
# ΠΡΟΓΡΑΜΜΑ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗΣ ΑΕΙ ΓΙΑ ΤΗΝ ΕΠΙΚΑΙΡΟΠΟΙΗΣΗ ΓΝΩΣΕΩΝ ΑΠΟΦΟΙΤΩΝ ΑΕΙ (ΠΕΓΑ)

*«Οι σύγχρονες τεχνικές βιο-ανάλυσης στην υγεία, τη γεωργία, το περιβάλλον και τη διατροφή»*

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Review

# Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization

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ABSTRACT

In both managed and natural ecosystems, beneficial plant-associated bacteria play a key role in supporting and/or increasing plant health and growth. Plant growth-promoting bacteria (PGPB) can be applied in agricultural production or for the phytoremediation of pollutants. However, because of their capacity to confer plant beneficial effects, efficient colonization of the plant environment is of utmost importance. The majority of plant-associated bacteria derives from the soil environment. They may migrate to the rhizosphere and subsequently the rhizoplane of their hosts before they are able to show beneficial effects. Some rhizoplane colonizing bacteria can also penetrate plant roots, and some strains may move to aerial plant parts, with a decreasing bacterial density in comparison to rhizosphere or root colonizing populations. A better understanding on colonization processes has been obtained mostly by microscopic visualisation as well as by analysing the characteristics of mutants carrying disfunctional genes potentially involved in colonization. In this review we describe the individual steps of plant colonization and survey the known mechanisms responsible for rhizosphere and endophytic competence. The understanding of colonization processes is important to better predict how bacteria interact with plants and whether they are likely to establish themselves in the plant environment after field application as biofertilisers or biocontrol agents.



## 1. Introduction

Since the elaboration of the rhizosphere concept by Lorenz Hiltner in 1904, many studies have reported that the soil environment attached to the root system is a hot spot of microbial abundance and activity due to the presence of root exudates and rhizodeposits (Hiltner, 1904; Smalla et al., 2006; Hartmann et al., 2008). Some rhizosphere microorganisms may be neutral or deleterious in regard to plant growth, whereas other microbes support their hosts (reviewed in Welbaum et al., 2004; Raaijmakers et al., 2008). Such plant growth-promoting bacteria (PGPB; Bashan and Holguin, 1998) or plant growth-promoting rhizobacteria (PGPR; Kloepper and Schroth, 1978) can stimulate plant growth, increase yield, reduce pathogen infection, as well as reduce biotic or abiotic plant stress, without conferring pathogenicity (Welbaum et al., 2004; van Loon and Bakker, 2005; Lugtenberg and Kamilova, 2009). Plant beneficial microorganisms are of interest for application in agriculture either as biofertilisers or as pesticides as well as for phytoremediation applications (reviewed in Sturz et al., 2000; Berg, 2009; Lugtenberg and Kamilova, 2009; Weyens et al., 2009). However, in many cases PGPB fail to induce the desired effects when applied in the field. This might be due to insufficient rhizosphere and/or plant colonization, which is as an important step required for exhibiting beneficial effects (Lugtenberg et al., 2001). Therefore, not only mechanisms responsible for plant growth promotion have to be investigated, but also a thorough understanding of all steps involved in plant colonization by PGPB is required to improve the efficiency and reliability of inoculant strains.

The rhizosphere is well known to host a variety of PGPB. In addition, some rhizosphere colonizers can enter plants as already postulated by Galippe in 1887 (Galippe, 1887). For a long time this was not recognized, although di Vestea (1888) confirmed Galippe's work. Other researchers at that time including Pasteur, Chamberland and Fernbach considered healthy plants to be free of microorganisms (reviewed in Smith, 1911). In the last decade it has been repeatedly demonstrated that the plant interior is colonized by a range of endophytes mostly deriving from the rhizosphere and many of them have been reported to improve plant growth or

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health (reviewed in Sturz and Nowak, 2000; Hardoim et al., 2008). Following rhizosphere colonization endophytes may colonize various plant organs (James et al., 2002; Compant et al., 2005b, 2008a). However, distinct microbial communities have been found in various plant organs such as roots, stem, leaves, flowers as well as fruits and seeds or even during plant development (Sessitsch et al., 2002; Berg et al., 2005b; Okunishi et al., 2005) indicating different capacities of bacterial strains to colonize various plant compartments.

In addition to understanding the mechanisms of the interaction between plants and microorganisms, colonization mechanisms and strategies represent an important aspect of the interaction. Successful colonization of a PGPB inoculant strain is a requirement to promote plant growth or health. In this paper we review the current understanding of the plant colonization process by bacteria including rhizosphere and subsequent endosphere colonization.

## 2. Rhizosphere and rhizoplane colonization

Since the 1980's, many studies have focused on the colonization by beneficial bacteria in the rhizosphere, i.e. the soil compartment, which influenced by rhizodeposits. Generally, approx. $10^7$–$10^9$ CFU culturable rhizosphere bacteria $g^{-1}$ of rhizosphere soil have been found (Benizri et al., 2001), whereas population densities in the rhizoplane range from $10^5$–$10^7$ CFU $g^{-1}$ of fresh weight (Benizri et al., 2001; Bais et al., 2006). By using gnotobiotic conditions and with the help of microscopic tools, which allow the detection of *gfp*- or *gusA*-labelled strains or of strains by immunomarkers or by fluorescence *in situ* hybridization, it has been demonstrated that bacterial cells first colonize the rhizosphere following soil inoculation (Gamalero et al., 2003). Then, bacterial cells have been visualized as single cells attached to the root surfaces, and subsequently as doublets on the rhizodermis, forming a string of bacteria (Fig. 1; Hansen et al., 1997). Colonization may then occur on the whole surface of some rhizodermal cells (Fig. 1) and bacteria can even establish as microcolonies or biofilms (Benizri et al., 2001). Rhizoplane colonization has been studied not only by using *in vitro* grown plants but also with plants grown in natural soil (Fig. 1), which is characterized by a high microbial diversity. Before being able to confer any plant beneficial effects, (inoculated) PGPB need to be rhizosphere and/or rhizoplane competent (Compant et al., 2005a), *i.e.* they have to be able to colonize the rhizosphere and/or the rhizoplane during an extended period characterized by strong microbial competition (Whipps, 2001). Many factors can be involved in rhizosphere and rhizoplane competence by PGPB. However, both in gnotobiotic systems and in natural soil, it is important to note that the root system is not colonized in a uniform manner. Different population densities were reported for the diverse root zones. This has been well described by Gamalero et al. (2004) with *Pseudomonas fluorescens* strain A6RI and tomato roots, where the distribution and density of the inoculant strain varied according to the root zone. Non-uniform bacterial colonization along the root can be explained by different factors such as varying root exudation patterns, bacterial quorum sensing effects as well as many others, which are summarized in Table 1 and described below in greater detail.

