Bacterial endophytes: who and where, and what are they doing there?[1]

Natalia Malfanova, Ben Lugtenberg, and Gabriele Berg

Abstract

Bacterial endophytes are ubiquitous colonizers of the inner plant tissues where they do not normally cause any substantial morphological changes and disease symptoms. In this chapter we will give an overview of which bacterial species can live as endophytes, and how they enter a plant and live inside. We will also describe various bacterial traits which are required for a successful colonization of the plant's interior by endophytes. Some endophytes can promote plant growth and/or protect their host against phytopathogens. Many mechanisms of their beneficial action are predicted, but we will focus on those for which experimental support *in planta* was reported. Genomic analysis can give a deeper insight into the capabilities of endophytes and their possible role in plant growth and health. We will end our chapter with a brief discussion of available postgenomic tools and their utility in understanding the functionality of endophytic bacteria in plants.

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Introduction

Virtually all plants are inhabited by diverse bacteria known as endophytes. Endophytic bacteria are referred to as those which can be detected at a particular moment within the tissues of apparently healthy plant hosts (Hallmann et al. 1997; Schulz and Boyle, 2006). Most of the endophytes colonize different compartments of the plant apoplast, including the intercellular spaces of the cell walls and xylem vessels. Some of them are able to colonize reproductive organs of plants, e.g. flowers, fruits and seeds. Inside a plant these bacteria do not normally cause any substantial morphological changes like root-nodule symbionts do. They also do not cause any disease symptoms, in contrast to phytopathogens. Many endophytic bacteria possess a number of plant-beneficial traits *in vitro*; few of those exhibit them *in planta* and only a small number of endophytes proved to be very effective plant-growth promoting and/or biocontrol agents under agricultural conditions (Scherwinski et al., 2008; Berg, 2009).

In the following paragraphs we will discuss a number of important issues about endophytes. We will begin with a description of which bacteria were found as endophytes. Subsequently, colonization strategies used by endophytes will be described. How do they get inside plants? Which molecular traits are important for endophytic colonization? How do they escape the plant's immune response? Once they have established themselves in a plant, some endophytes can have a number of beneficial effects on their hosts. What are the mechanisms of their beneficial influence on plants? Here we will focus on those mechanisms which have been verified *in planta*, e.g. by a mutational study. Finally, we will try to get a deeper insight into the capabilities of endophytic bacteria and their possible role in plant health and development by evaluating a genomic approach. The utility of metagenomic and postgenomic approaches to study the structure and function of the endophytic community will complete the discussion of this chapter

Which bacteria can be found as endophytes?

Since the first reliable reports about the isolation of endophytic bacteria from surface-sterilized plants (Samish et al., 1960; Mundt and Hinkle, 1976) more than 200 bacterial genera from 16 phyla have been reported as endophytes. These include both culturable and unculturable bacteria belonging to Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Cholorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospira, Planctomycetes, Proteobacteria, Spirochaetes and Verrucomicrobiae (Sun et al., 2006; Berg and

Hallmann, 2006; Mengoni et al., 2009; Manter et al., 2010; Sessitsch et al., 2012). However, the most predominant and studied endophytes belong to three major phyla (Actinobacteria, Proteobacteria and Firmicutes) and include members of *Azoarcus* (Krause et al., 2006), *Acetobacter* (renamed as *Gluconobacter*) (Bertalan et al., 2009), *Bacillus* (Deng et al., 2011), *Enterobacter* (Taghavi et al., 2010), *Burkholderia* (Weilharter et al., 2011), *Herbaspirillum* (Pedrosa et al. 2011), *Pseudomonas* (Taghavi et al., 2009), *Serratia* (Taghavi et al., 2009), *Stenotrophomonas* (Ryan et al., 2009) and *Streptomyces* (Suzuki et al., 2005). Species of these genera are ubiquitous in the soil/rhizosphere which represents the main source of endophytic colonizers (Hallmann and Berg, 2006). Other possible sources of endophytes include the phyllosphere, the anthosphere and seeds (Compant et al., 2010). Naturally occurring endophytes can be visualized by FISH (fluorescence *in situ* hybridization) combined with confocal laser scanning microscopy using specific probes (Amann et al. 1990; Loy et al. 2007). In **Fig. 1** examples are shown for the phyllosphere and rhizosphere of plants (Bragina et al., 2011 a, b).

