3 Sources, Niches and Routes of Colonization by Beneficial Bacterial Endophytes

Stéphane Compant[1]* and Corinne Vacher[2]

[1]*Bioresources Unit, Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH, Tulln, Austria;* [2]*BIOGECO, INRA, University of Bordeaux, Pessac, France*

Abstract

The plant individual is a holobiont as it hosts diverse microbial assemblages in and on vegetative, reproductive or disseminative organs. All plant compartments – roots, stems, leaves, flowers, fruits and seeds – have been shown to host microorganisms that can influence positively or negatively the plant performance. Some of these microorganisms thrive as endophytes inside plant tissues. Identifying the environmental sources of these microorganisms and the route they take to colonize plant tissues, visualizing their niches within their hosts and understanding how they make intimate associations with plants are of crucial importance to develop biocontrol and biofertilization approaches, both in organic and integrated protection systems. This chapter considers that the plant individual is part of a complex network of biotic interactions influenced by the environment in the phytobiome and provides a comprehensive review on the development of the interactions between plants and beneficial bacterial endophytes.

3.1 Introduction

Since the 19th century and the discovery of endophytes, i.e. microbes living inside plants, efforts have been made to identify which microbial taxa and assemblages inhabit plant tissues, depending on plant species and environmental conditions (Hardoim *et al.*, 2015). How the tissues are colonized, how the microorganisms are acquired from the environment and how they are transmitted from one generation to the next, and what influence they have on their hosts have been further revealed during the last decades (Compant *et al.*, 2010; Brader *et al.*, 2017; Kandel *et al.*, 2017). While some colonizers are known as pathogenic, others have been acknowledged as mutualists (Lemanceau *et al.*, 2017). The latter are of special interest for agriculture as they can improve agroecosystem health and productivity by alleviating abiotic stresses, reducing pathogen attacks and stimulating plant growth. A thorough understanding of the sources, niches and colonization routes of beneficial bacterial endophytes is required, however, for their successful application on crops and plantation forests (Turner *et al.*, 2013).

Most research performed so far on beneficial bacterial endophytes has focused on bacteria deriving from the rhizosphere and colonizing root tissues. However, other microenvironments on the plant surfaces, such as the anthosphere, carposphere, spermosphere, phyllosphere, calusphere, caulosphere or laimosphere, can also host beneficial microorganisms and constitute entry points toward internal plant tissues (Compant *et al.*, 2011,

---

* Corresponding author e-mail: stephane.compant@ait.ac.at

2016; Vacher *et al*., 2016; Lemanceau *et al*., 2017; Nelson *et al*., 2018). As plants interact both with their abiotic environment and complex communities of organisms (forming the phytobiome; Beans, 2017; Leach *et al*., 2017), beneficial endophytes can also come from further ways, such as neighbouring plants, and be transmitted by wind or animal vectors (Vacher *et al*., 2016). In this chapter, we first review the knowledge on below ground sources of colonization. We describe how the soil bacteria can reach the internal root tissues, and then eventually the above ground plant organs. We then review the knowledge on the above ground sources of colonization, which have been less studied so far. The colonization through natural openings in above ground vegetative organs and the role played by insect vectors are discussed. Finally, a focus on the colonization of reproductive and disseminative organs (flowers, fruits and seeds) and the possibility of transmission of beneficial bacteria to the plant offspring is provided.

3.2 Below ground Colonization Routes

3.2.1 From soil to inside roots

The soil is the main reservoir for bacterial endophytes. Many bacterial endophytes originate from the rhizosphere, the micro-environment surrounding roots, and are influenced by the presence of root exudates, rhizodeposits and microorganisms (Mendes *et al*., 2013). Up to 40% of the photosynthates produced by the plant can be released in the rhizosphere, thereby attracting many microorganisms from the surrounding bulk soil (Lugtenberg and Kamilova, 2009). Some bacteria can further colonize specific zones of the root surfaces (i.e. the rhizoplane) as single or several cells (Fig. 3.1A–C) or by forming biofilms (Benizri *et al*., 2001). Advanced visualization techniques revealed that strains can form lines along the grooves between root cells, multiply on them and then enter plant root tissues between two rhizodermal cells (Compant *et al*., 2010; Fig. 3.1A–C). It has further been shown that *Pseudomonas fluorescens* 95rkG5 can colonize tomato roots

in the root elongation zone (as single or dividing cells), in the root hair zone (as single or clustered cells) and in the collar zone, but not in the root tip zone (Gamalero *et al*., 2005). Other bacteria are able to colonize root tips before establishing subcommunities inside root tissues (Brader *et al*., 2017), as recently demonstrated for a strain isolated from grapevine plants and re-inoculated on *in vitro* plantlets (Lòpez-Fernàndez *et al*., 2016).

