilization) and providing protection from phytopathogens (biocontrol). This subset of the endophyte population is called plant-growth-promoting bacterial endophytes (PGPBEs).

(Hardoim et al., 2008). Obligate endophytes are unable to proliferate outside of plants and are likely transmitted via seed rather than originating from the rhizosphere (Hardoim et al., 2008). Facultative endophytes are free living in soil but will colonize plants when the opportunity arises, through coordinated infection (Hardoim et al., 2008). Most endophytes we will discuss relating to plant growth promotion belong to this group. The third group, the passive endophytes, do not actively seek to colonize the plant, but do so as a result of stochastic events, such as open wounds along the root hairs. This passive life strategy may cause the endophyte to be less competitive since the cellular machinery required for plant colonization is lacking (Verma et al., 2004; Rosenblueth and Martínez-Romero, 2006; Hardoim et al., 2008), and therefore may be less appropriate as plant growth promoters.

Endophyte distribution within plants depends on a combination of ability to colonize and the allocation of plant resources. Root endophytes often colonize and penetrate the epidermis at sites of lateral root emergence, below the root hair zone, and in root cracks (Dong et al., 2003b; Compant et al., 2005; Zakria et al., 2007). These colonizers are capable of establishing populations both inter- and intracellularly (Hurek et al., 1994; Zakria et al., 2007). After initial colonization, some endophytes can move to other areas of the plant by entering the vascular tissues and spreading systemically (Compant et al., 2005; Zakria et al., 2007; Johnston-Monje and Raizada, 2011). Using endophytes labeled with green-fluorescent-protein (GFP), Johnston-Monje and Raizada (2011) demonstrated the transport of the endophytes from seeds into plant roots and tissues, and endophytes injected into stems moved into the roots and rhizosphere, suggesting that there may be a continuing movement of organisms throughout the root microbiome.

The second factor influencing distribution is the allocation of resources throughout the plant. Different plant tissues can harbor compositionally distinct endophyte communities (Chi et al., 2005; Johnston-Monje and Raizada, 2011). For example, Garbeva et al. (2001) found that *Pseudomonas* spp. were more common in the stems than in the roots of potatoes (*Solanum tuberosum*) after 1 mo of growth. Surette et al. (2003) speculated that the higher endophyte concentration within carrot (*Daucus carota*) crowns compared with that in the metaxylem tissues was due to higher concentrations of photosynthate in crown regions, supplying more resources for a larger community to proliferate. While molecular studies can identify an observable distribution pattern of endophytes within plants, the mechanisms behind the establishment of the distribution patterns is not clear, and is a promising area for new research. Experiments characterizing transcriptome dynamics of endophytes and their host plants offer promising methods to discover some of the drivers of the plant–endophyte interactions.

***Plant growth promotion by endophytes*—**Plant-growth-promoting bacterial endophytes (PGPBEs) facilitate plant growth via three interrelated mechanisms: phytostimulation, biofertilization, and biocontrol (Bloemberg and Lugtenberg, 2001) (Fig. 2). These mechanisms are briefly outlined next.

Phytostimulation—Phytostimulation is the direct promotion of plant growth through the production of phytohormones (Bloemberg and Lugtenberg, 2001). The most highly studied example of phytostimulation involves lowering plant hormone ethylene levels by the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Several endophytes that release ACC deaminase have been shown to increase plant growth, including *Arthrobacter* spp. and *Bacillus* spp. in pepper plants (*Capsicum annuum*; Sziderics et al., 2007), as well as *Pseudomonas putida* and *Rhodococcus* spp. in peas (*Pisum sativum*; Belimov et al., 2001). The mechanism of plant growth promotion is unknown; however, ACC deaminase production may reduce abiotic stress by balancing plant ethylene-level production, because elevated ethylene levels inhibit cell division, DNA synthesis, and root/shoot growth (Burg, 1973). The production of other plant hormones including indole-3-acetic acid, jasmonates, and abscisic acid by bacterial strains may also stimulate plant growth (Patten and Glick, 2002; Forchetti et al., 2007).

Biofertilization—The promotion of plant growth by increasing the accessibility or supply of major nutrients is termed biofertilization (Bashan, 1998). A well-studied form of biofertilization is nitrogen fixation, which is the conversion of atmospheric nitrogen to ammonia (Bloemberg and Lugtenberg, 2001). Several PGPBEs have been studied extensively for their ability to fix nitrogen including *Azospirillum* spp. (Hill and Crossman, 1983), *Pantoea agglomerans* (Verma et al., 2001), and *Azoarcus* spp. (Hurek et al., 2002). Some PGPBEs can increase phosphorus availability to the plant through phosphorus solubilization. The release of low molecular weight acids can allow the chelation of the metal cation attached to phosphorus, making it more accessible to plants (Kpomblekou-A and Tabatabai, 2003). Forchetti et al. (2007) isolated, characterized, and quantified the phosphate solubilization abilities of endophytes in sunflower (*Helianthus annuus*), identifying *Achromobacter xiloxidans* and *Bacillus pumilus* as having the highest chelating capabilities. Yazdani and Bahmanyar (2009) showed that the use of PGPBEs in fertilizer treatments for corn (*Zea mays*) reduced the need for phosphorus application by 50% without significant loss in grain yield.

Biocontrol—The promotion of plant growth through protection from phytopathogens is known as biocontrol. Several mechanisms may be involved, including the production of siderophores or antibiotics. Siderophores, such as pyochelin and salicylic acid, chelate iron and can indirectly contribute to disease control by competing with phytopathogens for trace metals (Duffy and Défago, 1999). Antimicrobial metabolites produced by PGPBEs, such as 2,4-diacetylphloroglucinol (DAPG), can enhance disease suppression in plants. For example, eggplant wilt caused by *Ralstonia solanacearum* was reduced by 70% after seeds were inoculated with DAPG-producing endophytic isolates (Ramesh et al., 2008).

***Importance of endophytes*—**The potential of PGPBEs to improve plant health has led to a great number of studies examining their applied use as inoculants, primarily in agricultural crops (Kloepper and Schroth, 1978; Hallmann et al., 1997; Kuklinsky-Sobral et al., 2004). The potential for microbial inoculants to reduce the need for chemicals such as pesticides and fertilizers (Horrigan et al., 2002) makes them important in the development of sustainable agricultural practices. In the following sections, we will review drivers that determine endophyte community structure and factors that will need to be considered for applied use of PGPBEs in a field setting.

DRIVERS OF ENDOPHYTE MICROBIOME COMMUNITY STRUCTURE

Soil—Both large- and local-scale factors can determine endophyte community structure because of differences in species' optimal environmental conditions. These factors need to be considered when trying to successfully inoculate plants to improve plant health. Large-scale geographic and climatic factors provide external controls on endophyte communities by predetermining the microorganisms that can inhabit the bulk soil (Fig. 2). These factors can also influence plant physiology, thus altering plant–endophyte interactions (Fuentes-Ramirez et al., 1999). Latitude, elevation, temperature, and precipitation can interact and influence the endophyte composition in plants. For example, in sweet root (*Osmorhiza depauperata*), endophytes *Agrobacterium tumefaciens* and *Sinorhizobium meliloti* were more abundant at sites with higher precipitation and annual temperature, while *Paenibacillus* strains were more common at sites with higher latitudes and lower precipitation (Li et al., 2012). Rhizobial and non-rhizobial endophytes in red bladdervetch (*Sphaerophysa salsula*) were able to be clearly grouped by sample area, with distinct areas differing in precipitation, soil texture, and soil nutrient content (Deng et al., 2011).

Local edaphic conditions can be equally important in influencing endophyte community structure. The diversity of *Frankia* spp. communities was found to be highest in plants grown in intermediate soil moisture compared to those growing in arid and saturated environments (Benson and Dawson, 2007). In addition, endophytic nitrogen-fixing *Azoarcus* spp. are more abundant in rice (*Oryza sativa* spp.) and related grass species in flooded soils compared to dry soils, even though these bacteria are aerobic (Engelhard et al., 2000). Dry environments may select for drought-tolerant endophyte assemblages (Grönemeyer et al., 2011; Yandigeri et al., 2012); in a region of Namibia with a long dry season, several desiccation-resistant endophyte strains were found in pearl millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*), and maize (Grönemeyer et al., 2011). Similarly, cold environments select psychrophilic endophytes (Nissinen et al., 2012). Soil pH is a major determinant of bacterial species composition in bulk soil (Fierer and Jackson, 2006; Baker et al., 2009) and therefore influences the pool of potential endophytes available for plant recruitment. In legumes, increased soil acidity has resulted in lower endophyte richness and diversity and a greater abundance of acid-tolerant species of rhizobia (Lafay and Burdon, 1998; Bala et al., 2003). Soil type is also a major driver; organic soils have higher moisture retaining capacity, as well as more organic carbon and nutrients than their mineral counterparts, and support different communities. Root endophyte communities were more diverse in tobacco (*Nicotiana attenuata*) from organic soils compared to those grown in mineral soils (Long et al., 2010), and survival of *Pseudomonas* and *Pantoea* strains after they were applied to Italian ryegrass (*Lolium multiflorum*) and birdsfoot trefoil (*Lotus* spp.) was higher in loamy soil than sandy soil (Afzal et al., 2011). A study examining one variety of genetically modified canola (*Brassica napus*) grown at five field sites indicated differences in the endophyte community depending on the field site (Dunfield and Germida, 2001). Authors suggest that although plants play an important role in the composition of the root-associated microbial community, the soil has an overriding influence on this community; the plant can only associate with organisms that are already present in the bulk soil. Furthermore, soil type can interact with plant species. The endophyte microbial community is highly dependent on the field where the plant was grown (Dunfield and Germida, 2004). This finding was also observed in a more recent study examining culturable endophytes associated with the genetically modified *Bacillus thuringiensis* (Bt)-maize lines where the soil type, but not the genetic modification, significantly affected the endophyte community composition (Prischl et al., 2012).