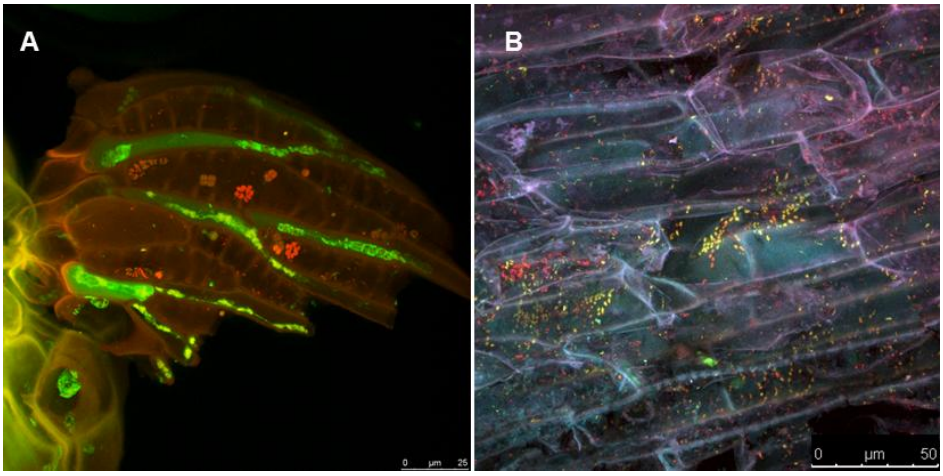

Fig. 1. Localization of endophytic bacteria by fluorescence *in situ* hybridization combined with confocal laser scanning microscopy in the phyllosphere of a moss gametophytes of *Sphagnum fallax* (A) and in the rhizosphere of *Lolium perenne* (B). Images show colonization of hyaline leave cells of *S. fallax* by Bacteria (red) and Alphaproteobacteria (yellow) (A) and of root cells of *L. perenne* by Bacteria (red), Alphaproteobacteria (pinkish), and Gammaproteobacteria (yellow) (B).

1. Colonization of plants by endophytic bacteria

There is a number of ways by which endophytic bacteria can get access to a plant's interior. In this section we will follow their main colonization route from the rhizosphere and give a brief description of alternative ways of plant colonization by endophytes.

1.1. Rhizoplane colonization

Colonization of the plant's interior by bacteria generally starts with their establishment in the rhizosphere. The early events of this process such as recognition and chemotaxis have been extensively reviewed by Lugtenberg et al. (2001) and Lugtenberg and Kamilova (2009). They will not be covered here. Following rhizosphere colonization, bacteria attach to the rhizoplane, i. e. the root surface. A number of mutational studies showed that attachment of bacterial cells to the root is a crucial step for subsequent endophytic establishment. Several bacterial surface components can be involved in this process. For *Azoarcus* sp. BH72, an endophytic diazotroph of rice, type IV pili encoded by *pil*AB are required for attachment to the root surfaces (Dörr et al., 1998). A mutant impaired in the expression of *pil*AB fails to successfully colonize roots and shoots of rice plants (Reinhold-Hurek et al., 2006). The attachment of another diazotrophic endophyte, *Herbaspirillum seropedicae*, to root surfaces of maize depends on LPS (liposaccharide) (Balsanelli et al., 2010). A mutant strain with changed monosaccharide composition in the core domain of LPS showed a hundred-fold lower root adhesion and endophytic spreading compared to the wild type. A similar study showed that EPS (exopolysaccharide) is necessary for rhizoplane and endosphere colonization of rice plants by *Gluconacetobacter diazotrophicus* (Meneses et al., 2011). Since none of these mutant strains completely lost their ability for adhesion, it can be expected that other bacterial surface components are also involved in this process.

1.2. Bacterial entry

The preferable sites of bacterial attachment and subsequent entry are the apical root zone with the thin-walled surface root layer such as the cell elongation and the root hair zone (zone of active penetration), and the basal root zone with small cracks caused by the emergence of lateral roots (zone of passive penetration) (**Fig. 2**). At these sites bacteria are often arranged in microcolonies comprising several hundreds of cells (Zachow et al., 2010). For active penetration, endophytic bacteria have to be well-equipped with cellulolytic enzymes which hydrolyze the plant's exodermal cell