Bacterial endophytes can actively or passively penetrate root tissues, depending on the strain and the root zone being colonized (James *et al*., 2001; Mercado-Blanco, 2015). Several bacterial traits can favour endophytism, such as pili, flagella, fimbriae, nod factors, quorum sensing, cell-wall-degrading enzymes, twitching motility, lipopolysaccharide, in addition to several traits required for rhizosphere competence (Compant *et al*., 2010). Interestingly, some (non-nodulating) bacteria have been visualized colonizing the interior of root hairs. For instance, *P. fluorescens* PICF7 and *Pseudomonas putida* PICP2 were observed inside root hairs of olive trees (Prieto *et al*., 2011). Bacteria were detected as either single cells or clusters (colonies) attached to inner membrane structures of a few root hairs, before they reached the cortical cell layers (Prieto *et al*., 2011). Once inside the rhizodermis, some bacteria colonize intercellular spaces while a few can be intracellular inside the cortical cell layers. James *et al*. (2002) showed, for instance, that a strain of *Herbaspirillum seropedicae* entered the roots through cracks at the point of lateral root emergence of rice plantlets. This strain subsequently colonized the root intercellular spaces, aerenchyma and cortical cells, and a few cells penetrated the stele and entered into the xylem vessels.

3.2.2 From roots to above ground organs

Some bacteria can further progress within root tissues. Some of them remain in the root cortex, while others can reach the endodermis barrier and pass to the central cylinder up to the xylem vessels (Compant *et al*., 2010; Fig. 3.1D). Once inside xylem vessels, some bacteria can pass from one element to the other (Fig. 3.1D) through the perforated
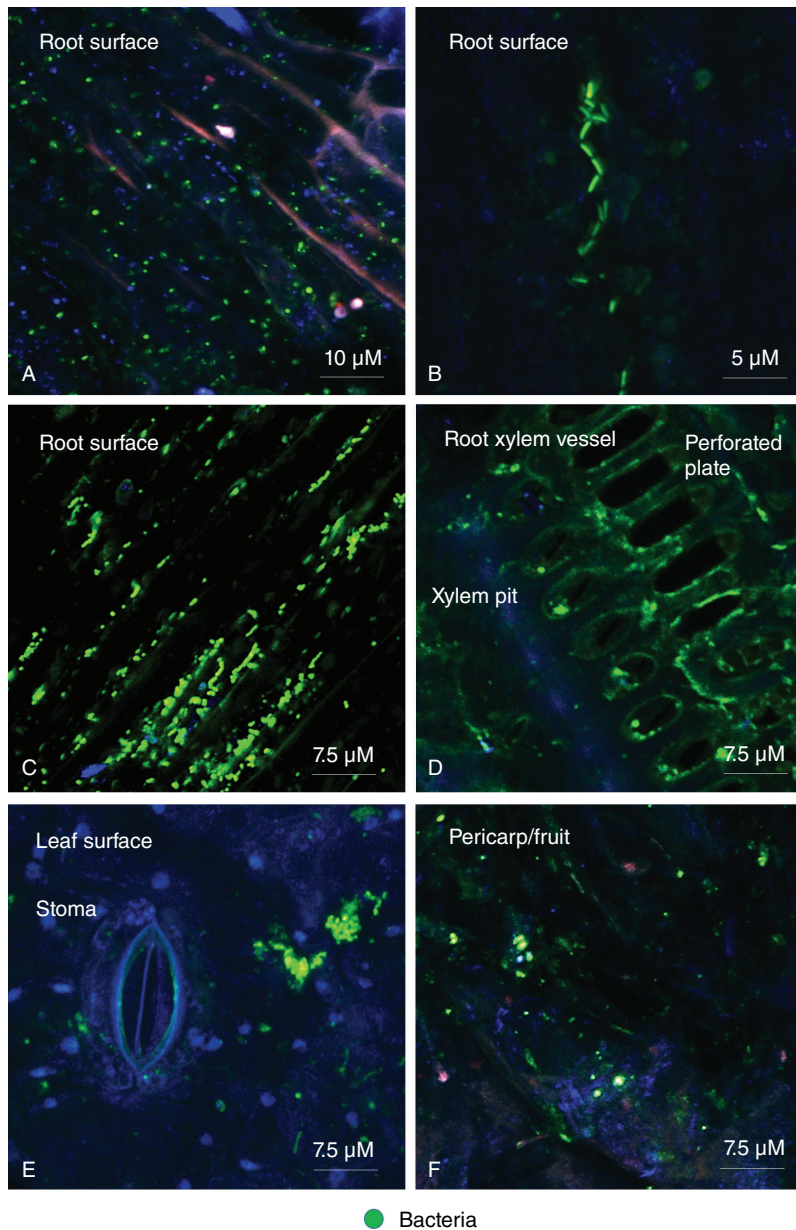
● Bacteria

Fig. 3.1. Confocal microscopy photographs of bacteria (green fluorescent stained with Syto9®) colonizing root surfaces of a plant (A–B), or being inside root tissues (D), on leaf surfaces (E) and inner fruit tissues (F) of grapevine natural microbial communities (A–B, D–F) or salad inoculated with a beneficial strain (C).