Anthropogenic management, such as fertilizer and pesticide application and soil tillage, can also influence the composition of an endophyte community. For example, maize amended with organic fertilizer showed a higher species richness of methanotrophic endophytes and a different overall endophyte composition compared to that found under mineral fertilizer application (Seghers et al., 2004). Hallmann et al. (1999) examined the use of chitin as a pesticide on cotton (*Gossypium hirsutum*) and found increased overall endophyte abundance and different dominant bacterial species compared to untreated soils. In contrast, chemical herbicides did not significantly alter endophyte species composition in maize (Seghers et al., 2004). In addition, the viability of endophyte communities in the soil can be affected by the soil disturbance associated with conventional tillage practices. Conservation tillage involves leaving plant litter from the crop harvest on the surface of a field, which can increase soil organic content, alter other soil properties, and inadvertently provide a habitat for both pathogenic and beneficial microorganisms (Peters et al., 2003). Carrots grown under conservation tillage had a higher abundance of endophytes than carrots under conventional tillage where plant litter was removed (Surette et al., 2003). Conservation tillage may also promote endophyte communities resistant to plant pathogens. For example, endophytes from potato under a 3-yr, low-tillage rotation had superior antifungal properties compared to those isolated from conventional tillage fields or shorter crop rotations (Peters et al., 2003).

Plants—The endophyte life cycle is inextricably linked with that of the host plant and is influenced by the plant species, plant life stage, and plant health. Some aspects of endophyte colonization may be stochastic, for example, if they take advantage of plant wounds, but plants also influence which endophytes are favored before and after root penetration (Rosenblueth and Martínez-Romero, 2006; Hardoim et al., 2008). This results in plants of different species grown on the same soil harboring distinct groups of endophytes (Olivares et al., 1996; Weber et al., 1999). In a study comparing five plant species, Dong et al. (2003b) found that monocots had a 100-fold higher number of *Klebsiella pneumonia* cells compared to dicots. The mechanisms behind such differential influences are unknown, but differences in root architecture, specific host/bacteria selection/recruitment, or the types of root exudates attracting specific endophytes may be factors. Root exudates influence microbial communities in the rhizosphere, determining the abundance and diversity of bacterial species that may colonize the host plant (Berendsen et al., 2012). As will be discussed more later, chemicals in root exudates of plants can act as signals for beneficial bacteria and allow bacteria to identify appropriate hosts (Mathesius and Mulders, 2003; Rosenblueth and Martínez-Romero, 2006; Rudrappa et al., 2008). In fact, within a single plant species, genotypic differences can foster different endophyte communities (Surette et al., 2003; Manter et al., 2010; Hardoim et al., 2011). Anthropogenic-directed changes in plant genotypes through selective breeding have been shown to inadvertently result in distinctive endophyte communities in traditional

and domesticated breeds of maize (Johnston-Monje and Raizada, 2011) and rice (Hardoim et al., 2011). Engelhard et al. (2000) found that modern strains of rice had a higher diversity of endophytic nitrogen-fixing bacteria compared with wild strains. These results indicate that the plant–endophyte relationship may be co-evolutionary, at least to some degree (Normand et al., 2007).

Spatiotemporal shifts inside the root microbiome—Spatiotemporal patterns in endophyte communities have been documented in a range of plant species. Endophytes can successfully colonize roots within a week of soil inoculation (Dong et al., 2003b; Compant et al., 2008) and increase in abundance in roots over time (Gagné et al., 1987; Chi et al., 2005; van Overbeek and van Elsas, 2008). The species composition of root endophytes changes as plants age (Roesti et al., 2006; Monteiro et al., 2011). van Overbeek and van Elsas (2008) showed that *Pseudomonas* and actinobacteria communities declined in abundance in potato roots over time. In vetiver (*Chrysopogon zizanioides*), changes in endophyte species composition were more noticeable in early growth stages (Monteiro et al., 2011). Changes in endophyte communities could reflect changes in plant physiology and hormonal changes with age (Taiz and Zeiger, 1998).

Little is currently known about how endophyte communities change over multiple years, such as during plant community succession. One study showed the persistence of identical *Nostoc* species over 4 yr in bryophytes at a site in Finland (Costa et al., 2001). Although bryophytes have rhizoids, which are not true roots, this type of work can be used to indicate trends that may also be seen in vascular plants. Many studies have investigated temporal changes in bacterial communities in the rhizosphere (e.g., Goddard et al., 2001; Buchan et al., 2010), but how this relates to changes within the plant is not clear. Endophytes can accumulate in the soil over time, which could be significant in agricultural systems due to the transfer of endophytes from one crop to the next in a crop rotation (Sturz and Christie, 1995; Sturz et al., 1998). For this reason, crop rotation has long been used in part to prevent repeat infections of crop-specific pathogens, although the same practice may discourage the colonization of beneficial endophytes (Sturz and Christie, 1995; Peters et al., 2003). Increasing our understanding of the drivers of endophyte community structure may allow researchers to develop methodologies that minimize the pathogen population while enhancing the beneficial population.

Microbes—Root colonization requires potential endophytes to reach the root surface through chemotactic response, outcompete other microbial species for insertion into the root surface, express genes in a coordinated effort for invasion of plant tissue with neighboring individuals, resist host plant immune responses, and secure a niche within the plant tissue (Bais et al., 2006; Rosenblueth and Martínez-Romero, 2006; Compant et al., 2010) (Fig. 2). Microbe–microbe signaling (Elasri et al., 2001; Faure et al., 2009) and microbe–plant signaling (Mathesius and Mulders, 2003; Rocha et al., 2007) are intricately involved at each step of the root colonization process. Root endophyte communities can differ significantly from those in the rhizosphere, suggesting recognition and selection of beneficial bacteria by root tissues (Compant et al., 2005). Plant–microbe cross talk begins with signaling through root exudates. Chemical signals and nutrients sent in root exudates allow plants to influence the microbial communities in the rhizosphere, determining the abundance and diversity of endophyte species that may colonize the host plant, and allow bacteria to identify appropriate hosts (Mathesius and Mulders, 2003; Rudrappa et al., 2008). In a study by Lemanceau et al. (1995), flax (*Linum usitatissinum*) and tomato (*Lycoperiscon esculentum*), attracted specific strains of *Pseudomonas* that were in low abundance in the bulk soil. *Arabidopsis thaliana* can also selectively recruit *Bacillus* spp. to enhance immunity and prevent pathogenic attack (Kloepper et al., 2004). In addition, certain plants have been shown to change their chemical responses when interacting with PGPBEs compared with nonbeneficial bacteria (Miché et al., 2006; Rocha et al., 2007).

Coordinated invasion by microbes on the root surface involves multiple signaling pathways and reciprocal signaling between plants and endophytes and between endophytes (as reviewed by Morris and Monier, 2003; Rosenblueth and Martínez-Romero, 2006; and shown by Rudrappa et al., 2008). A well-studied microbe–microbe signaling mechanism is quorum sensing (QS), a cell density-dependent regulator of microbial behavior (Teplitski, 2000). The QS system involves the production and perception of low molecular weight molecules, called autoinducers, which diffuse in and out of individual bacteria cells (Chernin, 2011). The QS system allows individual microbial cells to act in concert to increase fitness and survival of the microbial community because all individuals express genes together to increase colonization potential (Elasri et al., 2001). The most common QS signals in gram-negative bacteria are *N*-acyl homoserine lactones (AHLs) (Elasri et al., 2001). Bacterial populations can use distinct compounds to communicate within and between species (Steidle et al., 2001; Chen et al., 2002). This is most relevant when species share the same niche, or where bacteria are participating in interspecies cooperation, for example, to colonize root tissue (Faure et al., 2009). Elasri et al. (2001) illustrated that AHL production is more common among endophytic *Pseudomonas* spp. than soil-borne *Pseudomonas* spp. and that AHL-producing *Pseudomonas* strains are more numerous within plant tissues than in the rhizosphere. There is also evidence that signal compounds secreted by one species can induce density-dependent responses in other species (Pierson and Wood, 1998; Hosni et al., 2011; Marques et al., 2011).

In addition to communication between bacteria via extracellular molecules, many plants release host-specific compounds such as flavonoids that influence endophyte colonization (Balachandar et al., 2006). Multiple studies have shown plants secrete compounds that specifically stimulate or inhibit AHL-dependent QS responses through interactions with microbial AHL receptors (Teplitski, 2000; Gao and Teplitski, 2003). These QS mimics target different steps of QS regulation, including signal synthesis, signal stability and signal sensing (Teplitski, 2000; Gao and Teplitski, 2003; Rasmussen and Givskov, 2006). Gao and Teplitski (2003) identified 15–20 separable substances produced by barrel medic (*Medicago truncatula*) that affect QS regulation in endophytes. The release of specific flavonoids enables certain bacteria to preferentially colonize a host plant through the activation of gene expression required for colonization (Bais et al., 2004). The presence of flavonoids and certain growth hormones were also found to significantly improve the endophytic colonization ability of *Serratia* spp. in rice seedlings (Balachandar et al., 2006).

Endophytes have evolved ways to use plant hormone signaling pathways to their advantage. Preferential selection of PGPBEs with high ACC-deaminase activity by plants could benefit the plant and give ACC-deaminase containing endophytes a

competitive advantage in colonization (Hardoim et al., 2008). Endophytes use ACC-deaminase and synthesis of indole acetic acid (IAA) to direct their plant hosts through signaling pathways (Spaepen et al., 2007). For example, *Pseudomonas syringae* can induce IAA and abscisic acid biosynthesis in *Arabidopsis thaliana* (Schmelz and Engelberth, 2003; de Torres-zabala et al., 2007). These results illustrate that endophytes can effectively reprogram some plant signaling pathways and therefore influence endophyte community structure (Bianco et al., 2006; Spaepen et al., 2007).