### 2.1. Chemotaxis towards root exudates

Rhizosphere and rhizoplane colonization has been described to be linked to root exudation (Lugtenberg and Dekkers, 1999). Carbon fixed by plant photosynthesis is known to be partly translocated into the root zone and released as root exudates (Bais et al., 2006). Various carbohydrates, amino acids, organic acids, as well as other compounds, which provide a source of nutrients for root-associated bacteria, are released in the rhizosphere (Walker et al., 2003). Microorganisms are known to be chemoattracted and move towards exudates, allowing them to colonize and multiply both in the rhizosphere and the rhizoplane (Lugtenberg and Kamilova, 2009). A mutant of a plant growth-promoting *P. fluorescens* strain, which lacked the *cheA* gene responsible for chemotaxis showed reduced movement towards root exudates (or specific exudate components) in the tomato rhizosphere and also decreased root colonization (de Weert et al., 2002). In addition, genes known to be involved in recognition and chemotaxis to plant root exudates are involved. Mark et al. (2005) studied the transcriptomic response of *Pseudomonas aeruginosa* strain PAO1 to exudates of two cultivars of sugarbeet. This strain is an opportunistic human pathogen but was

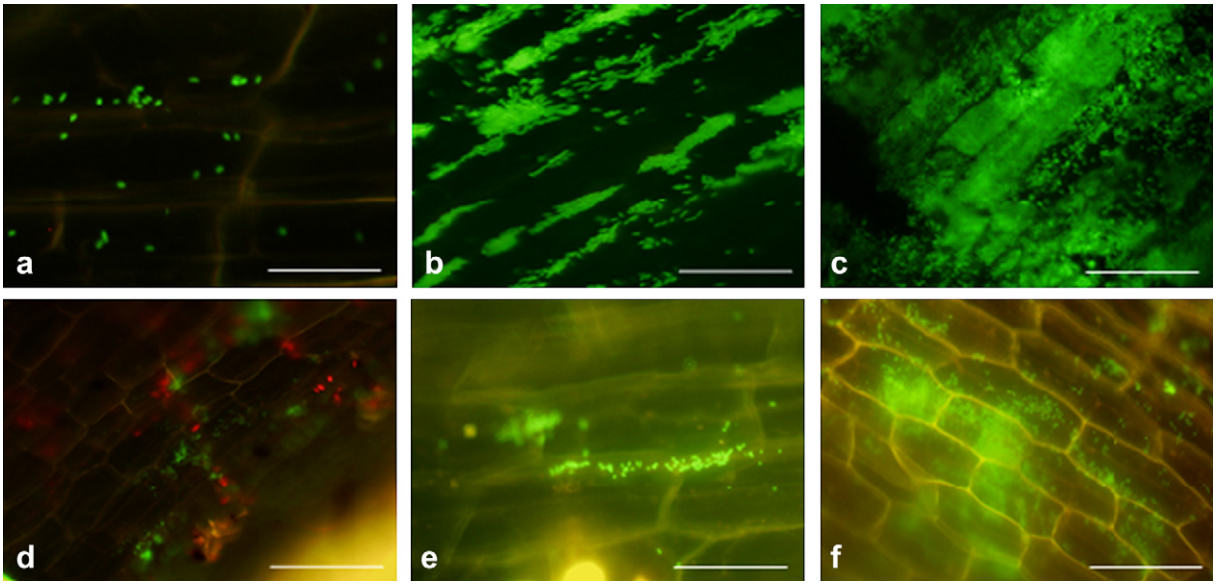


**Fig. 1.** Rhizoplane colonization under gnotobiotic (a–c) or non sterile conditions (d–f) of a beneficial bacteria, *Burkholderia phytofirmans* strain PsJN, tagged with *gfp* showing (a and d) single bacterial cells attached to the root surfaces of grapevine, (b and e) lines of bacteria or (c and f) bacteria colonizing the whole outline of some rhizodermal cells. Scale bars: (a) 15 μm, (b–d) 30 μm, (e) 15 μm and (f) 40 μm. Pictures by S. Compant.