plates of the xylem vessels, thus coloniz-ing the plant systemically (Compant *et al.*, 2005, 2008). For instance, *Paraburkholderia phytofirmans* strain PsJN was visualized from roots up to the infructescence tissues of grapevine plants, after being inoculated into the soil (Compant *et al.*, 2008). In rice, *Azorhizobium caulinodans* ORS571 has been observed colonizing rice from the roots up to some leaves (Chi *et al.*, 2005). In poplar, *Pseudomonas* sp. PopHV6 was found in cut-tings 10 weeks after cutting inoculation

(Germaine *et al*., 2004). However, not all bacteria are able to migrate from below ground to above ground plant organs. A strain of *Pantoea agglomerans* 33.1 tagged with the *gfp* was not recovered from inside leaf tissues after seedlings inoculation but was found inside root and stem tissues of *Eucalyptus grandis* and the hybrid *E. grandis* × *E. globulus* (Ferreira *et al*., 2008).

It is not yet clear why some bacteria are attracted by xylem vessels. However, an interesting study by Malfanova *et al*. (2013) demonstrated that some sugar compounds, especially l-arabinose, were present as traces inside xylem vessels of cucumber plants. These sugars could enable survival inside xylem of some endophytes such as *Pseudomonas* spp. strains. The time for bacterial colonization of above ground plant organs, e.g. weeks or months (James *et al*., 2002; Compant *et al*., 2005), suggests that the perforated plates slow down the spread of the endophytes. Nevertheless, some bacteria finally reach substomatal chambers in leaves, as they are close to xylem vessels inside leaf tissues (Compant *et al*., 2010). For instance, *P. phytofirmans* strain PsJN has been observed inside substomatal chambers of grapevine leaves after dissemination inside the plant (Compant *et al*., 2005). The cells even exited from stomata to attack the fungal pathogen *Botrytis cinerea* growing on leaves (Miotto-Vilanova *et al*., 2016). These observations suggest that the plant can select endophytes to improve its resistance to pathogen attacks (Berg, 2009). Other bacteria have been observed in substomatal chambers, such as the strain Z67 of *H. seropedicae* (James *et al*., 2001, 2002). However, in this case, the possibility of a bacterial colonization from the phylloplane was not excluded (James *et al*., 2001, 2002).

3.3 Above ground Colonization Routes

3.3.1 Colonization through stomata, other natural openings and wounds

Diversified bacterial assemblages thrive on the surface of the plant aerial organs. A multitude of bacteria colonize the stem external environment and its surface, the caulosphere, the one from the bud, the calusphere, and the leaf one, the phyllosphere (Vorholt, 2012; Vacher *et al*., 2016). These above ground bacteria are less studied than those of the rhizosphere and have long been neglected in plant ecology, despite substantial evidence of the link between phyllosphere microbial communities and crucial functional traits such as photosynthetic strategy, hydraulics, reproduction or defence (Rosado *et al*., 2018). For example, bacteria on the leaf surface can alter cuticular permeability and thus plant water loss through transpiration. They can also protect the plant against pathogens by competing with them or by priming the plant immune system (Remus-Emsermann and Schlechter, 2018). Phyllosphere microorganisms can be deposited on the leaf surface by wind, rainwater and irrigation water, or insects or they can colonize the flushing leaf after overwintering on twigs or in buds (Vacher *et al*., 2016). Those that survive the selection exerted by the leaf microclimate and foliar traits can multiply and enter the tissues via stomata, hydathodes or wounds (Vorholt, 2012; Vacher *et al*., 2016; Fig. 3.1E). Caulosphere bacteria can colonize stem internal tissues through lenticels, or stomata present on photosynthetic stems (Hardoim *et al*., 2015; Brader *et al*., 2017). Recently, a FISH analysis showed the aggregation of *Methylobacterium* PA1 cells in the substomatal chambers of *Arabidopsis thaliana*, after a phase of colonization of the phyllosphere (Peredo and Simmons, 2018). Another example of beneficial bacteria able to colonize the plant internal tissues through stomata is the nodulating bacteria belonging to the genus *Paraburkholderia*. These bacteria induce the formation of leaf nodules in about 450 dicotyledonous plant species, mostly growing in the tropics. They do not fix nitrogen but produce secondary metabolites that protect plants from herbivory. These endophytic bacteria are obligate symbionts and are maintained in a mucilage layer in buds and colonize young leaves through stomata. They are then transmitted to the plant offspring by colonizing inflorescences and then seeds (Pinto-Carbó *et al*., 2018).

