

Minireview

Bacterial seed endophytes: genera, vertical transmission and interaction with plants

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Summary

Although the importance of plant-associated microorganisms for plant growth and health was getting more recognition recently, the role of seed-associated microorganisms, and especially seed endophytic bacteria, still is underestimated. Nevertheless, these associations could be beneficial for germination and seedling establishment as seed endophytic bacteria are already present in these very early plant growth stages. Moreover, bacteria with beneficial characteristics can be selected by the plants and could be transferred via the seed to benefit the next generation. In this paper, the current literature concerning bacterial endophytes that have been isolated from seeds of different plant species is reviewed. Their colonization routes, localization inside seeds and mode of transmission as well as their role and fate during germination and seedling development are discussed. At the end, some examples of bacterial seed endophytes with applications as a plant growth-promoting or biocontrol agent are given.

Introduction

Seeds represent a remarkable phase in the life cycle of spermatophytes: they can persist for years in a dormant state and, when the appropriate conditions are met, develop into a new plant (Nelson, 2004). Seeds can benefit from seed-associated microorganisms as these can play a role in seed preservation and preparation of the environment for germination (Chee-Sanford *et al.*, 2006). When seeds start to germinate, they imbibe water and start to secrete exudates that attract bacteria, which colonize the spermosphere, rhizosphere and the seedling

where they can directly and indirectly promote plant growth and health (Nelson, 2004). Direct plant growth-promoting mechanisms involve nitrogen fixation, mobilization of nutrients such as phosphorus and iron by production of organic acids and siderophores, production of phytohormones such as auxins and cytokinins, and suppression of stress ethylene production by 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Weyens *et al.*, 2009). Indirect growth promotion occurs by preventing the growth or activity of phytopathogens through competition for space and nutrients, antibiosis, production of hydrolytic enzymes, inhibition of toxins and induction of plant defence mechanisms (Weyens *et al.*, 2009).

Apart from bacteria present in the seed surroundings, bacteria that are already present inside the seed can be equally important for the evolution of the microbial community of the seedling (Johnston-Monje and Raizada, 2011). Many seeds carry a diversity of endophytes (Table 1). Bacterial endophytes are described by Azevedo and Araújo (2007) as all bacteria that may or may not be successfully cultured, that internally colonize the plant and do not cause apparent damage and/or visible external structures.

Seed endophytes are of particular interest as they are transmitted from generation to generation. By being seed-borne, these endophytes assure their presence in new plants. This vertical transmission, defined as the direct transfer from parent to progeny, should select against pathogenicity and favour mutualism as these endosymbionts depend entirely on their host for survival and reproduction (Ewald, 1989; Rudgers *et al.*, 2009). At the same time, the host plant benefits by providing their progeny with beneficial endosymbionts (Ewald, 1989).

Many studies have focused on fungal endophytes in seeds, while reports concerning the presence and role of bacterial seed endophytes during germination and seedling development are less numerous. In this paper, an overview is given of the bacterial seed endophytes that have been isolated from different plant species. We discuss the colonization routes of these endophytes, their localization inside the seeds and their mode of transmission. Further, we review their role and fate during

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Table 1. Summary of the known bacterial seed endophytes isolated from different plant species.