**Table 1**
Known bacterial traits required in rhizosphere, rhizoplane and/or endophytic competence by beneficial bacteria.

| Rhizosphere and rhizoplane competence | Endophytic competence |
|---|---|
| Chemotaxis | Flagella |
| Bacterial growth rate | Nod genes |
| Quorum sensing | Cell-wall degrading enzymes |
| Amino acid synthesis | Detoxification mechanisms |
| Vitamin B1 synthesis | Type IV pili |
| NADH dehydrogenase I | Twitching motility |
| O-antigenic site of LPS | LPS |
| Flagella | |
| Fimbriae | |
| Outer membrane protein | |
| Agglutinin | |
| Type IV pili | |
| Antibiotic secretion | |
| Siderophore production | |
| Site specific recombinase | |

shown to be associated with several plants (Mark et al., 2005 and references therein). A commercial microarray facilitated the analysis of transcriptomic response to root exudates. Interestingly, different genes responded to the exudates of the two cultivars, and regulated genes included genes involved in aromatic compound catabolism, energy generation and amino biosynthesis and metabolism, type III secretion and various hypothetical proteins (Mark et al., 2005). The expression of genes involved in chemotaxis such as *cheY* encoding a two-component response regulator, *cheA* encoding a chemotaxis response regulator, and *pctA* encoding a chemotactic transducer protein, were down-regulated with exudates from one sugarbeet cultivar but were not affected by root exudates of the second cultivar (Mark et al., 2005). This is in contradiction with other data previously reported on gene expression or mutants involved in chemotaxis (de Weert et al., 2002), but could also reflect the physiological response of this strain at a given time point. Generally, chemotaxis is considered to play an important role for successful rhizosphere colonization (Walsh et al., 2001).

Host-bacteria associations can involve specific interactions and recognition processes (Benizri et al., 2001). The composition of exudates depends on the cultivar, the exposure of the plant to stress, the plant growth stage and may also show differences along the root structure resulting in differences in the composition of the various bacterial communities (Haichar et al., 2008). Differences in root exudate composition may also influence the colonization process (Lugtenberg et al., 2001). In addition, some exudates are known to have negative effects on bacterial strains. Differences between attractive or repulsive compounds that affect bacterial colonization (reviewed in Bais et al., 2006) are likely to have an effect on bacterial gene expression.

The root exudation process is known to be heterogeneous in space. Some exuded compounds are more concentrated in some root zones than in others. For instance, in the root collar and root hair zone high exudation occurs in comparison to root distal parts such as tips (Grayston et al., 1996). Due to different exudation patterns, some sites are better colonized by some rhizobacteria (Gamalero et al., 2004), potentially resulting in spatial differences of bacterial colonization. The amount of photosynthates secreted as root exudates varies also with the type of soil and the availability of nutrients (Kraffczyk et al., 1984; Paterson and Sim, 2000). This indicates that at different sites on the root as well as at different developmental stages, distinct rhizobacterial communities may establish and interact with their hosts.

The process of exudation is moreover not an unidirectional flux (Jones et al., 2009). Experiments under hydroponic conditions have revealed that plant roots can take up a range of exuded compounds from the rhizosphere into the roots and transfer them again to shoots (Jones and Darrah, 1994, 1995, 1996), which will also influence the type of root exudates available for root and rhizosphere colonizers.

Phytopathogen infection has been shown to influence root exudation and thereby potentially influences the composition and activity of rhizobacterial populations. Rudrappa et al. (2008) recently demonstrated that a plant may select specific rhizosphere colonizers *via* root exudation when its organs get infected by a plant pathogen. Using the *Arabidopsis thaliana*–*Pseudomonas syringae* pathosystem, the authors found enhanced exudation of malic acid in the rhizosphere after pathogen infection of the plant's leaves. Malic acid attracted a beneficial strain of *Bacillus subtilis*, which then colonized the rhizoplane of the same plant, formed a biofilm and protected roots against further aggression from the phytopathogen (Rudrappa et al., 2008). This study nicely demonstrated the interplay between the plant and different members of the associated microbial community.

In addition to root exudates, some bacteria are known to be attracted by root mucilage (Knee et al., 2001), i.e. hydrated polysaccharides sloughed off from the root tip. For instance, certain plant beneficial *Azospirillum* spp. strains are attracted by the root mucilage produced by maize (Mandimba et al., 1986). On the contrary, it has been reported that root mucilage prevents colonization by *P. fluorescens* strain SBW25 interacting with maize roots (Humphris et al., 2005). Different responses to root mucilage may further explain the spatial and temporal differences of bacterial colonization that are frequently observed along the root system.

### 2.2. Root colonization and biocontrol

Root exudates and mucilage-derived nutrients attract deleterious rhizobacteria as well as beneficial and neutral bacteria, fungi and other soil organisms (Walker et al., 2003). Consequently PGPB have to be highly competitive to successfully colonize the root zone. Secondary metabolites involved in biocontrol, which are known to confer the producing bacteria a selective and competitive advantage against other microorganisms, further contribute to their rhizocompetence and root site colonization (Compant et al., 2005a; Haas and Défago, 2005; Raaijmakers et al., 2008; Lugtenberg and Kamilova, 2009). Siderophores and lytic enzymes secreted by PGPB may reduce the growth of phytopathogens present in the rhizosphere. Moreover, some PGPB secrete antibiotics, which are particularly relevant for rhizosphere and rhizoplane colonization (van Loon and Bakker, 2005). Well known examples include 2, 4-diacetylphloroglucinol (DAPG), hydrogen cyanide, oomycin A, phenazine, pyoluteorin, pyrrolnitrin, thiotropocin, tropolone, as well as many others such as cyclic lipopeptides, rhamnolipids, oligomycin A, kanosamine, zwittermicin A, and xanthobaccin (Trust, 1975; Kintaka et al., 1984; Thomashow and Weller, 1988; Défago, 1993; Maurhofer et al., 1994; Milner et al., 1995, 1996; Kim et al., 1999; Nakayama et al., 1999; Nielsen et al., 2002; Raaijmakers et al., 2002; de Souza et al., 2003). Some bacterial strains may secrete one or more of these metabolites enabling them to better compete with the natural microflora residing in the rhizospheres and the rhizoplanes of plant hosts (Haas and Défago, 2005), further promoting their competitive ability in the root environment. Genome analysis further revealed that some bacteria such as the rhizosphere strains *Bacillus* amyloliquefaciens FZB42 (Chen et al., 2007) or *P. fluorescens* Pf-5 (Paulsen et al., 2005) possess large gene clusters responsible for the secretion of antibiotics and siderophores as well as for detoxification (which is also required during colonization), which explained in part their efficient colonization of the plant hosts.