The ability to colonize the leaf internal tissues from the leaf surface is restricted to

a few bacteria. Not all strains of non-pathogenic bacterial species can thrive as endophytes inside leaf internal tissues (Wilson *et al*., 1999; Sabaratnam and Beattie, 2003). Moreover, the penetration of bacteria into leaf internal tissues through stomata is a process regulated by both the plant and the environment. Stomata can close when pathogenic bacteria are recognized by the plant, but this mechanism of plant defence is less effective when the relative humidity is high (Panchal and Melotto, 2018). Water on the leaf surface is indeed a key factor of the colonization process, since it influences both leaf physiology and the development of phyllosphere microorganisms. Water promotes the diffusion of nutrients to the leaf surface, through the cuticle, and can form films that create a connection between the leaf surface and the substomatal chambers (Vacher *et al*., 2016; Dawson and Goldsmith, 2018). Heat can also influence the efficiency of colonization. It has been shown to reduce the number of leaf nodules in tropical plant species (Pinto-Carbó *et al*., 2018). Symbiosis establishment should be considered therefore as a tripartite interaction between the plant, the endophytic bacterium and the abiotic environment.

3.3.2 Introduction by animal vectors

Endophytic bacteria can also be introduced into the internal tissues of above ground plant organs by insect herbivores. For example, the leafhopper *Scaphoideus titanus*, which feeds on phloem sap in vines, is known to transmit the phytoplasma responsible for flavescence dorée. It has recently been shown that it can also transmit endophytic bacteria from one plant to the other by feeding on stems. The endophytic bacteria are then able to spread from the stems to the roots (Lòpez-Fernàndez *et al*., 2017). Similar results were obtained for the phloem-sucking insect *Hyalesthes obsoletus*. This insect species carries a bacterial species capable of colonizing the phloem and reducing symptoms caused by phytoplasmas. Interestingly, this protective bacterium can be effectively introduced into vine plants by spraying the leaves with a bacterial culture, confirming that the leaves are

a possible entry point for endophytic bacteria (Iasur-Kruh *et al*., 2018). Interestingly, the bacterium *Propionibacterium acnes* type Zappa has been observed further colonizing the bark, the pith, and xylem vessels of several grapevine plants using FISH microscopy. Its role is unknown, but it has established a subpopulation in grapevine since the neolith period and has diverged from human-associated populations of *P. acnes*, suggesting that not only insects but also other animals (including humans) can introduce endophytes inside plants (Campisano *et al*., 2014).

3.3.3 Transmission from plants to plants

Plant individuals are usually not isolated. They live in association with other plant individuals, belonging or not to the same species. Neighbouring plants can be a reservoir of plant pathogens, but also a reservoir of beneficial endophytes. Samad *et al*. (2017) showed, for instance, that similar microbial taxa were associated to with vines and weeds growing in the same vineyard and that some taxa had beneficial properties such as auxin, siderophore and HCN production and also some properties that would be of interest for biocontrol approaches. More information is currently needed, however, on the possibility for direct and indirect (through vectors) transmission of endophytic bacteria from one plant to its neighbour and on the influence of phylogenetic relatedness between plant species on the rate of transmission.

3.4 Colonization of Reproductive and Disseminative Organs and Vertical Transmission

3.4.1 Colonization of flowers

Flowers, fruits and seeds were considered as sterile up to the 2000s (Hallmann, 2001) due to very few isolates that could be isolated and cultivated, but there is increasing evidence that they are also colonized by beneficial bacterial endophytes. Flowers host diversified microbial assemblages, and endophytic

bacteria have been visualized in their ovaries, epidermis and xylem tissues. For instance, bacteria belonging to the *Pseudomonas* and *Bacillus* genera have been observed in grapevine flowers using FISH microscopy (Compant *et al*., 2011). Most of the bacterial colonizers of flowers derive from the anthosphere, the external microenvironment of flowers (Compant *et al*., 2011). Bacteria have been observed colonizing inner flower tissues through stomata present on the surfaces of preflower buds (Compant *et al*., 2011). Bacteria can also enter inside flower tissues throughout stigma, by using pollen as a vector (Escobar-Rodríguez *et al*., 2018). Fürnkranz *et al*. (2012) showed, for instance, the presence of bacteria on pollen of pumpkin flowers. Ambika Manirajan *et al*. (2016) further visualized the presence of bacteria on pollen of birch, rye, rapes and autumn crocus. Pollen grains support diverse bacterial communities, the composition of which depends on the plant species and pollination type (Manirajan *et al*., 2018). The role of these bacteria in the reproductive process and their ability to be transmitted vertically are, however, poorly understood. To date, only a few studies suggest a possibility of transmission of endophytic bacteria from pollen to offspring (reviewed by Franck *et al*., 2017). It is highly possible that pollinator insects also transport bacteria from flower to flower, and some bacteria could then penetrate into plant tissues (Junker *et al*., 2011).

3.4.2   Colonization of fruits

Bacteria have been observed in the pericarp zones of fruits, corresponding to exo-, meso- and endocarp tissues (Fig. 3.1E). A differential colonization was revealed, depending on strains, taxa and preflower colonization sites (Glassner *et al*., 2015). These authors studied the colonization routes of native bacteria within fruits of several *Cucurbitaceae* and suggested that they derive from flowers. Some of these strains have biocontrol properties against melon pathogens (Glassner *et al*., 2015). Fruits can also have microwounds due to external factors such as insects or wind, allowing some carpospheric and carpoplane microorganisms to endophytically colonize fruits. Some bacteria can further derive from the soil as they have been visualized inside xylem vessels albeit the soil being not the most important source of colonization of flowers and fruits (Compant *et al*., 2011).