METHODS TO STUDY THE MICROBIOME INSIDE THE ROOT

Surface sterilization of the root followed by tissue maceration is common to ensure isolation of endophytes from other rhizosphere and rhizoplane bacteria (Reissinger et al., 2001; Coombs and Franco, 2003; Hallmann et al., 2006). Culture-dependent methods of endophyte identification involve the isolation and growth of the bacteria from surface-sterilized root sections (Coombs and Franco, 2003; Hallmann et al., 2006; Qin et al., 2011), followed by characterization by a number of techniques, such as fatty acid or lipid (e.g., fatty acid methyl ester [FAME]) analysis, morphological, or enzymatic tests (Garbeva et al., 2001; Berg et al., 2005; Aravind et al., 2009). Despite the long history of culturing microorganisms, it is clear that most are, or will remain, unculturable in the laboratory setting (Schloss and Handelsman, 2005). Obligate endophytes by definition require the plant for survival and thus are often viable but nonculturable, which has widespread implications in identifying and measuring endophyte diversity and community structure. Culture-independent techniques based on modern molecular tools are now commonplace. These methods are often based on polymerase chain reaction (PCR), which amplifies a variable region of DNA, often from the 16S rRNA gene, and is followed by downstream methods to analyze the endophyte community, which may include cloning or other community fingerprinting techniques (recently reviewed by Gao and Tao, 2012). The current consensus has been to consider the complementarity of both culture-dependent and -independent methodologies, because there are biases inherent to each (Hardoim et al., 2008; Pereira et al., 2011; Reinhold-Hurek and Hurek, 2011). Complementary culture-dependent and -independent methods have been used to discover a vast number of as-yet uncultured or unculturable endophytes in many plant species (Garbeva et al., 2001; Conn and Franco, 2004; Pereira et al., 2011). These species could play a key functional role in the plant (Azevedo et al., 2000; Hurek et al., 2002; Sessitsch et al., 2012). New methodologies may enable researchers to further study these organisms despite their unculturable state (Thomas et al., 2008; Stewart, 2012).

Advances in tools for studying endophytes—Recently, endophyte research has been introduced to the expanding array of next-generation technologies in an effort to probe the vast amount of information in endophyte genomes, proteomes, and transcriptomes. With the availability of broader meta-approaches, discovering and characterizing the endophyte community structure and dynamics in its entirety is increasingly achievable. Microarray multiplex technology can target a wide variety of organisms to assess microbial diversity or analyze specific gene copy numbers or expression patterns, characterizing a multitude of species simultaneously from complex microbial environments (Gao and Tao, 2012). Microarrays have been used without *a priori* genome sequence information for genome comparisons between known endophytes and non-endophytic bacteria (Dong et al., 2001). However, this method might preclude many as-yet unknown genes. More recently, microarrays were used to characterize transcriptomes of endophytes to identify genes that are upregulated in the presence of host plants (Matilla et al., 2007; Shidore et al., 2012). These types of studies are helping us understand the genes involved in the intricately complex plant–microbe interactions.

High throughput sequencing (e.g., 454-pyrosequencing) is incredibly useful in investigating endophyte communities (Manter et al., 2010; Bulgarelli et al., 2012; Lundberg et al., 2012). A study of potato root extracts showed that five of the 10 most common genera had not been previously reported as potato endophytes (Manter et al., 2010). Similarly, a metagenomic analysis was used to describe metabolic processes, adaptations, and plant-growth-promoting characteristics in rice (Sessitsch et al., 2012). From this work, a wider range of novel endophyte phylogenetic lineages was discovered (Sun et al., 2008), underscoring a greater gap in understanding the total endophyte community in plants than previously realized. It is likely that without a complete understanding of the community composition, it may prove difficult to predict the success of selected endophytes to promote plant growth. Through the use of new molecular methodologies, the relative abundance of endophyte species can be determined more accurately, and factors influencing community structure can be distinguished and identified.

In addition to phylogenetic characterization, metagenomic sequencing is useful in the characterization of putative protein-coding sequences associated with colonization competence and plant growth promotion (Korf, 2004; Barret et al., 2011; Sessitsch et al., 2012). Genes involved in the detoxification of reactive oxygen species, protein secretion systems, and motility (flagella) have been reported as important determinants for successful plant colonization and microbial competition (Hérouart et al., 2002; Hardoim et al., 2008; Cheng et al., 2010). Genes involved in the production of siderophores, abscisic acid, indole acetic acid, and QS autoinducer genes have also been associated with biocontrol, phytostimulation, and colonization (Forchetti et al., 2007; Ramesh et al., 2008; Faure et al., 2009).

Proteomic studies can determine endophyte-induced changes in plant protein expression and/or plant-induced changes in endophyte protein expression (Pradet-Balade et al., 2001; Cheng et al., 2010). Understanding plant protein expression, such as hormone production and defense response, can improve our understanding of how plants modulate the phenotype of their endophytic partners before, during, and after colonization, and determine whether these changes are "push–pull" or single-partner controlled. Two-dimensional gel electrophoresis coupled with mass spectrometry (2-DE-MS) is a powerful technique that has been used for proteomic studies of endophytes. In rice inoculated with the PGPBE *Sinorhizobium meliloti*, 2-DE-MS was used to identify a number of plant defense-related proteins that were upregulated in roots and photosynthesis-related proteins that were upregulated in aerial tissues (Chi et al., 2010). Similarly, following inoculation of two sugarcane cultivars with the PGPBE *Gluconacetobacter diazotrophicus,* Lery et al. (2011) observed differences in defense response- and signaling-related protein expression, leading to differential colonization efficiency between cultivars. In addition, the plant host was inferred

to have influenced the expression of specific *G. diazotrophicus* genes that are related to colonization competency, thus highlighting the two-sided role of regulation in this plant–endophyte relationship. In similar work involving the kallar grass (*Leptochloa fusca*) endophyte *Azoarcus* sp., the regulation of certain genes was correlated to the presence of plant exudates when compared to a control endophyte not exposed to exudates (Shidore et al., 2012). The genes were identified and found to play roles in colonization success and rhizosphere competence of *Azoarcus* sp., thus confirming the potential role the host plant can play in effective colonization by bacterial endophytes. Using *Azoarcus* sp. BH72 as a model endophyte, Hauberg-Lotte et al. (2012) examined transcriptomic and proteomic data to identify expression patterns that are required for efficient colonization of plant surfaces and within roots. Certain genes, such as *pilAB* (type IV pilus, used in colonizing plant roots) were discovered to be regulated in a population density-dependent manner by an uncharacterized, secreted molecule. The secreted molecule is likely part of a novel and widespread microbial species-species communication system (Hauberg-Lotte et al., 2012). The levels of complexity of the genetic regulation associated with plant–endophyte interactions are still being discovered. Only by examining a wide range of host plants and bacterial endophytes will we determine whether there are useful trends that can be considered to identify endophytes for plant growth promotion. Proteomic and transcriptomic studies examining the metabolic response of endophytes within a native microbial community will also need to be explored, particularly for co-inoculation and competition studies. This area of research will advance our understanding of not only microbial interspecies communication, but also the factors influencing colonization success of potential endophyte inoculants.

Understanding the functional roles within the endophyte community is an important addition to sequencing projects. A technique known as nucleic acid-based stable isotope probing (SIP; Radajewski et al., 2000) has shown potential in advancing our knowledge and capabilities in this area. SIP-DNA and SIP-rRNA methods use an isotope-labeled plant assimilate, such as ^{13}C-CO_2. Microorganisms that use the photosynthate will have labeled genetic material that can be used for community profiling (Manefield et al., 2002; Schmid et al., 2006; Vandenkoornhuyse et al., 2007). Thus, SIP techniques enable researchers to explore plant specific effects on endophyte communities and identify active and dominant microbial populations within these communities, such as those known to promote plant growth (Hurek et al., 1994; Rasche et al., 2009; Spaepen et al., 2009). Stable isotopes have also been used in conjunction with endophyte inoculation to indicate and measure nitrogen fixation by diazotrophs such as *Klebsiella pneumoniae* in wheat plants (Iniguez et al., 2004). Using SIP-DNA, researchers have explored both rhizosphere (Lu et al., 2005) and stem endophytes (Rasche et al., 2009). In roots, SIP-rRNA has been used to explore the diversity of the primary bacterial consumers of plant-derived carbon and has identified new endophyte phylotypes (Vandenkoornhuyse et al., 2007). Future use of SIP technologies, such as SIP-mRNA, will undoubtedly continue to expand our functional knowledge of endophytes by using gene expression to identify organisms that assimilate plant substrate (Dumont et al., 2011). However, limitations have been identified due to biased abundances of certain gram-positive bacteria due to a high GC% content (Rasche et al., 2009) and difficulties in separating active from non-active cells based on an efficient labeling and accumulation activity (Schmid et al., 2006).