### 2.3. Other determinants involved in epiphytic colonization

In addition to chemotaxis to exudates and mucilage or biocontrol activities, characteristics like bacterial flagella, quorum sensing as well as the production of specific compounds/enzymes are involved in colonization processes. Flagella allow bacteria to get into contact with exudates and root mucilage components (Turnbull et al., 2001) and chemotaxis driven by flagella may thus play an important role in root colonization. However, in some cases flagella are not required for colonization, as it has been shown for fluorescent *Pseudomonas* and *Serratia* strains and wheat (Scher et al., 1988).

Cell density-dependent quorum sensing is known to regulate many bacterial functions, e.g. antibiotic production, nitrogen fixation as well as many others (Latour et al., 2008). As shown by Soto et al. (2006) quorum sensing can be also involved in the colonization of the rhizosphere and the rhizoplane. Quorum sensing-mediated root colonization was demonstrated with a derivative of *P. fluorescens* 2P24 carrying a mutation in a gene of the *LuxR-LuxI* family (Wei and Zhang, 2006). A *pcoI* mutant of *P. fluorescens* 2P24 showed reduced biocontrol activity against wheat take-all, biofilm formation as well as reduced root colonization (Wei and Zhang, 2006). However, other studies showed that *N*-acyl homoserine lactones (AHLs) mediating quorum sensing in Gram-negative bacteria are not always required for plant colonization. Mutants of *Serratia liquefaciens* or *Serratia plymuthica*, which do not produce AHLs, did not differ in their colonization ability of tomato and oilseed rape roots (Schuhegger et al., 2006; Müller et al., 2009). Similarly, impaired AHL synthesis of *Burkholderia phytofirmans* strain PsJN did not change colonization behaviour on and inside potato roots, although this mutant strain had a major, but cultivar-specific effect on stimulation of plant growth and physiology (Trognitz et al., unpub. results). However, it might be that quorum sensing affects the competitive ability of bacterial strains (e.g. by regulating antibiotic production), which will further influence colonization in the rhizosphere under natural conditions.

The ability of PGPB to synthesize amino acids, vitamin B1 and NADH dehydrogenase I (Simons et al., 1997; Camacho Carvajal et al., 2002), lipopolysaccharides (LPS) (de Weger et al., 1989; Dekkers et al., 1998a) or fimbriae (Vesper, 1987) have been additionally reported to be involved in root colonization. Furthermore, cell-surface proteins such as outer membrane proteins (de Mot et al., 1992) and agglutinin (Anderson et al., 1988), type IV pili (Dörr et al., 1998) as well as a site-specific recombinase involved in phase variation (Dekkers et al., 1998b) might be involved in the colonization process. In conclusion, the published information so far indicates that PGPB may employ an array of distinct mechanisms, either alone or in combination, to successfully colonize the root system.

## 3. Endophytic colonization

Several bacteria deriving from the rhizosphere do not only colonize the rhizosphere and/or the rhizoplane but can also enter plants and colonize internal tissues and many of them have shown plant growth-promoting effects (Hallmann, 2001; Compant et al., 2005b, 2008a; Sessitsch et al., 2004; Hallmann and Berg, 2007). As early as 1887, Victor Galippe postulated that soil microorganisms can penetrate tissues of healthy plants and that the involved colonization mechanisms needed to be investigated (Galippe, 1887). These early findings were, however, dismissed due to the general belief that microorganisms detected inside plants represent contaminants obtained during the isolation process (Smith, 1911). Several recent studies confirm that plants host diverse endophytic communities (Idris et al., 2004; Krechel et al., 2004; Berg et al., 2005b) and that endophytic bacteria mostly derive from the rhizosphere (Sessitsch et al., 2002; Compant et al., 2005a; Hardoim et al., 2008). Endophytes represent a subgroup of the rhizobacterial communities, which have the ability to enter the endorhiza (the root interior) of their hosts once the rhizoplane is colonized (reviewed in Gray and Smith, 2005; Rosenblueth and Martínez-Romero, 2006; Hallmann and Berg, 2007). In general, endophytes are more likely to show plant growth-promoting effects than bacteria exclusively colonizing the rhizosphere (Conn et al., 1997; Chanway et al., 2000).

### 3.1. Root endophytic colonization

Following rhizosphere and rhizoplane colonization, some soil-borne microorganisms can enter roots, and establish subpopulations ranging from $10^5$–$10^7$ CFU $g^{-1}$ FW (Hallmann, 2001). This involves specific traits required for endophytic competence (Table 1), *i.e.* the ability to successfully colonize the plant host. The penetration process does not necessarily involve active mechanisms and thus all rhizosphere bacteria can be expected to be endophytic at one stage of their life (Hardoim et al., 2008). Passive penetration can take place at cracks, such as those occurring at root emergence sites or created by deleterious microorganisms, as well as by root tips (Fig. 2; Reinhold-Hurek and Hurek, 1998). For certain bacteria specific adaptations have evolved, such as for nodulating bacteria or microbes, which have specific mechanisms for active penetration of the root system (reviewed in Hardoim et al., 2008). In some plant–rhizobia interactions such as in the symbiosis between the semi-aquatic legume *Sesbania rostrata* and *Azorhizobium caulinodans* (Goormachtig et al., 2004), invasion can happen through fissures at the lateral root base and by cortical, intercellular crack entry. For other rhizobia nodulating legumes, colonization occurs in the interior of hairy roots before infection threads are formed, they penetrate root tissues and subsequently specialized organs are developed by the plant, known as nodules (reviewed by Garg and Geetanjali, 2007). This specific phenomenon, currently known to be mediated by chemotaxis towards flavonoid exudates as well as by microbial signals such as nod factors, is required for the symbiotic lifestyle of nodule-forming bacteria. Sequenced genomes of nodule-forming bacteria such as *A. caulinodans* ORS 571, *Bradyrhizobium japonicum* USDA110 (Kaneko et al., 2002), *Burkholderia phymatum* strain STM815, *Cupriavidus taiwanensis* strain R1 (Amadou et al., 2008) and *Frankia* spp. strain CcI3 (Normand et al., 2007) have moreover confirmed the role of these *nod* genes in nodulation. Endophytic colonization apart from this specialized and frequently studied interaction between nodulating bacteria and legumes is less well understood.