3.4.3   Colonization of seeds and transmission of endophytic bacteria to the plant offspring

Bacteria have been visualized inside seeds as well, both inside the embryo (in the cotyledon and the root-hypocotyl tissues) and in the seed coat (Escobar-Rodríguez *et al*., 2018; Glassner *et al*., 2018). For instance, Compant *et al*. (2011) located bacteria by FISH analyses inside the tegument of seeds of grapevine. Non-culturable endophytic bacteria were also detected in seeds of cactus *Mammillaria fraileana* by scanning electron microscopy, and FISH enabled to locate bacteria inside the seed coat and embryo (Lopez *et al*., 2011). Glassner *et al*. (2018) further visualized bacteria inside the seed coat, cotyledon and root-hypocotyl tissues of melon seeds, and Escobar-Rodríguez *et al*. (2018) also described the presence of bacteria inside tomato seeds, especially on the root surface of the embryo, the cotyledon and the seed coat. Similar findings were obtained for kernels of wheat (Escobar-Rodríguez *et al*., 2018) and other plants such as *Anadenanthera colubrina* (Alibrandi *et al*., 2018).

These seed endophytes can derive from the tissues of either flowers or fruits, depending on their location. Bacteria present inside the seed coat might derive from the fruit and colonize the seed when it is still immature. Bacteria inside the cotyledon and root-hypocotyl embryo might derive from the colonized ovule at flowering. This could be the same for the perisperm/endosperm envelope surrounding the embryo. This thin layer can be massively colonized by bacteria as it is rich in nutritive compounds that can be used by bacteria (Glassner *et al*., 2018), and we can expect that among all bacteria some could be beneficial for their hosts.

Interestingly, the routes of colonization from flowers to seeds have been further revealed by using a beneficial strain of *P. phytofirmans*. This strain, PsJN, has been found in seeds (including the embryo) of cereals and other plants after flower inoculation (Mitter *et al*., 2017) and can promote plant growth of the offspring. However, the strain was not recovered in the seeds of the offspring. This is not surprising, however, as different sources of colonization exist, and due to various environmental conditions and plant status, different routes of colonization can lead to different bacterial assemblages inside seeds (Escobar-Rodríguez *et al*., 2018). Seeds harbour, however, a core microbiome with some bacteria being transmitted from one generation to the next with some of them helping the new plant generation (Nelson, 2018).

Once seeds reach the soil and germinate, some bacteria present from the soil can colonize the spermosphere, the microenvironment surrounding the seed once the seed has germinated. Similar to the rhizosphere, this zone contains exudates and other compounds attracting microbes (Nelson, 2018). After colonizing the spermoplane, the microbial colonizers then can enter inside plant tissues at the root, stem and crown levels using the same routes described before from the soil to inner root tissues. Bacteria have been also visualized as entering breaches due to stem and root development from the seeds. However, some bacteria can also exit from the seed when it germinates and colonize the soil surrounding the plant. Yang *et al*. (2017) showed that the seed microbiota has an early impact on the soil microbiota composition, while later on other colonizers replace the original population. They also demonstrated that some seed endophytes, belonging to the *Enterobacteriaceae* and *Paenibacillaceae* families, were abundant in plant roots in axenic systems but became less abundant when plants were grown in natural soil. Recently, Rahman *et al*. (2018) showed, with a FISH approach on young roots of barley plants grown under sterile conditions and from surface-sterilized seeds, a dense bacterial colonization from the root tip to the root hair zone after germination, suggesting the vertical transmission of some bacteria.

3.5 Conclusions

Microorganisms isolated from the internal tissues of plants (that are endophytes) have long been considered as a contaminant. However, several decades of research demonstrated that these endophytes readily interact with the plant host and may or may not improve its performance, depending on the physiological and genetic status of the plant, the microorganism, soil characteristics and the abiotic environmental conditions. Albeit some of them could be beneficial for the plant, many bacterial endophytes do not provide the expected beneficial effects once applied in the field, due to screening bias or ineffective colonization caused by non-appropriate environmental conditions and microhabitats (Compant *et al*., 2010). A current challenge is to place the beneficial bacteria at the right place and time to improve agroecosystem health and productivity. To do so, knowledge on the environmental sources of beneficial bacteria, their routes of colonization and their niches within the plant is required. Here, we showed that beneficial bacterial endophytes can derive from the soil, the surfaces of flowers, fruits and seeds and can be also transmitted by insects and other animals, other plants and humans. Interestingly, some bacterial endophytes originate from the soil, enter into the internal tissues of plant roots and then move up to the aerial plant organs through xylem vessels. These bacteria constitute a functional linkage between the below ground and above ground subsystems that should be included in future network analyses aimed at predicting the response of phytobiomes to global change (Ramirez *et al*., 2018). Overall, future research on beneficial bacterial endophytes should take into account the various components of phytobiomes. It should go beyond the understanding of the molecular interactions enabling the plant to recognize the bacterial endophytes and of the mechanisms enabling the bacteria to benefit the plant. Research at the community and ecosystem levels is needed. For instance, the environmental reservoirs of beneficial bacterial endophytes should be better explored. The impact of the abiotic environment on

the success of colonization and on the output of the interaction should be further better assessed.