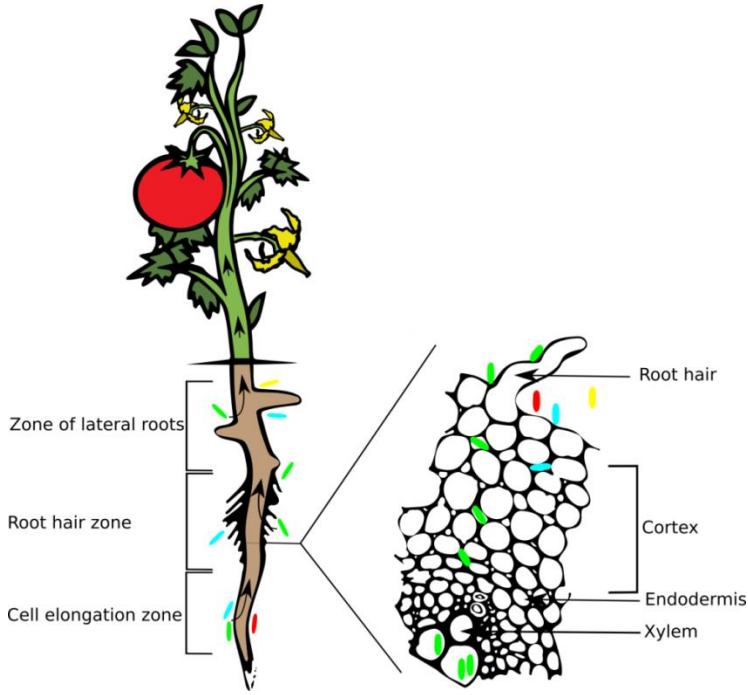

Fig. 2. The main plant colonization routes by endophytic bacteria. Bacteria can enter a plant at several root zones as indicated above. Endophytes can either remain at the site of entry (indicated in blue) or move deeper inside and occupy the intercellular space of the cortex and xylem vessels (indicated in green). Red and yellow represent rhizospheric bacteria which are unable to colonize inner plant tissues.

walls. *In vitro* production of these enzymes has been reported for many endophytes (Compant et al., 2005; Reinhold-Hurek et al., 2006). The expression of endoglucanase, the main cellulase responsible for hydrolysis of β(1→4) linkage in cellulose, was detected *ad planta* at the primary sites of entry of *Azoarcus* sp. BH72 (Reinhold-Hurek et al., 2006). Moreover, the role of endoglucanase in its endophytic colonization has been confirmed by mutational analysis. An *egl*A mutant failed to efficiently invade plant cells and to systemically colonize the plant, in contrast to the wild type strain and the mutant complemented with *egl*A.

Bacterial cell-wall degrading enzymes are also known to be involved in the elicitation of defense pathways in plants as many proteins which are involved in defense and repair are associated with plant cell walls (Norman-Setterblad et al., 2000). Induction of such a response usually results in decreasing the spread of pathogens inside a plant (Iniguez et al., 2005). Since this is not the case for endophytes, endophytic bacteria must be able to escape the plant immune response or even reduce it to some extent. Genomic analysis of sequenced endophytes

confirmed this notion (see section Genomic and postgenomic view of plant-endophyte interactions). The exact mechanism of this process remains to be elucidated.

By entering a plant through natural cracks at the region where the lateral roots appear, bacteria remain "invisible" for the plant's immune system. This mode of entry (often combined with active penetration) has been suggested for *Azoarcus* sp. BH72 (Reinhold-Hurek and Hurek, 1998) and *Burkholderia vietnamiensis* (Govindarajan et al., 2007) in rice, *B. phytofirmans* PsJN in grape (Compant et al., 2005), *B. subtilis* Lu144 (Ji et al., 2008) and *B. cepacia* Lu10-1 (Ji et al., 2010) in mulberry, *Gluconacetobacter diazotrophicus* Pal5 in sugar cane (James et al., 1994) and *Herbaspirillum seropedicae* Z67 in rice (James et al., 2002).

1.3. Colonization of the plant cortex

Once bacterial cells have crossed the exodermal barrier, they can remain at the site of entry as it has been shown for *Paenibacillus polymyxa* in *Arabidopsis* (Timmusk et al., 2005) or move deeper inside and occupy the intercellular space of the cortex (James et al., 1994; Roncato-Maccari et al., 2003; Compant et al., 2005; Gasser et al., 2011) (**Fig. 2**). It is uncommon for endophytic bacteria to penetrate plant cells and cause formation of specific morphological structures like root-nodule bacteria do. However, recently Huang et al. (2011) showed that *Bacillus subtilis* GXJM08 colonizes the root of the leguminous plant *Robinia pseudoacacia* L. in a mode similar to that used by rhizobia. The most dramatic changes include (i) deformation of the root hair (swelling, dichotomous branching), (ii) development of infection threads with bacteria between the cell walls of root cortical cells, and (iii) formation of bacteroids inside plant cortical cells. It is unknown whether this strain could fix N like the root-nodule bacteria do. It would also be of interest to determine whether other non-symbiotic bacteria can induce similar morphological changes in this plant.