Tracking endophytes—One of the most widespread techniques used in the localization of indigenous bacterial endophytes, in endophyte competition and colonization studies, and in identifying active bacteria, is green fluorescent protein (GFP) labeling (see reviews by Schmid et al., 2006; Ramos et al., 2011). Insertion of GFP requires conjugation of the target organism with a carrier organism (e.g., *E. coli* containing a plasmid with GFP and the Tn5 transposon) (Bhatia et al., 2002). The transconjugant can then be visualized by microscopy *in planta*. GFP-labeling has been successful in the study of colonization patterns of bacterial endophytes in poplar trees (Germaine et al., 2004; Taghavi et al., 2009), plant-growth-promoting *Burkholderia* sp. in *Vitis vinifera* (Compant et al., 2005), and the diazotroph *Klebsiella pneumoniae* strains in maize (Chelius and Triplett, 2000). The colonization and persistence of human pathogenic bacterial endophytes has implications to food security in agriculture. In this respect, work has been done with *E. coli* and/or *Salmonella* strains in alfalfa (*Medicago sativa*) (Dong et al., 2003a), *A. thaliana* (Cooley et al., 2003), lettuce (*Lactuca sativa*) (Franz et al., 2007), and tomato (*Solanum lycopersicum*) (Guo et al., 2002). Antibody labeling of enzyme proteins has also been used in functional diversity and colonization studies (see reviews by Reinhold-Hurek and Hurek, 1998; Schmid et al., 2006). Using GFP and immunogold labeling, Egener et al. (1999) discovered that the rice root endophyte *Azoarcus* spp. expressed high levels of nitrogenase in the aerenchyma, helping to establish our understanding of localization and nitrogen fixation within rice roots. GFP-based techniques may form the foundation for measuring and tracking inoculant success. Subsequently, PCR-based (e.g., quantitative and reverse-transcription PCR) methods should be developed to track and quantify endophytes based on specific genetic markers. And finally, as adoption of high-throughput sequencing methods becomes widespread, a detailed global approach can be made to study the abundance of the inoculant within the greater endophytic community.

Implications of new methodologies—An important question regarding plant growth promotion by endophytes is whether the success of the plant–endophyte interaction can be predicted by genetic analyses. For example, genome sequencing has identified genetic systems common to pathogenic, symbiotic, and commensal bacteria relating to plant colonization (Pühler et al., 2004). These genes could be used to determine the genetic or phenotypic differences that distinguish phytopathogens from symbiotic or commensal endophytes (Pühler et al., 2004; Monteiro et al., 2012). It is unclear whether certain bacterial species may live interchangeably as phytopathogens or symbiotic endophytes, but this has been recently discovered in the fungal antagonist *Fusarium oxysporum* in lettuce (Moretti et al., 2012). Future studies distinguishing potential environmental factors or cues may eventually elucidate these overlapping life strategies. In recent work by Monteiro et al. (2012), the endophyte *Herbaspirillum seropedicae* and the phytopathogen *H. rubrisubalbicans* were compared using suppressive subtractive hybridization (SSH) and partial genome sequencing. *Herbaspirillum rubrisubalbicans* causes mottled stripe disease in sugarcane; however, *H. seropedicae* does not (Olivares et al., 1997). SSH library comparisons showed there were major differences in genes relating to cellulose biosynthesis, with gene clusters coding for lipopolysaccharide and adhesins more highly expressed in *H. rubrisubalbicans* and hypothesized to have a role in outcompeting *H. seropedicae* for root colonization and binding sites (Monteiro

et al., 2012). Additionally, Haapalainen et al. (2012) have identified proteins produced by phytopathogens in the rhizosphere that are not induced *in planta* that are involved in outcompeting organisms such as yeast and bacteria.

Examining contextual factors, including environmental cues or stressors on the plant, should reveal the integrity of the plant–endophyte relationship. These types of studies will not only further our understanding of endophyte (and PGPBE) survival and colonization traits, but also lay the technical foundation to study transient expression differences before, during, and after colonization. Identifying key phenotypic changes in the plant and proteomic or gene expression differences in either the plant or PGPBE may indicate a switch toward a pathogenic state or diminished plant growth promotion. These tests, once developed, should play a significant role in monitoring the efficacy of PGPBEs.

IMPORTANCE OF CONSIDERING ENDOPHYTE COMMUNITY ECOLOGY FOR PLANT GROWTH PROMOTION

In the field, the internal root microbiome is made up of an entire community of endophytes that interact with each other and with the plant. It is important to understand the ecological impacts of adding novel strains of endophytes to pre-existing communities that have already been shaped by ecological and evolutionary factors. The success of an endophyte strain depends on its ability to survive and compete within the endophyte community colonizing the plant (Bent and Chanway, 1998; Rojas et al., 2001; Conn and Franco, 2004). The strain of bacteria needs to be considered (Berg et al., 2005; Forchetti et al., 2007; Trivedi et al., 2010). A mixture of naturally cohabitating endophytes may be a better alternative than applying an individual endophyte species, because different species may fulfill different ecological niches. Niche complementarity has been demonstrated in the field. Black (*Avicennia germinans*), white (*Laguncurlaria racemosa*), and red (*Rhizophora mangle*) mangroves that were simultaneously inoculated with phosphate-solubilizing *Bacillus licheniformis* and nitrogen-fixing *Phyllobacterium* sp. showed higher nitrogen fixation and phosphorus solubilization rates than after inoculation with only one of the species (Rojas et al., 2001). In addition, Roesti et al. (2006) showed that co-inoculation of wheat with *Pseudomonas* sp. and arbuscular mycorrhizal fungi native to that plant variety resulted in increased yield and grain quality compared with uninoculated plants and plants inoculated solely with either organism.

Endophyte community interactions—Given that endophyte populations interact closely with one another, the predicted influence of an endophyte on plant growth needs to be considered within the context of the resident endophyte community. A few studies have considered the impact of adding bacterial strains on the resident endophytes. Conn and Franco (2004) demonstrated that inoculation of wheat (*Triticum aestivum*) with a mixture of endophytic actinobacteria that had been previously identified as biocontrol agents resulted in a 50% decrease in the diversity of the native endophytic actinobacteria community. They suggested that this result is likely due to the inoculated strains being competitive. Several studies by Andreote et al. (2004, 2006, 2009, 2010) have examined the impact of endophyte inoculation on indigenous endophyte community structure. The application of a genetically modified *Enterobacter cloacae* strain and *Methylobacterium mesophilicum* strain SR1.6/6 both induced shifts in the bacterial endophyte community of citrus seedlings in two separate studies (Andreote et al., 2004, 2006). Similarly, Andreote et al. (2009) observed differences in the diversity of the root-associated bacterial communities in plants colonized by a bacterial endophyte, *Pseudomonas putida* strain P9, compared with plants that were not inoculated. In particular, the abundance of *Pseudomonas* species that were already present on the surface and internally in the roots were most reduced (Andreote et al., 2009). In a further study (Andreote et al., 2010), multivariate analysis indicated that alphaproteobacteria and *Paenibacillus* communities on the surface of and within potato roots were significantly affected by inoculation with two bacterial endophytes, *Paenibacillus* sp. strain E119 and *M. mesophilicum* strain SR1.6/6 (Andreote et al., 2010). Authors point out the importance of understanding the ecological impacts of inoculant strains to obtain insight into their performance in the field.

While it seems clear from these studies that endophyte inoculation can shift endophyte community structure, only a few studies have examined the subsequent effect that the changes in endophyte community structure can have on plant growth or health (Bent and Chanway, 1998; Bent et al., 2001; Ardanov et al., 2012). Changes in endophyte community structure after inoculation with a beneficial microbe was related to increased pathogen resistance in potato, pine (*Pinus sylvestris*), and tomato varieties (Ardanov et al., 2012). Bent and Chanway (1998) observed that the ability of a PGPBE *Bacillus polymyxa* strain to increase plant root growth was inhibited when a non-plant-growth-promoting endophyte, *Curtobacterium flaccumfaciens,* was applied as a co-inoculant. In follow-up work, they showed that the plant-growth-promoting ability of some rhizobacteria can be significantly reduced in the presence of another rhizobacterium, even when individually both strains can benefit plant growth (Bent et al., 2001). Collectively, these results suggest that the composition of the native endophyte community could influence the effectiveness of a PGPBE. As yet, it is difficult to predict how the community-level interactions could benefit or harm plant growth. We believe that more studies need to consider changes in bacterial endophyte community structure in association with inoculation and the subsequent impact that these changes have on plant growth and health.

Conclusions—The ubiquity of beneficial and nonbeneficial, naturally occurring bacterial endophytes in plant roots is undisputed. It is less clear what determines absolutely whether the endophytes will be beneficial for the host plant or not, the external factors or cues involved, and what shapes the dynamics of the plant–endophyte relationship. Endophytes tested in isolation may indicate different life strategies and plant-growth-promoting characteristics compared to similar species or co-inoculating multiple strains. In a similar vein, external factors may contribute to altered life strategies of endophytes, such as the conditions imposed upon them via the host plant including soil and geographic factors, and anthropogenic management of the crops. These factors drive the overall structure and function in the root interior microbiome. As with all species assemblages, the internal root microbiome is dynamic and determined by many interacting abiotic and biotic factors that occur at various spatial and temporal scales. Endophyte community dynamics remain an important area of future research. Researchers now have the tools to more fully explore the interactions of abiotic and biotic factors that influence these communities and the subsequent impact of these changes on plant health. A particularly relevant question

is how these populations will be affected in an environment that is under the influence of climate change. Novel molecular methods have highlighted the limitations of the past, increased our overall understanding of the nature of plant–microbe and microbe–microbe interactions, and have helped outline new questions for the future. Future application of this research in combination may lead to the development of an optimal PGPBE inoculant strain that is robust, such that slight variations in external environmental factors and in the plant will not affect the efficacy of plant growth promotion.

LITERATURE CITED

AFZAL, M., S. YOUSAF, T. G. REICHENAUER, M. KUFFNER, AND A. SESSITSCH. 2011. Soil type affects plant colonization, activity and catabolic gene expression of inoculated bacterial strains during phytoremediation of diesel. *Journal of Hazardous Materials* 186: 1568–1575.