References

Alibrandi, P., Cardinale, M., Rahman, M.M., Strati, F., Ciná, P. *et al*. (2018) The seed endosphere of *Anadenanthera colubrina* is inhabited by a complex microbiota, including *Methylobacterium* spp. and *Staphylococcus* spp. with potential plant-growth promoting activities. *Plant and Soil* 422(1–2), 81–99. doi:10.1007/s11104-017-3182-4

Ambika Manirajan, B., Ratering, S., Rusch, V., Schwiertz, A., Geissler-Plaum, R. *et al*. (2016) Bacterial microbiota associated with flower pollen is influenced by pollination type, and shows a high degree of diversity and species-specificity. *Environmental Microbiology* 18, 5161–5174. doi:10.1111/1462-2920.13524

Beans, C. (2017) Core concept: Probing the phytobiome to advance agriculture. *Proceedings of the National Academy of Sciences of the United States of America* 22, 114. doi:10.1073/pnas.1710176114

Benizri, E., Baudoin, E. and Guckert, A. (2001) Root colonization by inoculated plant growth rhizobacteria. *Biocontrol Science and Technology* 11, 557–574.

Berg, G. (2009) Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology* 84, 11–18. doi:10.1007/s00253-009-2092-7

Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F. *et al*. (2017) Ecology and genomic insights of plant-pathogenic and -nonpathogenic endophytes. *Annual Review of Phytopathology* 55, 61–83. doi:10.1146/annurev-phyto-080516-035641

Campisano, A., Ometto, L., Compant, S., Pancher, M., Antonielli, L. *et al*. (2014) Interkingdom transfer of the acne causing agent, *Propionibacterium acnes*, from human to grapevine. *Molecular Biology and Evolution* 31, 1059–1065. doi:10.1093/molbev/msu075

Chi, F., Shen, S.H., Cheng, H.P., Jing, Y.X., Yanni, Y.G. *et al*. (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(11), 7271–7278.

Compant, S., Clément, C. and Sessitsch, A. (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants. Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* 42, 669–678.

Compant, S., Kaplan, H., Sessitsch, A., Nowak, J., Ait Barka, E. *et al*. (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., Mitter, B., Coli-Mull, J.G., Gangl, H. and Sessitsch, A. (2011) Endophytes of grapevine flowers, berries, and seeds: Identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microbial Ecology* 62, 188–197. doi:10.1007/s00248-011-9883-y

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C. *et al*. (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology* 71, 1685–1693.

Compant, S., Saikkonen, K., Mitter, B., Campisano, A. and Mercado-Blanco, J. (2016) Editorial special issue: Soil, plants and endophytes. *Plant and Soil* 405(1), 1–11. doi:10.1007/s11104-016-2927-9

Dawson, T.E. and Goldsmith, G.R. (2018) The value of wet leaves. *New Phytologist* 219(4), 1156–1169. doi:10.1111/nph.15307

Escobar-Rodríguez, C., Mitter, B., Barret, M., Sessitsch, A. and Compant, S. (2018) Commentary: Seed bacterial inhabitants and their routes of colonization. *Plant and Soil* 422(1–2), 129–134. doi:10.1007/s11104-017-3368-9

Ferreira, A., Quecine, M., Lacava, P., Oda, S., Azevedo, J. *et al*. (2008) Diversity of endophytic bacteria from *Eucalyptus* species seeds and colonization of seedlings by *Pantoea agglomerans*. *FEMS Microbiology Letters* 287, 8–14. doi:10.1111/j.1574-6968.2008.01258.x

Franck, A.C., Saldierna Guzmán, J.P. and Shay, J.E. (2017) Transmission of bacterial endophytes. *Microorganisms* 5(4), 70. doi:10.3390/microorganisms5040070

Fürnkranz, M., Lukesch, B., Müller, H., Huss, H., Grube, M. *et al*. (2012) Microbial diversity inside pumpkins: Microhabitat-specific communities

display a high antagonistic potential against phytopathogens. *Microbial Ecology* 63, 418–428. doi:10.1007/s00248-011-9942-4

Gamalero, E., Lingua, G., Tombolini, R., Avidano, L., Pivato, B. *et al*. (2005) Colonization of tomato root seedling by *Pseudomonas fluorescens* 92rkG5: Spatio-temporal dynamics localization, organization, viability and culturability. *Microbial Ecology* 50, 289–297.