Lipopolysaccharides, flagella, pili, and twitching motility (Duijff et al., 1997; Dörr et al., 1998; Böhm et al., 2007) have been shown to affect endophytic colonization and bacterial mobility within host plants. In addition, the secretion of cell-wall degrading enzymes (CWDEs) is involved in bacterial penetration (reviewed in Lodewyckx et al., 2002; Fig. 2) and spreading within the plant (see below). The latter function has been confirmed by genome analysis of the non-nodulating endophyte *Azoarcus* sp. BH72 (Krause et al., 2006) possessing genes encoding CWDEs such as cellulases and polygalactorunases. Within the genome of the endophyte *Klebsiella pneumoniae* Kp342, which colonizes maize, alfalfa as well as many other crops, it has been demonstrated moreover that several additional genes are important for colonization (Fouts et al., 2008). These not only include genes involved in chemotaxis, the formation of flagella and pili but also various metabolic pathways and transport systems. In particular, those enabling the recognition and catabolism of plant compounds such as the uptake and degradation of plant-derived polysaccharides have been described to be

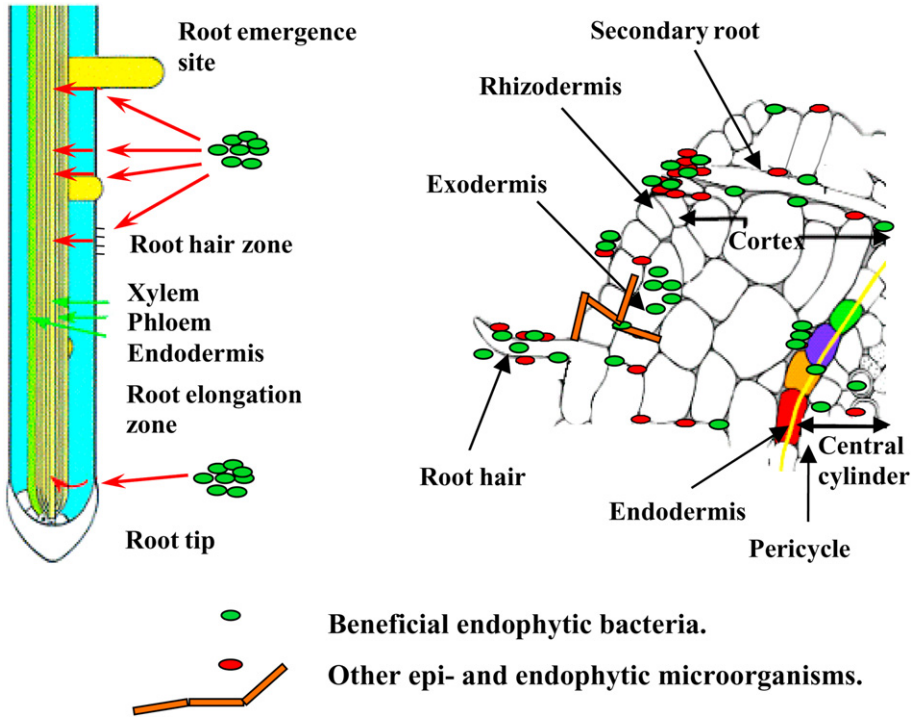


**Fig. 2.** Sites of plant colonization by endophytic bacteria. Drawing modified from Reinhold-Hurek and Hurek (1998) and Compant (2007).

involved in this process (Fouts et al., 2008). Recent work by Rasche et al. (2009) further showed that endophytes have the capacity to metabolize photosynthetic plant products.

Active or passive mechanisms have been reported for translocation processes of endophytic bacteria inside their plant hosts allowing them to progress from the rhizoplane to the cortex of the root system. Once a bacterium reaches the root cortical zone, a barrier such as the endodermis can block further colonization as only few bacteria are able to pass through the endodermis (Gregory, 2006). It is likely that endophytes able to pass through the endodermis can secrete CWDEs allowing them to continue colonization inside the endorhiza (James et al., 2002). Alternatively, some bacteria may passively enter as a portion of this endodermal cell layer is often disrupted, such as during the growth of secondary roots, which derive from the pericycle, situated just below the endodermis barrier (Gregory, 2006). Under natural conditions, some deleterious bacteria can moreover disrupt the endodermis, allowing endophytic bacteria at the same time to pass into the central cylinder (Fig. 2). After passing through the endodermis barrier, endophytic bacteria have to penetrate the pericycle to further reach the root xylem vessels of their hosts (Figs. 2 and 3). This has been shown for example for *Herbaspirillum seropedicae* Z67 in rice (James et al., 2002), for *B. phytofirmans* strain PsJN in grapevine (Compant et al., 2005b, 2008a), and will be the case for many additional endophytic bacteria. Even if some endophytes can colonize root internal tissues and continue their progression until the root xylem vessels, active penetration of the endophytes is known to induce defence mechanisms of the host plants (reviewed in Rosenblueth and Martínez-Romero, 2006). Different defence reactions have been often described during plant–endophyte interactions. Strengthening of cell walls, establishment of surrounding material inside the cortex or xylem as well as gum formation inside vessels have been observed (James et al., 2002; Compant et al., 2005b; Miché et al., 2006). However, in constrast to the plant response to phytopathogens only few defence responses have been described in plant response to endophytes. These differences can be probably explained by the secretion of different compounds or by the amount of secreted metabolites, which may be very low in the case of endophytes (discussed in James et al., 2002). However, it has been also reported that plants may show defence reactions controlling endophytic colonization (Iniguez et al., 2005). Dicotyledonous plants are known to use salicylic acid (SA) and ethylene as signalling molecules, which control colonization by some endophytes, as demonstrated under laboratory conditions (Iniguez et al., 2005). By contrast, in monocotyledonous plants such as rice, the addition of jasmonic acid (JA) but not ethylene was shown to interfere with the colonization of the diazotroph *Azoarcus* sp., suggesting that plant defence responses involving the JA signalling pathway might also control endophytic colonization inside the root system (Miché et al., 2006). However, in a compatible endophytic association, JA-associated plant responses were less pronounced and did not restrict endophytic colonization (Miché et al., 2006).