ANDREOTE, F. D., U. N. DA ROCHA, W. L. ARAÚJO, J. L. AZEVEDO, AND L. S. VAN OVERBEEK. 2010. Effect of bacterial inoculation, plant genotype and developmental stage on root-associated and endophytic bacterial communities in potato (*Solanum tuberosum*). *Antonie van Leeuwenhoek* 97: 389–399.

ANDREOTE, F. D., W. L. DE ARAÚJO, J. L. DE AZEVEDO, J. D. VAN ELSAS, U. N. DA ROCHA, AND L. S. VAN OVERBEEK. 2009. Endophytic colonization of potato (*Solanum tuberosum* L.) by a novel competent bacterial endophyte, *Pseudomonas putida* strain P9, and its effect on associated bacterial communities. *Applied and Environmental Microbiology* 75: 3396–3406.

ANDREOTE, F. D., M. J. M. GULLO, A. O. DE SOUZA LIMA, W. M. JÚNIOR, J. L. AZEVEDO, AND W. L. ARAÚJO. 2004. Impact of genetically modified *Enterobacter cloacae* on indigenous endophytic community of *Citrus sinensis* seedlings. *Journal of Microbiology (Seoul, Korea)* 42: 169–173.

ANDREOTE, F. D., P. T. LACAVA, C. S. GAI, W. L. ARAÚJO, W. MACCHERONI JR., L. S. VAN OVERBEEK, J. D. VAN ELSAS, AND J. L. AZEVEDO. 2006. Model plants for studying the interaction between *Methylobacterium mesophilicum* and *Xylella fastidiosa. Canadian Journal of Microbiology* 52: 419–426.

ARAVIND, R., A. KUMAR, S. J. EAPEN, AND K. RAMANA. 2009. Endophytic bacterial flora in root and stem tissues of black pepper (*Piper nigrum* L.) genotype: Isolation, identification and evaluation against *Phytophthora capsici. Letters in Applied Microbiology* 48: 58–64.

ARDANOV, P., A. SESSITSCH, H. HÄGGMAN, N. KOZYROVSKA, AND A. M. PIRTTILÄ. 2012. *Methylobacterium*-induced endophyte community changes correspond with protection of plants against pathogen attack. *PLoS ONE* 7: e46802.

AZEVEDO, J. L., W. MACCHERONI JR., J. O. PEREIRA, AND W. L. DE ARAÚJO. 2000. Endophytic microorganisms: A review on insect control and recent advances on tropical plants. *Electronic Journal of Biotechnology* 3: 40–65.

BAIS, H. P., S.-W. PARK, T. L. WEIR, R. M. CALLAWAY, AND J. M. VIVANCO. 2004. How plants communicate using the underground information superhighway. *Trends in Plant Science* 9: 26–32.

BAIS, H. P., T. L. WEIR, L. G. PERRY, S. GILROY, AND J. M. VIVANCO. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* 57: 233–266.

BAKER, K. L., S. LANGENHEDED, G. W. NICOL, D. RICKETTS, K. KILLHAM, C. D. CAMPBELL, AND J. I. PROSSER. 2009. Environmental and spatial characterisation of bacterial community composition in soil to inform sampling strategies. *Soil Biology & Biochemistry* 41: 2292–2298.

BALA, A., P. MURPHY, AND K. E. GILLER. 2003. Distribution and diversity of rhizobia nodulating agroforestry legumes in soils from three continents in the tropics. *Molecular Ecology* 12: 917–929.

BALACHANDAR, D., G. S. SANDHIYA, T. C. K. SUGITHA, AND K. KUMAR. 2006. Flavonoids and growth hormones influence endophytic colonization and in planta nitrogen fixation by a diazotrophic *Serratia* sp. in rice. *World Journal of Microbiology & Biotechnology* 22: 707–712.

BARRET, M., J. P. MORRISSEY, AND F. O'GARA. 2011. Functional genomics analysis of plant growth-promoting rhizobacterial traits involved in rhizosphere competence. *Biology and Fertility of Soils* 47: 729–743.

BASHAN, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. *Science* 16: 729–770.

BELIMOV, A. A., ET AL. 2001. Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. *Canadian Journal of Microbiology* 47: 642–652.

BENSON, D. R., AND J. O. DAWSON. 2007. Recent advances in the biogeography and genecology of symbiotic *Frankia* and its host plants. *Physiologia Plantarum* 130: 318–330.

BENT, E., AND C. CHANWAY. 1998. The growth-promoting effects of a bacterial endophyte on lodgepole pine are partially inhibited by the presence of other rhizobacteria. *Canadian Journal of Microbiology* 44: 980–988.

BENT, E., S. TUZUN, C. P. CHANWAY, AND S. ENEBAK. 2001. Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. *Canadian Journal of Microbiology* 47: 793–800.

BERENDSEN, R. L., C. M. J. PIETERSE, AND P. A. H. M. BAKKER. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science* 17: 478–486.

BERG, G., A. KRECHEL, M. DITZ, R. A. SIKORA, A. ULRICH, AND J. HALLMANN. 2005. Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiology Ecology* 51: 215–229.

BERG, G., AND K. SMALLA. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology* 68: 1–13.

BHATIA, R., R. C. DOGRA, AND P. K. SHARMA. 2002. Construction of green fluorescent protein (GFP)-marked strains of *Bradyrhizobium* for ecological studies. *Journal of Applied Microbiology* 93: 835–839.

BIANCO, C., E. IMPERLINI, AND R. CALOGERO. 2006. Indole-3-acetic acid improves *Escherichia coli*'s defences to stress. *Archives of Microbiology* 185: 373–382.

BLOEMBERG, G. V., AND B. J. J. LUGTENBERG. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology* 4: 343–350.

BUCHAN, A., B. CROMBIE, AND G. M. ALEXANDRE. 2010. Temporal dynamics and genetic diversity of chemotactic-competent microbial populations in the rhizosphere. *Environmental Microbiology* 12: 3171–3184.

BULGARELLI, D., M. ROTT, K. SCHLAEPPI, E. VER LOREN VAN THEMAAT, N. AHMADINEJAD, F. ASSENZA, P. RAUF, ET AL. 2012. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 488: 91–95.

BULGARELLI, D., K. SCHLAEPPI, S. SPAEPEN, E. VER LOREN VAN THEMAAT, AND P. SCHULZE-LEFERT. 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* 64: 807–838. doi:10.1146/annurev-arplant-050312-120106

BURG, S. P. 1973. Ethylene in plant growth. *Proceedings of the National Academy of Sciences, USA* 70: 591–597.

CARROLL, G. 1988. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. *Ecology* 69: 2–9.

CHELIUS, M. K., AND E. W. TRIPLETT. 2000. Immunolocalization of dinitrogenase reductase produced by *Klebsiella pneumoniae* in association with *Zea mays* L. *Applied and Environmental Microbiology* 66: 783–787.

CHEN, X., S. SCHAUDER, AND N. POTIER. 2002. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* 415: 545–549.

CHENG, Z., B. J. MCCONKEY, AND B. R. GLICK. 2010. Proteomic studies of plant–bacterial interactions. *Soil Biology & Biochemistry* 42: 1673–1684.

CHERNIN, L. S. 2011. Quorum-sensing signals as mediators of PGPRs' beneficial traits. *In* D. K. Maheshwari [ed.], Bacteria in agrobiology: Plant nutrient management, 209–236. Springer, Berlin, Germany.

CHI, F., S.-H. SHEN, H.-P. CHENG, Y.-X. JING, Y. G. YANNI, AND F. B. DAZZO. 2005. Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice

growth physiology. *Applied and Environmental Microbiology* 71: 7271–7278.

CHI, F., P. YANG, F. HAN, Y. JING, AND S. SHEN. 2010. Proteomic analysis of rice seedlings infected by *Sinorhizobium meliloti* 1021. *Proteomics* 10: 1861–1874.

COMPANT, S., C. CLÉMENT, AND A. SESSITSCH. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology & Biochemistry* 42: 669–678.

COMPANT, S., H. KAPLAN, A. SESSITSCH, J. NOWAK, E. AIT BARKA, AND C. CLÉMENT. 2008. Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: From the rhizosphere to inflorescence tissues. *FEMS Microbiology Ecology* 63: 84–93.

COMPANT, S., B. REITER, A. SESSITSCH, J. NOWAK, C. CLEMENT, AND E. AIT BARKA. 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology* 71: 1685–1693.

CONN, V. M., AND C. M. FRANCO. 2004. Analysis of the endophytic actinobacterial population in the roots of wheat (*Triticum aestivum* L.) by terminal restriction fragment length polymorphism and sequencing of 16S rRNA clones. *Applied and Environmental Microbiology* 70: 1787–1794.

COOLEY, M. B., W. G. MILLER, AND R. E. MANDRELL. 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157: H7 and competition by *Enterobacter asburiae*. *Applied and Environmental Microbiology* 69: 4915–4926.

COOMBS, J. T., AND C. M. M. FRANCO. 2003. Isolation and identification of actinobacteria from surface-sterilized wheat roots. *Applied and Environmental Microbiology* 69: 5603–5608.

COSTA, J.-L., P. PAULSRUD, J. RIKKINEN, AND P. LINDBLAD. 2001. Genetic diversity of *Nostoc* symbionts endophytically associated with two bryophyte species. *Applied and Environmental Microbiology* 67: 4393–4396.

DE TORRES-ZABALA, M., M. H. BENNETT, J. W. MANSFIELD, R. EGEA, L. BO, AND M. GRANT. 2007. *Pseudomonas syringae* pv. tomato hijacks the *Arabidopsis* abscisic acid signalling pathway to cause disease. *EMBO Journal* 26: 1434–1443.