Germaine, K., Keogh, E., Borremans, B., Lelie, D., Barac, T. *et al*. (2004) Colonisation of poplar trees by *gfp* expressing bacterial endophytes. *FEMS Microbiology Ecology* 48, 109–118. doi:10.1016/j.femsec.2003.12.009

Glassner, H., Zchori-Fein, E., Compant, S., Sessitsch, A., Katzir, N. *et al*. (2015) Characterization of endophytic bacteria from cucurbit fruits with potential benefits to agriculture in melons (*Cucumis melo* L.). *FEMS Microbiology Ecology* 91(7), pii: fiv074. doi:10.1093/femsec/fiv074

Glassner, H., Zchori-Fein, E., Yaron, S., Sessitsch, A., Sauer, U. *et al*. (2018) Bacterial niches inside seeds of *Cucumis melo* L. *Plant and Soil* 422(1–2), 101–113. doi:10.1007/s11104-017-3175-3

Hallmann, J. (2001) Plant interactions with endophytic bacteria. In Jegger, M.J. and Spence, N.J. (eds) *Biotic Interactions in Plant–Pathogen Associations*. CAB International, Wallingford, UK, pp. 87–119.

Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirtillä, A.M., Compant, S. *et al*. (2015) The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews* 79(3), 293–320. doi:10.1128/MMBR. 00050-14

Iasur-Kruh, L., Zahavi, T., Barkai, R., Freilich, S., Zchori-Fein, E. *et al*. (2018) *Dyella*-like bacterium isolated from an insect as a potential biocontrol agent against grapevine yellows. *Phytopathology* 108, 336–341. doi:10.1094/PHYTO-06-17-0199-R

James, E.K., Gyaneshwar, P., Manthan, N., Barraquio, W.L., Reddy, P.M. *et al*. (2002) Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. *Molecular Plant-Microbe Interactions* 15, 894–906.

James, E.K., Olivares, F.L., de Oliveira, A.L.M., dos Reis, F.B. Jr, da Silva, L.G. *et al*. (2001) Further observations on the interaction between sugar cane and *Gluconacetobacter diazotrophicus* under laboratory and greenhouse conditions. *Journal of Experimental Botany* 52, 747–760.

Junker, R.R., Loewel, C., Gross, R., Dötterl, S., Keller, A. *et al*. (2011) Composition of epiphytic bacterial communities differs on petals and leaves. *Plant Biology* 13(6), 918–924. doi:10. 1111/j.1438-8677.2011.00454.x

Kandel, S.L., Joubert, P.M. and Doty, S.L. (2017) Bacterial endophyte colonization and distribution within plants. *Microorganisms* 5(4), pii: E77. doi:10.3390/microorganisms5040077

Leach, J.E., Triplett, L.R., Argueso, C.T. and Trivedi, P. (2017) Communication in the phytobiome. *Cell* 169(4), 587–596. doi:10.1016/j.cell.2017. 04.025

Lemanceau, P., Barret, M., Mazurier, S., Mondy, S., Pivato, B. *et al*. (2017) Plant communication with associated microbiota in the spermosphere, rhizosphere and phyllosphere. *Advances in Botanical Research* 82, 101–133.

Lopez, B.R., Bashan, Y. and Bacilio, M. (2011) Endophytic bacteria of *Mammillaria fraileana*, an endemic rock-colonizing cactus of the southern Sonoran Desert. *Archives in Microbiology* 193(7), 527–541. doi:10.1007/s00203-011-0695-8

Lòpez-Fernàndez, S., Compant, S., Vrhosek, U., Bianchedi, P.L., Sessitsch, A. *et al*. (2016) Grapevine colonization by endophytic bacteria shifts secondary metabolism and suggests activation of defense pathways. *Plant and Soil* 405(1), 155–175. doi:10.1007/s11104-015-2631-1

Lòpez-Fernàndez, S., Mazzoni, V., Pedrazzoli, F., Pertot, I. and Campisano, A. (2017) A phloem-feeding insect transfers bacterial endophytic communities between grapevine plants. *Frontiers in Microbiology* 8, 834. doi:10.3389/fmicb. 2017.00834

Lugtenberg, B. and Kamilova, F. (2009) Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* 63, 541–556.

Malfanova, N., Kamilova, F., Validov, S., Chebotar, V. and Lugtenberg, B. (2013) Is l-arabinose important for the endophytic lifestyle of *Pseudomonas* spp.? *Archives of Microbiology* 195(1), 9–17. doi:10.1007/s00203-012-0842-x

Manirajan, B.A., Maisinger, C., Ratering, S., Rusch, V., Schwiertz, A. *et al*. (2018) Diversity, specificity, co-occurrence and hub taxa of the bacterial-fungal pollen microbiome. *FEMS Microbiology Ecology* 94, 8. doi:10.1093/femsec/fiy112

Mendes, R., Garbeva, P. and Raaijmakers, J.M. (2013) The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews* 37(5), 634–663. doi:10. 1111/1574-6976.12028