1.4. Colonization of the xylem

Only a few bacteria can penetrate the endodermal barrier and invade the xylem vessels (James et al., 2002; Roncato-Maccari et al., 2003; Compant et al., 2005; Gasser et al., 2011) **(Fig. 2).** This usually happens through unsuberized endodermal cells in the apical root zone and/or in the basal root zone, where the emerging lateral roots interrupt the continuity of the Casparian band in the wall of endodermal cells. The long-distance transport of water, ions and low-molecular weight organic compounds, such as sugars, organic and amino acids, takes place in the xylem (Sattelmacher, 2001).

Though the concentration of available nutrients is relatively low and represents 0.006 - 0.034 µmol/g of fresh weight for some sugars (Madore and Webb, 1981), it has been calculated that they are sufficient to support the growth of endophytic bacteria (Sattelmacher, 2001; Bacon and Hinton, 2006). Direct evidence that bacterial endophytes feed on plant nutrients came from several radioactive labeling experiments. For example, after incubation of potato plants with $^{13}CO_2$, Rasche et al. (2009) detected the isotope label first in the plant's photosynthetic metabolites and subsequently in diverse bacterial endophytes.

Several attempts were made to find carbon sources which might be important or crucial for the endophytic lifestyle (Shishido et al., 1999; Krause et al., 2011; Malfanova et al., 2013). Shishido et al. (1999) compared carbon oxidation profiles of the endophytic *Paenibacillus polymyxa* strain Pw-2R and *Pseudomonas fluorescens* Sm3-RN with those of rhizospheric strains, which were unable to colonize spruce endophytically. Strains Pw-2R and Sm3-RN were able to metabolize D-sorbitol and D-galacturonic acid while their rhizospheric colleagues could not. In our recent study (Malfanova et al., 2013) we found that, in contrast to most rhizospheric *Pseudomonas* spp., endophytic pseudomonads isolated from cucumber plants were able to utilize L-arabinose, one of the most abundant available sugars in the xylem fluid of various plants (Iwai et al., 2003). In another study Krause et al. (2011) detected induced expression of several bacterial alcohol dehydrogenases inside rice roots during their colonization by *Azoarcus* sp. BH72. Mutant strains with disrupted genes coding for alcohol dehydrogenases colonized the root interior less efficiently than the wild type. Since ethanol is abundant in waterlogged rice, these data suggest that it might be one of the major carbon sources for strain BH72 cells inside the plant. Taking together, these studies show that the ability of bacteria to utilize certain plant metabolites might be a prerequisite for their successful endophytic establishment.

1.5. Colonization of the reproductive organs

It is likely that the concentration of available nutrients in xylem is decreasing along the plant axis. This can explain the facts that the diversity and population density of endophytic bacteria decreases with the distance from the root and that only a small number of bacteria reaches the upper parts of shoots, the leaf apoplast and reproductive organs, such as flowers, fruits and seeds (Compant et al., 2010; Fürnkranz et al. 2011). The presence of endophytic bacteria in reproductive organs of plants was confirmed by cultivation (Samish and Etinger-Tulczynska, 1963; Mundt and Hinkle,

1976; Graner et al., 2003; Okunishi et al., 2005; Fürnkranz et al., 2011) and by microscopic visualization (Coombs and Franco, 2003; Compant et al., 2011). Most likely, bacterial cells enter the reproductive organs through the plant's vascular tissues. For example, many bacterial and fungal phytopathogens infect the developing seeds via vascular tissues of the funiculus and chalaze region as well as via the stigma and micropyle (Agarwal and Sinclair, 1996). It is also possible that if one of the reproductive cells (egg cell or male gametes) carries a microbe, the resulting embryo and endosperm may be colonized. This could explain the transfer of endophytes from plants to seeds. However, so far the invasion of reproductive tissues (ovule, megaspore mother cell, stamens, and pollen mother cells) has been shown only for viruses (Agarwal and Sinclair, 1996). The exact mechanism of transmission of endophytic bacteria from the vascular tissues to the reproductive organs and subsequently to the new plant generation still remains to be established.