DENG, Z. S., ET AL. 2011. Diversity of endophytic bacteria within nodules of the *Sphaerophysa salsula* in different regions of Loess Plateau in China. *FEMS Microbiology Ecology* 76: 463–475.

DONG, Y., J. D. GLASNER, F. R. BLATTNER, AND E. W. TRIPLETT. 2001. Genomic interspecies microarray hybridization: Rapid discovery of three thousand genes in the maize endophyte, *Klebsiella pneumoniae* 342, by microarray hybridization with *Escherichia coli* K-12 open reading frames. *Applied and Environmental Microbiology* 67: 1911–1921.

DONG, Y., A. L. INIGUEZ, B. M. M. AHMER, AND E. W. TRIPLETT. 2003a. Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of *Medicago sativa* and *Medicago truncatula*. *Applied and Environmental Microbiology* 69: 1783–1790.

DONG, Y., A. L. INIGUEZ, AND E. W. TRIPLETT. 2003b. Quantitative assessments of the host range and strain specificity of endophytic colonization by *Klebsiella pneumoniae* 342. *Plant and Soil* 257: 49–59.

DUFFY, B. K., AND G. DÉFAGO. 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Applied and Environmental Microbiology* 65: 2429–2438.

DUMONT, M. G., B. POMMERENKE, P. CASPER, AND R. CONRAD. 2011. DNA-, rRNA-and mRNA-based stable isotope probing of aerobic methanotrophs in lake sediment. *Environmental Microbiology* 13: 1153–1167.

DUNFIELD, K., AND J. GERMIDA. 2001. Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. *FEMS Microbiology Ecology* 38: 1–9.

DUNFIELD, K., AND J. GERMIDA. 2004. Impact of genetically modified crops on soil-and plant-associated microbial communities. *Journal of Environmental Quality* 33: 806–815.

EGENER, T., T. HUREK, AND B. REINHOLD-HUREK. 1999. Endophytic expression of *nif* genes of *Azoarcus* sp. strain BH72 in rice roots. *Molecular Plant-Microbe Interactions* 12: 813–819.

ELASRI, M., S. DELORME, P. LEMANCEAU, G. STEWART, B. LAUE, E. GLICKMANN, P. M. OGER, AND Y. DESSAUX. 2001. Acyl-homoserine lactone production is more common among plant-associated *Pseudomonas* spp. than among soilborne *Pseudomonas* spp. *Applied and Environmental Microbiology* 67: 1198–1209.

ENGELHARD, M., T. HUREK, AND B. REINHOLD-HUREK. 2000. Preferential occurrence of diazotrophic endophytes, *Azoarcus* spp., in wild rice species and land races of *Oryza sativa* in comparison with modern races. *Environmental Microbiology* 2: 131–141.

FAURE, D., D. VEREECKE, AND J. H. LEVEAU. 2009. Molecular communication in the rhizosphere. *Plant and Soil* 321: 279–303.

FIERER, N., AND R. B. JACKSON. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences, USA* 103: 626–631.

FORCHETTI, G., O. MASCIARELLI, S. ALEMANO, D. ALVAREZ, AND G. ABDALA. 2007. Endophytic bacteria in sunflower (*Helianthus annuus* L.): Isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Applied Microbiology and Biotechnology* 76: 1145–1152.

FRANZ, E., A. A. VISSER, A. D. VAN DIEPENINGEN, M. M. KLERKS, A. J. TERMORSHUIZEN, AND A. H. C. VAN BRUGGEN. 2007. Quantification of contamination of lettuce by GFP-expressing *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. *Food Microbiology* 24: 106–112.

FUENTES-RAMÍREZ, L. E., J. CABALLERO-MELLADO, J. SEPÚLVEDA, AND E. MARTÍNEZ-ROMERO. 1999. Colonization of sugarcane by *Acetobacter diazotrophicus* is inhibited by high N-fertilization. *FEMS Microbiology Ecology* 29: 117–128.

GAGNÉ, S., C. RICHARD, H. ROUSSEAU, AND H. ANTOUN. 1987. Xylem-residing bacteria in alfalfa roots. *Canadian Journal of Microbiology* 33: 996–1000.

GAO, D., AND Y. TAO. 2012. Current molecular biologic techniques for characterizing environmental microbial community. *Frontiers of Environmental Science & Engineering* 6: 82–97.

GAO, M., AND M. TEPLITSKI. 2003. Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. *Molecular Plant-Microbe Interactions* 16: 827–834.

GARBEVA, P., L. S. OVERBEEK, J. W. L. VUURDE, AND J. D. ELSAS. 2001. Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microbial Ecology* 41: 369–383.

GERMAINE, K., E. KEOGH, G. GARCIA-CABELLOS, B. BORREMANS, D. VAN DER LELIE, T. BARAC, L. OEYEN, ET AL. 2004. Colonisation of poplar trees by *gfp* expressing bacterial endophytes. *FEMS Microbiology Ecology* 48: 109–118.

GERMIDA, J. J., S. D. SICILIANO, J. RENATO DE FREITAS, AND A. M. SEIB. 1998. Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). *FEMS Microbiology Ecology* 26: 43–50.

GODDARD, V. J., M. J. BAILEY, P. DARRAH, A. K. LILLEY, AND I. P. THOMPSON. 2001. Monitoring temporal and spatial variation in rhizosphere bacterial population diversity: A community approach for the improved selection of rhizosphere competent bacteria. *Plant and Soil* 232: 181–193.

GOTTEL, N. R., H. F. CASTRO, M. KERLEY, Z. YANG, D. A. PELLETIER, M. PODAR, T. KARPINETS, et al. 2011. Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. *Applied and Environmental Microbiology* 77: 5934–5944.

GRAYSTON, S. J., S. WANG, C. D. CAMPBELL, AND A. C. EDWARDS. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology & Biochemistry* 30: 369–378.

GRÖNEMEYER, J. L., C. S. BURBANO, T. HUREK, AND B. REINHOLD-HUREK. 2011. Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia. *Plant and Soil* 356: 67–82.

GUO, X., M. VAN IERSEL, AND J. CHEN. 2002. Evidence of association of *salmonellae* with tomato plants grown hydroponically in inoculated nutrient solution. *Applied and Environmental Microbiology* 68: 3639–3643.

HAAPALAINEN, M., H. MOSORIN, F. DORATI, R. WU, E. ROINE, S. TAIRA, R. NISSINEN, ET AL. 2012. Hcp2, a secreted protein of the phytopathogen

Pseudomonas syringae pv. tomato DC3000, is required for fitness for competition against bacteria and yeasts. *Journal of Bacteriology* 194: 4810–4822.

HALLMANN, J., AND G. BERG. 2006. Spectrum and population dynamics of bacterial root endophytes. *In* B. Schulz, C. Boyle, and N. Sieber [eds.], Soil biology, vol. 9, 15–31. Springer, Berlin, Germany.

HALLMANN, J., G. BERG, AND B. SCHULZ. 2006. Isolation procedures for endophytic microorganisms. *In* B. Schulz, C. Boyle, and N. Sieber [eds.], Soil biology, vol. 9, 299–319. Springer, Berlin, Germany.

HALLMANN, J., A. QUADT-HALLMANN, W. F. MAHAFFEE, AND J. W. KLOEPPER. 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* 43: 895–914.

HALLMANN, J., R. RODRÍGUEZ-KÁBANA, AND J. W. KLOEPPER. 1999. Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. *Soil Biology & Biochemistry* 31: 551–560.

HARDOIM, P. R., F. D. ANDREOTE, B. REINHOLD-HUREK, A. SESSITSCH, L. S. VAN OVERBEEK, AND J. D. VAN ELSAS. 2011. Rice root-associated bacteria: Insights into community structures across 10 cultivars. *FEMS Microbiology Ecology* 77: 154–164.

HARDOIM, P. R., L. S. VAN OVERBEEK, AND J. D. VAN ELSAS. 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends in Microbiology* 16: 463–471.

HARTMANN, A., M. ROTHBALLER, AND M. SCHMID. 2008. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil* 312: 7–14.

HAUBERG-LOTTE, L., H. KLINGENBERG, C. SCHARF, M. BÖHM, J. PLESSL, F. FRIEDRICH, U. VÖLKER, ET AL. 2012. Environmental factors affecting the expression of *pilAB* as well as the proteome and transcriptome of the grass endophyte *Azoarcus* sp. strain BH72. *PLoS ONE* 7: e30421.

HÉROUART, D., E. BAUDOIUN, P. FRENDO, J. HARRISON, R. SANTOS, A. JAMET, G. V. DE SYPE, ET AL. 2002. Reactive oxygen species, nitric oxide and glutathione: A key role in the establishment of the legume–*Rhizobium* symbiosis? *Plant Physiology and Biochemistry* 40: 619–624.

HILL, A., AND S. M. CROSSMAN. 1983. Characterization of N_2-fixing bacteria associated with sweet potato roots. *Canadian Journal of Microbiology* 29: 860–862.

HORRIGAN, L., R. LAWRENCE, AND P. WALKER. 2002. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental Health Perspectives* 110: 445–456.

HOSNI, T., C. MORETTI, AND G. DEVESCOVI. 2011. Sharing of quorum-sensing signals and role of interspecies communities in a bacterial plant disease. *The ISME Journal* 5: 1857–1870.

HUREK, T., L. L. HANDLEY, B. REINHOLD-HUREK, AND Y. PICHÉ. 2002. *Azoarcus* grass endophytes contribute fixed nitrogen to the plant in an unculturable state. *Molecular Plant-Microbe Interactions* 15: 233–242.

HUREK, T., B. REINHOLD-HUREK, M. VAN MONTAGU, AND E. KELLENBERGER. 1994. Root colonization and systemic spreading of *Azoarcus* strain BH72 in grasses. *Journal of Bacteriology* 176: 1913–1923.