### *3.2. Bacterial colonization of xylem vessels and endophytic translocation to vegetative plant parts*

Although not frequently investigated it is well known that endophytes may spread systemically inside the plant and colonize stems and leaves (Hardoim et al., 2008), where their cultivable population densities may reach $10^3$–$10^4$ CFU $g^{-1}$ of fresh weight under natural conditions (reviewed in Hallmann, 2001). It is not clear, whether endophytes colonizing roots or above ground plant tissues have different effects on the plant or whether root colonization is sufficient for conferring beneficial effects.

Some endophytes colonize nutrient-rich intercellular spaces of plant hosts using them to spread inside host plants (Cavalcante and Dobereiner, 1988; Dong et al., 1994). Some systemic bacterial colonizers can also use the lumen of xylem vessels to spread throughout the plant (James et al., 2001; Compant et al., 2005b, 2008a). Lumen colonization of xylem vessels has been however more frequently reported as a route of spreading of endophytic

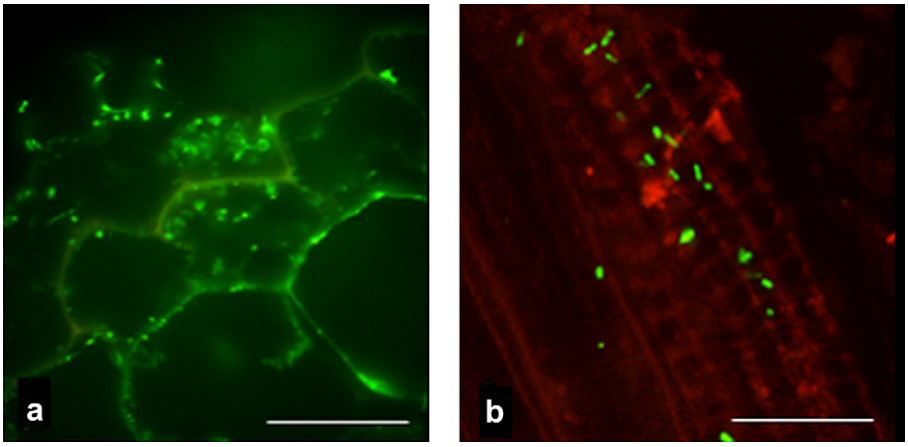


**Fig. 3.** Endophytic colonization of a plant growth-promoting bacterium (*Burkholderia phytofirmans* PsJN) observed *via* fluorescence *in situ* hybridization (a) or with *gfp* tagging (b) showing bacterial cells as green rods inside cortex (a) or xylem vessels (b) with green fluorescence. Scale bars: (a) 25 μm, and (b) 10 μm. Pictures by S. Compant. Picture b from Compant et al. (2008a) with permission.

bacteria to reach vegetative plant parts, probably because they are open conduits, whereas migration along intercellular spaces requires the secretion of active CWDEs. Although at the beginning of the 1990's it was strongly argued, that lumen xylem colonization is a property of phytopathogens (McCully, 2001), it is nowadays known that non-phytopathogenic endophytes can spread inside plants in the same manner (Fig. 4). Beneficial bacteria can pass from one xylem element to another using the perforated plates (Fig. 4). The size of the plate holes allows the passage of bacteria without requiring the activity of CWDEs (Bartz, 2005). Bacterial flagella and/or the plant transpiration stream seem to further support their movements inside plants (James et al., 2002; Compant et al., 2005b). However, only few endophytes are able to colonize aerial vegetative plants parts (Hallmann, 2001) as they have to pass over several barriers as well as need to possess the physiological requirements to establish in different plant niches. Those migrating to the above ground parts are thus well adapted to this particular endophytic environment.

### 3.3. Endophytic colonization of flowers, fruits and seeds

A few studies reported that some endophytic bacteria colonize flowers, fruits and seeds (reviewed in Hallmann, 2001). However, under natural conditions the majority of flowers and fruits does not contain endophytic bacteria at all or only very low densities (Hallmann, 2001), reaching population densities up to $10^2$–$10^3$ CFU $g^{-1}$ of fresh weight (S. Compant, unpub. results). It is likely that only specialized endophytic strains are able to colonize and survive in reproductive plant organs. Mundt and Hinkle (1976) as well as Misaghi and Donndelinger (1990) detected endophytic colonizers inside ovaries and fruits of some plants. Moreover, some strains belonging to *Pseudomonas* and/or *Bacillus* as well as to other genera, which also show plant growth promoting abilities, were observed and isolated from the interior of flowers, fruits and seeds of grapevine (Compant et al., unpub. results; Fig. 5). Few species were isolated from sterilized rice seeds (Okunishi et al., 2005). Strains belonging to *Pseudomonas* and *Rahnella* genera were additionally isolated from Norway spruce (Cankar et al., 2005) as well as from yellow lupine seeds (Barac et al., 2004), and from some other plants, providing some information on the type of microorganisms colonizing plant reproductive organs. However, their ecology, functioning and their origin have been poorly investigated.