1.6. Other ways of plant colonization

Although the rhizosphere is assumed to be the main source of endophytic colonizers, other sites of entry cannot be ignored. Some bacteria are able to enter a plant through stomata as has been shown for *Gluconobacter diazotrophicus* on sugarcane (James et al., 2001) and for *Streptomyces galbus* on rhododendron (Suzuki et al., 2005). In the latter case, production of non-specific wax-degrading enzymes might have facilitated the leaf surface colonization and the subsequent endophytic establishment of this microbe (Suzuki et al., 2005). Bacteria can also enter a plant through flowers, fruits and seeds. However this is mostly known for specialized phytopathogens and was not shown for (non-pathogenic) bacterial endophytes.

2. Beneficial endophytic bacteria and their effects on a plant

After establishing in a plant, endophytes can positively influence plant growth and its resistance to different stresses. For detailed overviews of their beneficial actions the reader is referred to Ryan et al. (2008), Hardoim et al. (2008) and Berg (2009). In the following section we will restrict ourselves to the plant growth-promoting effects mediated by endophytic bacteria. These can be grouped as direct PGP (plant growth promotion) and biocontrol of phytopathogens. A variety of PGP and biocontrol mechanisms can be expected for endophytic bacteria based on those described for rhizobacteria (see Chapters 3 and 4, respectively). However, only a few mechanisms have been proven to occur *in planta* (**Fig. 3**).

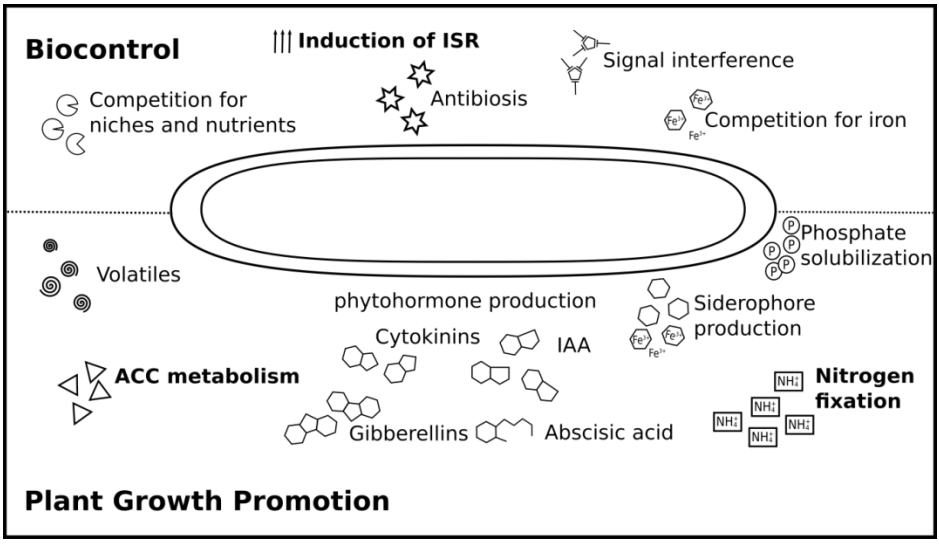

Fig. 3. Illustration of the main mechanisms of PGP and BC mediated by endophytic plant-beneficial bacteria. Indicated in bold are the mechanisms used by endophytes as shown by experimental studies. Other mechanisms are putatively involved based on genomic data.

2.1. Plant growth promotion by endophytic bacteria

PGP has been shown for many endophytic bacteria (Zachow et al., 2010; Gasser et al., 2011; Malfanova et al., 2011). Direct PGP mediated by endophytes is mostly based on providing essential nutrients to plants and production and/or regulation of phytohormones.

After water, nitrogen is the major limiting compound for crop production. Many plants can obtain nitrogen through a process known as BNF (biological nitrogen fixation). For details see Chapter 3. BNF by legumes is based on a symbiosis with root-nodule nitrogen-fixing bacteria while other agriculturally important plants such as maize, rice, sugar cane and wheat can benefit from the association with diverse endophytic diazotrophs. The best studied endophytic diazotrophs include members of *Azoarcus*, *Burkholderia*, *Gluconobacter, Herbaspirillum* and *Klebsiella* (James, 2000).