Mercado-Blanco, J. (2015) Life of microbes inside the plant. In: Lugtenberg, B.J.J. (ed.) *Principles of Plant-Microbe Interactions*. Springer, Berlin, pp. 25–32. doi:10.1007/978-3-319-08575-3_5

Miotto-Vilanova, L., Jacquard, C., Courteaux, B., Wortham, L., Michel, J. *et al*. (2016) *Burkholderia*

phytofirmans PsJN confers grapevine resistance against *Botrytis cinerea* via a direct antimicrobial effect combined with a better resource mobilization. *Frontiers in Plant Science* 7, 1236. doi:10.3389/fpls.2016.0123

Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L. *et al*. (2017) A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology* 8, 11. doi:10.3389/fmicb.2017.00011

Nelson, E.B. (2018) The seed microbiome: Origins, interactions, and impacts. *Plant and Soil* 422(1–2), 7–34. doi:10.1007/s11104-017-3289-7

Nelson, E.B., Simoneau, P., Barret, M., Mitter, B. and Compant, S. (2018) Editorial special issue: The soil, the seed, the microbes and the plant. *Plant and Soil* 422(1–2), 1–5. doi:10.1007/s11104-018-3576-y

Panchal, S. and Melotto, M. (2018) Stomate-based defense and environmental cues. *Plant Signaling & Behavior* 12(9), e1362517. doi:10.1080/15592324.2017.1362517

Peredo, E.L. and Simmons, S.L. (2018) Leaf-FISH: Microscale imaging of bacterial taxa on phyllosphere. *Frontiers in Microbiology* 8, 2669. doi:10.3389/fmicb.2017.02669

Pinto-Carbó, M., Gademann, K., Eberl, L. and Carlier, A. (2018) Leaf nodule symbiosis: Function and transmission of obligate bacterial endophytes. *Current Opinion in Plant Biology* 44, 23–31. doi:10.1016/j.pbi.2018.01.001

Prieto, P., Schiliro, E., Maldonado-Gonzalez, M.M., Valderrama, R., Barroso-Albarracin, J.B. *et al*. (2011) Root hairs play a key role in the endophytic colonization of olive roots by *Pseudomonas* spp. with biocontrol activity. *Microbial Ecology* 62, 435–445. doi:10.1007/s00248-011-9827-6

Rahman, M.M., Flory, E., Koyro, H.W., Abideen, Z., Schikora, A. *et al*. (2018) Consistent associations with beneficial bacteria in the seed endosphere of barley (*Hordeum vulgare* L.). *Systematic and Applied Microbiology* 41(4), 386–398. doi:10.1016/j.syapm.2018.02.003

Ramirez, K.S., Geisen, S., Morriën, E., Snoek, B.L. and van der Putten, W.H. (2018) Network analyses can advance above-belowground ecology. *Trends in Plant Science* 23(9), 759–768. doi:10.1016/j.tplants.2018.06.009

Remus-Emsermann, M.N.P. and Schlechter, R.O. (2018) Phyllosphere microbiology: At the interface between microbial individuals and the plant host. *New Phytologist* 218(4), 1327–1333. doi:10.1111/nph.15054

Rosado, B.H.P., Almeida, L.C., Alves, L.F., Lambais, M.R., Oliveira, R.S. (2018) The importance of phyllosphere on plant functional ecology: a phyllo trait manifesto. *New Phytologist* 219(4), 1145–1149. doi: 10.1111/nph.15235.

Sabaratnam, S. and Beattie, G.A. (2003) Differences between *Pseudomonas syringae* pv. syringae B728a and *Pantoea agglomerans* BRT98 in epiphytic and endophytic colonization of leaves. *Applied and Environmental Microbiology* 69, 1220–1228.

Samad, A., Trognitz, F., Compant, S., Antonielli, L. and Sessitsch, A. (2017) Shared and host-specific microbiome diversity and functioning of grapevine and accompanying weed plants. *Environmental Microbiology* 19(4), 1407–1424. doi:10.1111/1462-2920.13618

Turner, T.R., James, E.K. and Poole, P.S. (2013) The plant microbiome. *Genome Biology* 14(6), 209–219. doi:10.1186/gb-2013-14-6-209

Vacher, C., Hampe, A., Porte, A.J., Sauer, U., Compant, S. *et al*. (2016) The phyllosphere: Microbial jungle at the plant-climate interface. *Annual Review of Ecology, Evolution, and Systematics* 47, 1–24.

Vorholt, J.A. (2012) Microbial life in the phyllosphere. *Nature Reviews Microbiology* 10(12), 828–840. doi:10.1038/nrmicro2910

Wilson, M., Hirano, S.S. and Lindow, S.E. (1999) Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf. *Applied and Environmental Microbiology* 65(4), 1435–1443.

Yang, L., Danzberger, J., Schöler, A., Schröder, P., Schloter, M. *et al*. (2017) Dominant groups of potentially active bacteria shared by barley seeds become less abundant in root associated microbiome. *Frontiers in Plant Science* 8, 1005. doi:10.3389/fpls.2017.01005