The migration of the endophyte *B. phytofirmans* strain PsJN from the rhizosphere to inflorescence tissues of grapevine was studied to explain colonization of endophytes throughout the plant including reproductive organs. This strain, known for its plant growth-promoting effects on potato, tomato, grapevine, chickpea, barley and other plants (Sessitsch et al., 2005) has been described to colonize upper grapevine organs, especially berries of cv. Chardonnay following soil inoculation (Compant et al., 2008a). Although only a low density of bacterial cells has been detected inside fruits, these experiments indicated that some PGPB can be translocated (via xylem colonization; Fig. 4; Compant et al., 2008a). These reports moreover demonstrated that some endophytes colonizing infructescences may derive from the rhizosphere, and provided information about the putative niche of a microbial inoculant derived from the soil. Furthermore, field studies have been performed and showed, that some strains isolated from plant reproductive organs are the same as those reported as colonizing the rhizosphere and the endorhiza, especially in grapevine (S. Compant, unpub. results). This indicates that even under natural conditions some rhizosphere colonizers can spread inside the plant and finally colonize inflo/infructescence tissues.

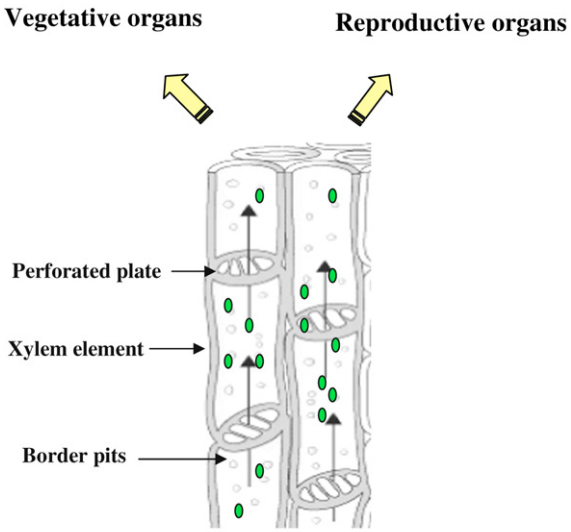


**Fig. 4.** Bacterial spread inside xylem vessels in aerial plant parts. Arrows show the colonization process. Drawing of xylem vessels modified from The American Heritage® Student Science Dictionary (2002).

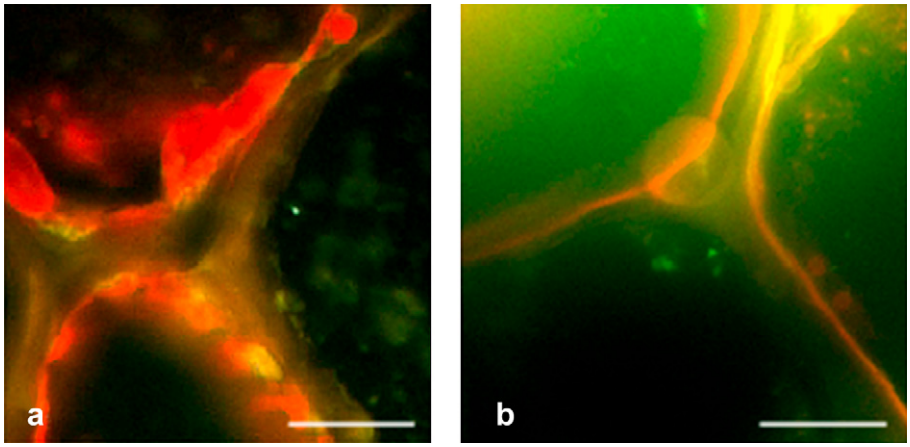


**Fig. 5.** Bacterial presence inside cells of berries of grapevine plants visualized by fluorescence *in situ* hybridization (a and b) showing green fluorescent bacterial cells. Scale bars: (a) 20 μm, and (b) 15 μm. Pictures by S. Compant.

Limited knowledge exists on the function of endophytes colonizing flowers, fruits and seeds. Recently, Mastretta et al. (2009) reported that some endophytic bacteria, isolated from seeds of *Nicotiana tabacum* grown in the field under metal stress, induced plant growth promotion as well as reduced cadmium phytotoxicity during the early growth of new plants. van Oevelen et al. (2003) suggested that an endophyte, a *Burkholderia* sp. strain obtained from *Psychotria* sp. (belonging to the Rubiaceae) can be transmitted from seed to seed, as plants without the endosymbiotic *Burkholderia* sp. strain could not grow and eventually died. This suggests that a plant may support seed colonization of endophytes with specific functions. Considering the observation that reproductive organs contain (if at all) low numbers of endophyte cells it seems that these low numbers might be sufficient for further vertical transmission. Once established in the new plant, cell densities may increase and be involved in plant growth promotion. However, this needs to be confirmed. It is moreover unknown if all seed endophytes can be successfully transmitted to the next plant generation or whether they all derive from the soil/rhizosphere environment.

### 3.4. Other sources of endophytic bacteria

For a long time, any other source of beneficial endophytes than soil has been questioned. Using cultivation-based approaches, some endophytes have been isolated exclusively from above ground plant parts, but were not found in the rhizosphere, the rhizoplane or inside roots of potato (Berg et al., 2005b) or grapevine (S. Compant, unpub. results). This has been confirmed using cultivation-independent techniques, which detected some strains in aerial plant parts but not in soil or inside roots (Berg et al., 2005b). Alternative sources might be the caulosphere for stem endophytes, the phyllosphere for leaf endophytes, the anthosphere for the ones residing inside flowers as well as the carposphere for those colonizing fruits.