The ability of endophytic diazotrophs to fix N_2 *in planta* was demonstrated in several studies. This was done by monitoring the expression of nitrogenase genes in nitrogen-fixing cells at the endophytic stage (Egener et al., 1999; Roncato-Maccari et al., 2003; You et al., 2005) and by isotope analysis (Sevilla et al., 2001; Elbeltagy et al., 2001). $^{15}N_2$ incorporation experiments showed that sugar cane plants inoculated with *G. diazotrophicus* Pal5 obtained up to 0.6% of total N from BNF over a 24-h period (Sevilla et al., 2001); for rice plants harboring *Herbaspirillum* sp. B501 this value was

0.14% (Elbeltagy et al., 2001), indicating that diazotrophic endophytes can contribute a significant amount of N to a plant. Other studies suggested that plants can get up to 70% of the required nitrogen through BNF mediated by endophytic diazotrophs (James, 2000).

Nitrogen fixation is regulated by the concentration of oxygen and the availability of nitrogen. In *Herbaspirillum* sp. B501 the expression of nitrogenase was repressed in free air (21% O_2) and induced under microoxic conditions (2% O_2) (You et al., 2005) suggesting that the plant's interior is a suitable environment for BNF. Sevilla et al. (2001) have demonstrated that under N-deficient conditions sugarcane plants inoculated with wild type *G. diazotrophicus* Pal5 have significantly greater shoot mass and N content than plants inoculated with a mutant unable to fix N_2, suggesting that BNF is the likely cause of PGP. It is interesting to note that N starvation can also derepress the biosynthesis of the plant hormone IAA (indole-3-acetic acid). For example, Brandi et al. (1996) demonstrated that IAA synthesis in the culture supernatant of *Erwinia herbicola* 299R was over 10-fold higher under nitrogen-limiting conditions. IAA was detected in the culture supernatant of *G. diazotrophicus* (Fuentes-Ramirez et al., 1993; Bastian et al., 1998). Therefore, it is likely that some diazotrophic bacteria stimulate plant growth both by supplying N and by production of phytohormones, in particular IAA. This possibility is further supported by the observation that when N was not limiting, both wild type *G. diazotrophicus* Pal5 and its fix^- mutant strains were able to increase the biomass of sugar cane (Sevilla et al., 2001). The *in vitro* production of IAA and its possible involvement in PGP has been reported for many other endophytic bacteria (Govindarajan et al., 2008; Rothballer et al., 2008; Jha and Kumar, 2009; Malfanova et al., 2011). However, the principal role of IAA in PGP was confirmed only for rhizobacteria, using mutational studies (Patten and Glick, 2002; Spaepen et al., 2008). For a more detailed overview of the microbial production of auxins and its role in the interaction with plants the reader is referred to Spaepen and Vanderleyden (2011).

Many IAA-producing endophytes possess ACC (1-aminocyclopropane-1-carboxylate)-deaminase activity which is involved in lowering the level of plant ethylene (Long et al., 2008). Elevated levels of ethylene caused by some stresses (see Chapter 3) are known to inhibit root elongation and lateral root emergence (Ivanchenko et al., 2008). According to the model proposed by Glick (2005) bacterial IAA activates ACC-synthase of plants resulting in the production of ACC, the ethylene precursor. Some bacteria can use ACC as a nutrient source and thereby decrease the

synthesis of ethylene in plants. ACC-deaminase activity was described for plant growth-promoting endophytic strains of *Burkholderia* (Sun et al., 2009; Gasser et al., 2011), *Herbaspirillum* (Rothballer et al., 2008) and *Pseudomonas* (Long et al., 2008). The role of ACC-deaminase in plant growth promotion has been further confirmed in a mutational study by Sun et al. (2009). Deletion of the *acd*S gene, coding for ACC-deaminase, in *B. phytofirmans* PsJN resulted in a decrease of the root length of canola seedlings by 32%.

Other phytohormones produced by endophytic bacteria include ABA (abscisic acid) (Cohen et al., 2008), cytokinins (Sgroy et al., 2009) and GBs (gibberellins) (Lucangeli and Bottini, 1997; Malfanova et al., 2011). Inoculation of maize with a GB-producing endophytic *Azospirillum* spp. increased the level of GA3 in plant roots and resulted in promotion of plant growth (Lucangeli and Bottini, 1997). An enhanced ABA content in plants has been detected after inoculation of *A. thaliana* with an ABA-producing strain of *Azospirillum* (Cohen et al., 2008). However, whether endophytic bacteria directly contribute to the increase of the plant phytohormone pool remains to be elucidated.