INIGUEZ, A. L., Y. DONG, AND E. W. TRIPLETT. 2004. Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. *Molecular Plant-Microbe Interactions* 17: 1078–1085.

JOHNSTON-MONJE, D., AND M. N. RAIZADA. 2011. Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS ONE* 6: e20396.

KLOEPPER, J., AND M. N. SCHROTH. 1978. Plant growth-promoting rhizobacteria on radishes. *In* Fourth International Conference on Plant Pathogenic Bacteria, vol. II, 879–882. Gibert Clarey, Tours, France.

KLOEPPER, J. W., C.-M. RYU, AND S. ZHANG. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94: 1259–1266.

KORF, I. 2004. Gene finding in novel genomes. *BMC Bioinformatics* 5: 59.

KPOMBLEKOU-A, K., AND M. A. TABATABAI. 2003. Effect of low-molecular weight organic acids on phosphorus release and phytoavailabilty of phosphorus in phosphate rocks added to soils. *Agriculture Ecosystems & Environment* 100: 275–284.

KUKLINSKY-SOBRAL, J., W. L. ARAÚJO, R. MENDES, I. O. GERALDI, A. A. PIZZIRANI-KLEINER, AND J. L. AZEVEDO. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environmental Microbiology* 6: 1244–1251.

LAFAY, B., AND J. J. BURDON. 1998. Molecular diversity of rhizobia occurring on native shrubby legumes in southeastern Australia. *Applied and Environmental Microbiology* 64: 3989–3997.

LEMANCEAU, P., T. CORBERAND, L. GARDAN, X. LATOUR, AND G. LAGUERRE. 1995. Effect of two plant species, flax (*Linum usitatissinum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations of fluorescent *Pseudomonads. Applied and Environmental Microbiology* 61: 1004–1012.

LERY, L. M. S., A. S. HEMERLY, E. M. NOGUEIRA, W. M. A. VON KRÜGER, AND P. M. BISCH. 2011. Quantitative proteomic analysis of the interaction between the endophytic plant-growth-promoting bacterium *Gluconacetobacter diazotrophicus* and sugarcane. *Molecular Plant-Microbe Interactions* 24: 562–576.

LI, L., H. SINKKO, L. MONTONEN, G. WEI, K. LINDSTRÖM, AND L. A. RÄSÄNEN. 2012. Biogeography of symbiotic and other endophytic bacteria isolated from medicinal *Glycyrrhiza* species in China. *FEMS Microbiology Ecology* 79: 46–68.

LONG, H. H., D. G. SONNTAG, D. D. SCHMIDT, AND I. T. BALDWIN. 2010. The structure of the culturable root bacterial endophyte community of *Nicotiana attenuata* is organized by soil composition and host plant ethylene production and perception. *New Phytologist* 185: 554–567.

LONG, S. R. 1996. *Rhizobium* symbiosis: Nod factors in perspective. *Plant Cell* 8: 1885–1898.

LU, Y., T. LUEDERS, M. W. FRIEDRICH, AND R. CONRAD. 2005. Detecting active methanogenic populations on rice roots using stable isotope probing. *Environmental Microbiology* 7: 326–336.

LUNDBERG, D. S., S. L. LEBEIS, S. H. PAREDES, S. YOURSTONE, J. GEHRING, S. MALFATTI, J. TREMBLAY, ET AL. 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 488: 86–90.

MANEFIELD, M., A. WHITELEY, R. I. GRIFFITHS, AND M. J. BAILEY. 2002. RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Applied and Environmental Microbiology* 68: 5367–5373.

MANTER, D. K., J. A. DELGADO, D. G. HOLM, AND R. A. STONG. 2010. Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. *Microbial Ecology* 60: 157–166.

MARQUES, J., P. LAMOSA, AND C. RUSSELL. 2011. Quorum-sensing signal autoinducer-2 (AI-2) characterization of phospho-(*S*)-4, 5-dihydroxy-2,3-pentanedione isomerization by LsrG protein. *Journal of Biological Chemistry* 286: 18331–18343.

MATHESIUS, U., AND S. MULDERS. 2003. Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proceedings of the National Academy of Sciences, USA* 100: 1444–1449.

MATILLA, M. A., M. ESPINOSA-URGEL, J. J. RODRÍGUEZ-HERVA, J. L. RAMOS, AND M. I. RAMOS-GONZÁLEZ. 2007. Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. *Genome Biology* 8: R179.

MENDES, R., M. KRUIJT, I. DE BRUIJN, E. DEKKERS, M. VAN DER VOORT, J. H. M. SCHNEIDER, Y. M. PICENO, ET AL. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332: 1097–1100.

MICHÉ, L., F. BATTISTONI, S. GEMMER, M. BELGHAZI, AND B. REINHOLD-HUREK. 2006. Upregulation of jasmonate-inducible defense proteins and differential colonization of roots of *Oryza sativa* cultivars with the endophyte *Azoarcus* sp. *Molecular Plant-Microbe Interactions* 19: 502–511.

MIETHLING, R., G. WIELAND, H. BACKHAUS, AND C. TEBBE. 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. *Microbial Ecology* 41: 43–56.

MONTEIRO, J. M., R. E. VOLLÚ, M. R. R. COELHO, A. FONSECA, S. C. GOMES NETO, AND L. SELDIN. 2011. Bacterial communities within the rhizosphere and roots of vetiver (*Chrysopogon zizanioides* (L.) Roberty) sampled at different growth stages. *European Journal of Soil Biology* 47: 236–242.

MONTEIRO, R., E. BALSANELLI, T. TULESKI, H. FAORO, L. M. CRUZ, AND R. WASSEM. 2012. Endophyte *Herbaspirillum seropedicae* SmR1 and the phytopathogen *Herbaspirillum rubrisubalbicans* M1 by suppressive subtractive hybridization and partial genome. *FEMS Microbiology Ecology* 80: 441–451.

MORETTI, M., D. MINERDI, P. GEHRIG, A. GARIBALDI, M. L. GULLINO, AND K. RIEDEL. 2012. A bacterial–fungal metaproteomic analysis enlightens an intriguing multicomponent interaction in the rhizosphere of *Lactuca sativa. Journal of Proteome Research* 11: 2061–2077.

MORRIS, C., AND J. MONIER. 2003. The ecological significance of biofilm formation by plant-associated bacteria. *Annual Review of Phytopathology* 41: 429–453.

MUNDT, J. O., AND N. F. HINKLE. 1976. Bacteria within ovules and seeds. *Applied and Environmental Microbiology* 32: 694–698.

NISSINEN, R. M., M. K. MÄNNISTÖ, AND J. D. VAN ELSAS. 2012. Endophytic bacterial communities in three arctic plants from low arctic fell tundra are cold-adapted and host-plant specific. *FEMS Microbiology Ecology* 82: 510–522.

NORMAND, P., P. LAPIERRE, L. S. TISA, J. P. GOGARTEN, N. ALLOISIO, E. BAGNAROL, C. A. BASSI, ET AL. 2007. Genome characteristics of facultatively symbiotic *Frankia* sp. strains reflect host range and host plant biogeography. *Genome Research* 17: 7–15.

OLIVARES, F., V. BALDANI, AND V. REIS. 1996. Occurrence of the endophytic diazotrophs *Herbaspirillum* spp. in roots, stems, and leaves, predominantly of Gramineae. *Biology and Fertility of Soils* 21: 197–200.

OLIVARES, F., E. JAMES, J. I. BALDANI, AND J. DÖBEREINER. 1997. Infection of mottled stripe disease-susceptible and resistant sugar cane varieties by the endophytic diazotroph *Herbaspirillum. New Phytologist* 135: 723–737.

PATTEN, C. L., AND B. R. GLICK. 2002. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Applied and Environmental Microbiology* 68: 3795–3801.

PEREIRA, P., F. IBÁÑEZ, M. ROSENBLUETH, M. ETCHEVERRY, AND E. MARTINEZ-ROMERO. 2011. Analysis of the bacterial diversity associated with the roots of maize (*Zea mays* L.) through culture-dependent and culture-independent methods. *ISRN Ecology* 2011: Article ID 938546, 10 pp. doi:10.5402/2011/938546.

PETERS, R. D., A. V. STURZ, M. R. CARTER, AND J. B. SANDERSON. 2003. Developing disease-suppressive soils through crop rotation and tillage management practices. *Soil & Tillage Research* 72: 181–192.

PIERSON, E., AND D. WOOD. 1998. Interpopulation signaling via *N*-acylhomoserine lactones among bacteria in the wheat rhizosphere. *Molecular Plant-Microbe Interactions* 11: 1078–1084.

PRADET-BALADE, B., F. BOULMÉ, AND H. BEUG. 2001. Translation control: Bridging the gap between genomics and proteomics? *Trends in Biochemical Sciences* 26: 225–229.

PRISCHL, M., E. HACKL, M. PASTAR, S. PFEIFFER, AND A. SESSITSCH. 2012. Genetically modified Bt maize lines containing *cry3Bb1, cry1A105* or *cry1Ab2* do not affect the structure and functioning of root-associated endophyte communities. *Applied Soil Ecology* 54: 39–48.

PÜHLER, A., M. ARLAT, A. BECKER, M. GÖTTFERT, J. P. MORRISSEY, AND F. O'GARA. 2004. What can bacterial genome research teach us about bacteria–plant interactions? *Current Opinion in Plant Biology* 7: 137–147.

QIN, S., K. XING, J.-H. JIANG, L.-H. XU, AND W.-J. LI. 2011. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. *Applied Microbiology and Biotechnology* 89: 457–473.