Following phyllosphere inoculation of grapevine plants cv. Chardonnay with *B. phytofirmans* strain PsJN, bacteria were found inside leaf internal tissues using *in vitro* grown plantlets (Compant et al., unpublished results). Beattie and Lindow (1995) assumed that only phytopathogenic bacteria can colonize leaves endophytically following phyllosphere inoculation. This was elaborated after testing both, pathogenic and beneficial bacterial strains (Beattie and Lindow, 1995). However, contrasting results have been reported as well. For example, inoculation of sugarcane leaves with a *Gluconacetobacter diazotrophicus* strain resulted in successful colonization of leaf xylem vessels (James et al., 2001), although this bacterium is well known to systemically spread inside the plant following soil or rhizosphere inoculation (James et al., 2001). It is thus possible that endophytic bacteria may also colonize leaves endophytically following phyllosphere inoculation, but data on survival or further transmission to other tissues of endophytes entering from the phyllosphere are lacking.

Although experimental evidence is missing, the anthosphere or carposphere might represent potential sources of endophytes. It can be assumed that flowers or fruits with small injuries allow the entry of some endophytes. This would explain the colonization of some strains in inflo/infructescences but their absence in roots and soils. Another explanation might be the presence of viable but not cultivable bacteria (VBNC) in some tissues. This has been reported for instance for *Azoarcus* sp. BH72 colonizing grasses, which has been shown to fix nitrogen in an unculturable state following soil inoculation (Hurek et al., 2002).

## 4. Conclusions and future prospects

Many plant-associated bacteria are well known for their capacity to confer plant growth promotion and to increase resistance towards various diseases as well as abiotic stresses. Nevertheless, they often fail to confer these beneficial effects when applied in the field, which is often due to insufficient rhizo- and/or endosphere colonization. The lack of various characteristics, which are important for efficient colonization of the plant environment and which have been outlined in this review, could explain poor plant host colonization by rhizosphere and endophytic bacteria. A better understanding on how beneficial bacteria colonize different plant niches will not only result in increased knowledge on plant–microbe interactions but will also lead to a more successful and reliable use of bacterial inoculants.

Several issues require further research. For example, some rhizosphere-restricted bacteria as well as some endophytes have been detected on and/or inside plants as VBNC cells (Hurek et al., 2002; Gamalero et al., 2004, 2005), but it is currently unknown why cells switch to this state. Switching to a VBNC state on and inside plants might indicate that bacteria experience stress during host colonization, but this phenomenon is still poorly understood. Additionally, most rhizo- and endophytic bacteria cannot be cultivated, either because they enter a VBNC state or because their cultivation conditions are unknown. Little information is available on the functional activities or the effects on plant performance of uncultivated bacteria. Metagenomic approaches and other cultivation-independent techniques might in the future reveal more information on not yet cultivated microorganisms.

Research performed so far has been mostly related to plant growth promotion and/or to rhizosphere or root endophytic colonization. Although novel root colonizers are being detected (e.g. Andreote et al., 2009), the functioning and contribution to plant growth of endophytes localized in aerial parts is rather poorly understood. Correlation between colonization and beneficial effects as well as genomic comparison of bacteria colonizing different plant tissues will help to better understand the role of these endophytes. Of particular interest are endophytes that colonize fruits as well as seeds (inside fruits), as they are likely to (i) show specific functions, (ii) important for plant health and growth, and are (iii) vertically transmitted.

An issue of concern, which has not been addressed in this review, is that plants may host various human pathogens (Berg et al., 2005a; Allerberger and Sessitsch, 2009). It has been reported that some of them can even exhibit plant growth-promoting effects or may improve plant health and colonize the rhizosphere as well as plant internal tissues (Berg et al., 2005a). However, we are at the onset of understanding the differences between plant colonizers and clinical isolates. A recent paper on *Stenotrophomonas maltophilia*, a species known for clinical infection as well as for plant colonization (Ryan et al., 2009), revealed some information on differences, which evolved among isolates derived from the different niches. Some genomic islands typically found in clinical strains are absent in endophytic strains, on the other hand some endophytes and clinical isolates show extremely high homology. Genome plasticity could explain differences between isolates possibly allowing adaptation to a different environment (Ryan et al., 2009). However, some genes known to be involved in pathogenicity were found in strains obtained from both, the plant and the clinical environment (Ryan et al., 2009). Furthermore, the mechanisms responsible for the colonization of plants and for the antagonistic activity of *S. maltophilia* strains against plant pathogens might be similar to those that are responsible for the colonization of human tissues and for pathogenicity. This knowledge necessitates careful selection of inoculant strains to be applied and released into the environment (Berg et al., 2005a). Similarly, Burkholderiales such as *Burkholderia* spp. (Compant et al., 2008b) and *Enterobacteriaceae* that can be potent pathogens and which use the plant as an internal reservoir (Holden et al., 2009). By investigating further the genetic differences between plant and animal/human colonizers it might be soon possible to better predict the pathogenicity potential of microbial inoculants.

Further analysis of sequenced genomes, the characterization of yet unknown genes and the identification of genes expressed during colonization will help to improve our understanding on the colonization process and the interaction of beneficial microbes with plants. Matilla et al. (2007) showed by using microarrays that two selective forces of different nature are important for beneficial bacteria to colonize the rhizosphere: stress adaptation and the availability of particular nutrients, as demonstrated with maize rhizosphere and a *Pseudomonas putida* strain. New bacterial traits conferring strain survival in this niche have been found and opened a way to better understand specific signalling and regulatory processes governing the plant-beneficial bacteria association (Matilla et al., 2007). It will not be surprising if some new factors, functions, as well as genes required for rhizosphere and endophytic lifestyle of such microorganisms will be identified in the near future. However, we will need to separate common traits required for colonization and specific factors involved during the interaction, as differences in specific adaptation and recognition might be involved in different plant-bacteria associations. Generally, a more comprehensive understanding of plant colonization by bacteria has to be developed in order to better predict how bacteria interact with plants and whether they are likely to establish themselves in the plant environment after field application as biofertilisers or biocontrol agents.

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