2.2. Biocontrol of phytopathogens by endophytic bacteria

While the biocontrol effect of endophytic bacteria is well known (Berg and Hallmann, 2006; Scherwinski et al., 2008; Malfanova et al., 2011), the mechanisms of biocontrol mediated by endophytes are less well elucidated. Biocontrol of phytopathogens can be based on several mechanisms which include antibiosis, CNN (competition for nutrients and niches) and ISR (induced systemic resistance) (**Fig. 3**). For more mechanisms, see Chapter 4. So far, only the role of ISR in biocontrol mediated by endophytes has been confirmed *in planta*. This was done by microscopic observations of endophytic bacteria inside the plant, where they induce morphological changes associated with ISR and reduce disease symptoms at locations where the endophyte itself is absent. For example, Melnick et al. (2008) evaluated the ability of several Bacilli to colonize cacao plants and reduce the symptoms of black pod rot caused by *Phytophthora capsici*. Inoculation of leaves with a suspension of vegetative cells resulted in a local colonization of plants. A small subpopulation (5-15%) of bacteria was recovered from the inner leaf tissues and no bacteria were detected in vascular tissues or in newly-developed leaves, indicating that bacteria were unable to systemically colonize the plant. Significant biocontrol was observed 26 days after inoculation on newly developed, non-colonized leaves, suggesting the induction of systemic resistance of cacao plants by bacilli.

Colonization of plants by biocontrol endophytes induces several cell-wall modifications, such as deposition of callose, pectin, cellulose and phenolic compounds leading to the formation of a structural barrier at the site of potential attack by phytopathogens (Benhamou et al., 1998; Benhamou et al., 2000). Another common response of bacterized plants challenged with a pathogen is an induction of defense-related proteins such as peroxidases, chitinases and β-1,3-glucanases (Fishal et al., 2010). These reactions result in a substantial reduction of pathogen spreading in a plant. For example, in *Pythium*-infected cucumber plants the hyphal growth was mainly restricted to the outer root tissue five days after oomycete inoculation (Benhamou et al., 2000). Moreover, 80% of the oomycete hyphae which penetrated the epidermis barrier were distorted. Significant disease suppression was also reported for wheat plants endophytically colonized with *B. subtilis* (Liu et al., 2009) and for banana plants pre-inoculated with endophytic *Pseudomonas* and *Burkholderia* (72 days before pathogen challenge) (Fishal et al., 2010).

Most likely, a combination of several mechanisms is exhibited by many biocontrol endophytic bacteria. This notion is supported by the fact that some antimicrobial compounds are involved in both antibiosis and triggering ISR (Ongena et al., 2007). The presence of other mechanisms such as competition for iron and for colonization sites is proposed for some endophytes based on the analysis of their genomes (see below). However this has not yet been confirmed *in planta.*

3.Genomic and postgenomic view of plant-endophyte interactions

In recent years a number of genomes of endophytic bacteria has been sequenced (**Table 1**). All beneficial traits which are discussed above (N fixation, IAA, ACC deaminase, etc.) are reflected in their genomes. Moreover, analysis of their genomes also revealed the existence of a high number of genes involved in iron uptake and metabolism. For example, the genome of *Enterobacter* sp. 638 has nine ABC transporters for siderophore complexes in contrast to four in *E. coli* K12 (Taghavi et al., 2010). *Azoarcus* sp. BH72 has 22 iron TonB receptor genes, which is twice as much as its free-living soil colleague EbN1 (Krause et al., 2006). These data suggest that endophytic bacteria are well-equipped to survive in a low-iron environment and can efficiently compete for this element with other microorganisms, including phytopathogens.