RADAJEWSKI, S., P. INESON, N. R. PAREKH, AND J. C. MURRELL. 2000. Stable-isotope probing as a tool in microbial ecology. *Nature* 403: 646–649. doi:10.1038/35001054

RAMESH, R., A. JOSHI, AND M. P. GHANEKAR. 2008. *Pseudomonads*: Major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). *World Journal of Microbiology & Biotechnology* 25: 47–55.

RAMOS, H. J. O., M. G. YATES, F. O. PEDROSA, AND E. M. SOUZA. 2011. Strategies for bacterial tagging and gene expression in plant-host colonization studies. *Soil Biology & Biochemistry* 43: 1626–1638.

RASCHE, F., T. LUEDERS, M. SCHLOTER, S. SCHAEFER, F. BUEGGER, A. GATTINGER, R. C. HOOD-NOWOTNY, AND A. SESSITSCH. 2009. DNA-based stable isotope probing enables the identification of active bacterial endophytes in potatoes. *New Phytologist* 181: 802–807.

RASMUSSEN, T., AND M. GIVSKOV. 2006. Quorum sensing inhibitors: A bargain of effects. *Microbiology* 152: 895–904.

REINHOLD-HUREK, B., AND T. HUREK. 1998. Interactions of gramineous plants with *Azoarcus* spp. and other diazotrophs: Identification, localization, and perspectives to study their function. *Critical Reviews in Plant Sciences* 17: 29–54.

REINHOLD-HUREK, B., AND T. HUREK. 2011. Living inside plants: Bacterial endophytes. *Current Opinion in Plant Biology* 14: 435–443.

REISSINGER, A., V. VILICH, AND R. A. SIKORA. 2001. Detection of fungi in planta : Effectiveness of surface sterilization methods. *Mycological Research* 105: 563–566.

ROCHA, F., R. S. PAPINI-TERZI, M. Y. NISHIYAMA, R. Z. N. VÊNCIO, R. VICENTINI, R. D. C. DUARTE, V. E. DE ROSA, ET AL. 2007. Signal transduction-related responses to phytohormones and environmental challenges in sugarcane. *BMC Genomics* 8: 71.

ROESTI, D., R. GAUR, B. JOHRI, AND G. IMFELD. 2006. Stage, fertiliser management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure. *Soil Biology & Biochemistry* 38: 1111–1120.

ROJAS, A., G. HOLGUIN, B. R. GLICK, AND Y. BASHAN. 2001. Synergism between *Phyllobacterium* sp. (N_2-fixer) and *Bacillus licheniformis* (P-solubilizer), both from a semiarid mangrove rhizosphere. *FEMS Microbiology Ecology* 35: 181–187.

ROSENBLUETH, M., AND E. MARTÍNEZ-ROMERO. 2006. Bacterial endophytes and their interactions with hosts. *Molecular Plant-Microbe Interactions* 19: 827–837.

RUDRAPPA, T., K. CZYMMEK, P. W. PARÉ, AND H. P. BAIS. 2008. Root-secreted malic acid recruits beneficial soil bacteria. *Plant Physiology* 148: 1547–1556.

SCHLOSS, P. D., AND J. HANDELSMAN. 2005. Metagenomics for studying unculturable microorganisms: Cutting the Gordian knot. *Genome Biology* 6: 229.

SCHMELZ, E., AND J. ENGELBERTH. 2003. Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. *Proceedings of the National Academy of Sciences, USA* 100: 10552–10557.

SCHMID, M. D., M. SELESI, M. ROTHBALLER, N. SCHLOTER, E. LEE, E. KANDELER, AND A. HARTMANN. 2006. Localization and visualization of microbial community structure and activity in soil microhabitats. *In* B. Schulz, C. Boyle, and T. Sieber [eds.], Soil biology, vol. 6, 439–461. Springer, Berlin, Germany.

SCHULZ, B., C. BOYLE, AND T. SIEBER. 2006. Soil biology, vol. 9. Springer, Berlin, Germany.

SEGHERS, D., L. WITTEBOLLE, E. M. TOP, W. VERSTRAETE, AND S. D. SICILIANO. 2004. Impact of agricultural practices on the *Zea mays* L. endophytic community. *Applied and Environmental Microbiology* 70: 1475–1482.

SESSITSCH, A., P. HARDOIM, A. WEILHARTER, A. KRAUSE, AND T. WOYKE. 2012. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. *Molecular Plant-Microbe Interactions* 25: 28–36.

SHIDORE, T., T. DINSE, J. OHRLEIN, A. BECKER, AND B. REINHOLD-HUREK. 2012. Transcriptomic analysis of responses to exudates reveal genes required for rhizosphere competence of the endophyte *Azoarcus* sp. strain BH72. *Environmental Microbiology* 14: 2775–2787.

SMITH, S. E., AND D. J. READ. 2008. Mycorrhizal symbiosis, 3rd ed. Academic Press, San Diego, California, USA.

SPAEPEN, S., J. VANDERLEYDEN, AND Y. OKON. 2009. Plant growth-promoting actions of rhizobacteria. *Advances in Botanical Research* 51: 283–320.

SPAEPEN, S., J. VANDERLEYDEN, AND R. REMANS. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews* 31: 425–448.

STEIDLE, A., K. SIGL, AND R. SCHUHEGGER. 2001. Visualization of *N*-acylhomoserine lactone-mediated cell–cell communication between bacteria colonizing the tomato rhizosphere. *Applied and Environmental Microbiology* 67: 5761–5770.

STEWART, E. J. 2012. Growing unculturable bacteria. *Journal of Bacteriology* 194: 4151–4160.

STURZ, A. V., AND B. R. CHRISTIE. 1995. Endophytic bacterial systems governing red clover growth and development. *Annals of Applied Biology* 126: 285–290.

STURZ, A. V., B. R. CHRISTIE, AND B. G. MATHESON. 1998. Associations of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. *Canadian Journal of Microbiology* 44: 162–167.

SUN, L., F. QIU, X. ZHANG, X. DAI, X. DONG, AND W. SONG. 2008. Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microbial Ecology* 55: 415–424.

SURETTE, M. A., A. V. STURZ, R. R. LADA, AND J. NOWAK. 2003. Bacterial endophytes in processing carrots (*Daucus carota* L. var. *sativus*): Their localization, population density, biodiversity and their effects on plant growth. *Plant and Soil* 253: 381–390.

SZIDERICS, A. H., F. RASCHE, F. TROGNITZ, A. SESSITSCH, AND E. WILHELM. 2007. Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.). *Canadian Journal of Microbiology* 53: 1195–1202.

TAGHAVI, S., C. GARAFOLA, S. MONCHY, L. NEWMAN, A. HOFFMAN, N. WEYENS, T. BARAC, ET AL. 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Applied and Environmental Microbiology* 75: 748–757.

TAIZ, L., AND E. ZEIGER. 1998. Plant physiology, 2nd ed. Sinauer, Sunderland, Massachussetts, USA.

TEPLITSKI, M. 2000. Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Molecular Plant-Microbe Interactions* 13: 637–648.

THAKORE, Y. 2006. The biopesticide market for global agricultural use. *Industrial Biotechnology* 2: 194–208.

THOMAS, P., G. K. SWARNA, P. PATIL, AND R. D. RAWAL. 2008. Ubiquitous presence of normally non-culturable endophytic bacteria in field shoot-tips of banana and their gradual activation to quiescent cultivable form in tissue cultures. *Plant Cell, Tissue and Organ Culture* 93: 39–54.

TRIVEDI, P., Y. DUAN, AND N. WANG. 2010. Huanglongbing, a systemic disease, restructures the bacterial community associated with citrus roots. *Applied and Environmental Microbiology* 76: 3427–3436.

VAN OVERBEEK, L., AND J. D. VAN ELSAS. 2008. Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (*Solanum tuberosum* L.). *FEMS Microbiology Ecology* 64: 283–296.

VANDENKOORNHUYSE, P., S. MAHÉ, P. INESON, P. STADDON, N. OSTLE, J. B. CLIQUET, A. J. FRANCEZ, et al. 2007. Active root-inhabiting microbes identified by rapid incorporation of plant-derived carbon into RNA. *Proceedings of the National Academy of Sciences, USA* 104: 16970–16975.

VERMA, S. C., A. SINGH, S. P. CHOWDHURY, AND A. K. TRIPATHI. 2004. Endophytic colonization ability of two deep-water rice endophytes, *Pantoea* sp. and *Ochrobactrum* sp. using green fluorescent protein reporter. *Biotechnology Letters* 26: 425–429.

VERMA, S. C. C., J. K. K. LADHA, AND A. K. K. TRIPATHI. 2001. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *Journal of Biotechnology* 91: 127–141.

WANG, D., S. YANG, F. TANG, AND H. ZHU. 2012. Symbiosis specificity in the legume: Rhizobial mutualism. *Cellular Microbiology* 14: 334–342.

WEBER, O., V. BALDANI, AND K. TEIXEIRA. 1999. Isolation and characterization of diazotrophic bacteria from banana and pineapple plants. *Plant and Soil* 210: 103–113.

WILSON, D. 1995. Endophyte: The evolution of a term, and clarification of its use and definition. *Oikos* 73: 274–276.

YANDIGERI, M. S., ET AL. 2012. Drought-tolerant endophytic actinobacteria promote growth of wheat (*Triticum aestivum*) under water stress conditions. *Plant Growth Regulation* 68: 411–420.

YAZDANI, M., AND M. BAHMANYAR. 2009. Effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn (*Zea mays* L.). *World Academy of Science, Engineering and Technology* 49: 90–92.

ZAKRIA, M., J. NJOLOMA, Y. SAEKI, AND S. AKAO. 2007. Colonization and nitrogen-fixing ability of *Herbaspirillum* sp. strain B501 gfp1 and assessment of its growth-promoting ability in cultivated rice. *Microbes and Environments* 22: 197–206.