In addition to the above-mentioned plant beneficial traits, a number of genes involved in QS (quorum sensing) have been identified in the endophytic genomes. For

Table 1. Sequenced bacterial endophytes and mechanisms of their beneficial action

Endophyte	Phylum	Plant of origin	Mechanisms of beneficial action[a]	References
Azoarcus sp. BH72	ß-Proteobacteria	Kallar grass	**BNF** and competition for iron	Krause et al., 2006
Azospirillum sp. B510	α-Proteobacteria	Rice	ACC metabolism, BNF, ISR, production of IAA and siderophores	Kaneko et al., 2010
Bacillus subtilis BSn5	Firmicuta	Konjac	Antibiosis *in vitro*, production of lipopeptides and polyketides	Deng et al., 2011
Burkholderia phytofirmans PsJN	ß-Proteobacteria	Onion	**ACC metabolism**, production of IAA and siderophores	Weilharter et al., 2011
Enterobacter sp. 638	Y-Proteobacteria	Poplar	Competition for iron, production of antimicrobials, IAA, siderophores and volatiles	Taghavi et al., 2010
Gluconacetobacter diazotrophicus Pal5	α-Proteobacteria	Sugarcane	Antibiosis, **BNF**, phosphate and zinc solubilization, production of GB, IAA and volatiles	Bertalan et al., 2009
Herbaspirillum seropedicae SmR1	ß-Proteobacteria	Sorghum	ACC metabolism, BNF, competition for iron, production of IAA	Pedrosa et al., 2011
Klebsiella pneumoniae 342	Y-Proteobacteria	Maize	BNF	Fouts et al. 2008
Methylobacterium populi BJ001	α-Proteobacteria	Poplar	Unknown	Copeland et al., *unpublished*
Pseudomonas putida W619	Y-Proteobacteria	Poplar	Production of IAA	Taghavi et al., 2009
Pseudomonas stutzeri A1501	Y-Proteobacteria	Rice	BNF	Yan et al., 2008
Serratia proteamaculans 568	Y-Proteobacteria	Poplar	Production of volatiles	Taghavi et al., 2009
Stenotrophomonas maltophilia R551-3	Y-Proteobacteria	Poplar	Antibiotic production	Taghavi et al., 2009
Variovorax paradoxus S110	ß-Proteobacteria	Potato	ACC metabolism, competition for iron, signal interference	Han et al., 2011

[a] Both confirmed (in bold) and suspected from genomic analysis and experimental studies

example, 24 *lux*R QS genes are present in the genome of *Serratia proteamaculans* 568. In the related endophytic strain *S. plymuthica* G3 QS controls important colonization-related traits such as swimming motility and biofilm formation (Liu et al., 2011). Interestingly, in some free-living *Serratia* spp. these traits are QS-independent, suggesting that the precise role of QS depends on the bacterium's lifestyle.

Further genome analysis revealed genes which might be important for the endophytic lifestyle. For example, the genome of diazotrophic *K. pneumoniae* (Kp) 342 contains genes for superoxide dismutases, putative catalases, peroxidases and reductases which are involved in the protection of bacterial cells against plant ROS (reactive oxygen species) (Fouts et al., 2008). Additionally, genome analysis revealed the ability of Kp342 to metabolize a wide range of plant sugars, carbohydrates and hemicellulosic substrates. Furthermore, a comparison of the genome of Kp342 with that of the clinical isolate MGH78578 revealed a major difference in their metabolism, surface attachment and secretion. These data suggest that Kp342 is well adapted to escape plant defense reactions and successfully establish itself inside a plant.

Metagenomic analysis of the most abundant endophytic bacteria of rice verified traits which are shared among endophytes and are therefore potentially important for their interactions with plants (Sessitsch et al., 2012). These include (i) a whole set of specialized secretion systems, except the type III secretion system which was not highly conserved among rice endophytes, (ii) cellulolytic and pectinolytic enzymes, (iii) flagellins, (iv) enzymes involved in ROS degradation, (v) receptors and transporters for iron uptake, (vi) QS systems, (vii) metabolic pathways for degradation of plant compounds, and (viii) numerous plant-growth promoting and biocontrol traits (ACC-deaminase activity, BNF, production of antimicrobial compounds, phytohormones and volatiles).

Applying postgenomic approaches, such as metaproteomics, metaproteogenomics and metatranscriptomics, can link the genomic potential with function and therefore give a deeper insight into plant-endophyte interactions. These tools deal with global expression of proteins (metaproteomics) or mRNA (metatranscriptomics) from microbial communities. Metaproteogenomics links the proteome and the genome of the environmental sample. This allows identification of more proteins (functions) than proteomics alone. Recently, a metaproteogenomic approach was used to study microbial communities in the phyllosphere and rhizosphere of rice (Knief et al., 2011). The results showed that despite the presence of *nifH* genes in both microenvironments, dinitrogenase reductase was exclusively identified in the

rhizosphere. If such an approach could be applied to study the endosphere, more significant data regarding the endophyte functionality can be collected.

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