Plant	Actinobacteria	Bacteroidetes	Firmicutes	Proteobacteria				References
				α	β	γ	δ	
Norway spruce (<i>Picea abies</i>)	0	0	0	0	0	2	0	Cankar <i>et al.</i> , 2005
Grape vine (<i>Vitis vinifera</i>)	0	0	3	0	0	0	0	Compant <i>et al.</i> , 2011
Eucalyptus (<i>Eucalyptus</i>)	1	0	3	3	0	0	0	Ferreira <i>et al.</i> , 2008
Rice (<i>Oryza sativa</i>)	12	2	5	5	3	13	0	Mukhopadhyay <i>et al.</i> , 1996; Ebeltagy <i>et al.</i> , 2000; Bacilio-Jiménez <i>et al.</i> , 2001; Cottyn <i>et al.</i> , 2001; Okunishi <i>et al.</i> , 2005; Mano <i>et al.</i> , 2006; Tripathi <i>et al.</i> , 2006; Kaga <i>et al.</i> , 2009; Ruiz <i>et al.</i> , 2011; Hardoim <i>et al.</i> , 2012
Bean (<i>Phaseolus vulgaris</i>)	4	0	4	3	0	2	0	Pleban <i>et al.</i> , 1995; Perez-Ramirez <i>et al.</i> , 1998; López-López <i>et al.</i> , 2010; Rosenblueth <i>et al.</i> , 2010
Tobacco (<i>Nicotiana tabacum</i>)	1	0	1	0	0	4	0	Mastretta <i>et al.</i> , 2009
Cactus (<i>Pachycereus pringlei</i>)	0	0	2	0	0	3	0	Puente <i>et al.</i> , 2009b
Coffee (<i>Coffea arabica</i>)	3	0	1	0	1	5	0	Vega <i>et al.</i> , 2005
Rapeseed (<i>Brassica napus</i>)	1	1	2	0	2	4	0	Granér <i>et al.</i> , 2003
Maize (<i>Zea mays</i>)	18	5	14	16	17	33	1	Dunleavy, 1989; Rijavec <i>et al.</i> , 2007; Rosenblueth <i>et al.</i> , 2010; Johnston-Monje and Raizada, 2011; Liu <i>et al.</i> , 2012; Liu <i>et al.</i> , 2013
Ash (<i>Fraxinus</i>)	3	0	4	0	0	3	0	Donnarumma <i>et al.</i> , 2010
Soybean (<i>Glycine max</i>)	1	1	1	2	0	2	1	Kremer, 1987; Dunleavy, 1989; Oehrle <i>et al.</i> , 2000
Wheat (<i>Triticum aestivum</i> ; <i>Elymus trachycaulus</i> ; <i>Agropyron fragile</i>)	3	0	2	0	0	4	0	Coombs and Franco, 2003; Ringelberg <i>et al.</i> , 2012
Sugar beet (<i>Beta vulgaris</i>)	0	0	1	0	0	0	0	Dent <i>et al.</i> , 2004
Garden pea (<i>Pisum sativum</i>)	0	0	1	0	0	0	0	Smerda <i>et al.</i> , 2005
Pumpkin (<i>Cucurbita pepo</i>)	0	0	1	0	0	1	0	Fürnkranz <i>et al.</i> , 2012
Peanut (<i>Arachis hypogaea</i>)	0	0	2	0	0	1	0	Sobolev <i>et al.</i> , 2013
Cauliflower (<i>Brassica oleracea</i>)	0	0	1	0	0	0	0	Pleban <i>et al.</i> , 1995
Wild mustard (<i>Sinapis arvensis</i>)	0	0	1	0	0	0	0	Pleban <i>et al.</i> , 1995
Tomato (<i>Lycopersicon esculentum</i>)	0	0	1	0	0	0	0	Xu <i>et al.</i> , 2014
Strawberry (<i>Fragaria</i>)	0	0	0	0	0	1	0	Kukkurainen <i>et al.</i> , 2005
Thale cress (<i>Arabidopsis thaliana</i>)	1	0	3	5	2	1	0	Truyens <i>et al.</i> , 2013
Grasses (<i>Lolium multiflorum</i> ; <i>Phleum pretense</i> ; <i>Panicum virgatum</i> ; <i>Agrostis capillaris</i>)	1	0	3	0	0	3	0	Ikeda <i>et al.</i> , 2006; Gagne-Bourgue <i>et al.</i> , 2013; Truyens <i>et al.</i> , 2014
Broadleaf weed species	0	1	1	0	1	7	0	Kremer, 1987
Herbaceous and woody species	5	2	3	0	2	7	0	Mundt and Hinkle, 1976

germination and seedling development. Finally, examples of seed endophytes with direct plant growth-promoting capacities or biocontrol properties are discussed.

Frequently reported bacterial seed endophytes

The conditions within the seed change during seed maturation and this can affect which bacteria will be able to inhabit the seed. By consequence, some characteristics are typically found in seed endophytes and not in endophytes isolated from other plant tissues such as roots or shoots. The accumulation of starch and loss of water during the seed maturation process seem to favour endophytes that are tolerant to a high osmotic pressure (Ebeltagy *et al.*, 2000; Mano *et al.*, 2006). Endospore formation can also be an important feature for seed colonizers as this protects them from the changes that are happening inside the seed (Mano *et al.*, 2006; Compant *et al.*, 2011). Seed endophytes often seem to possess amylase activity in order to utilize starch and resume growth after long-term survival inside the seeds (Mano *et al.*, 2006). Some seed endophytes were also reported to be able to use phytate, which is the main storage form of phosphorus in seeds, as a source of phosphate (López-López *et al.*, 2010). Moreover, Okunishi and colleagues (2005) and Ebeltagy and colleagues (2000) found that almost all the seed endophytes from rice seeds were motile, which enables them to migrate into the seeds before they harden. Johnston-Monje and Raizada (2011) reported that most of the bacterial isolates from seeds of different maize genotypes were able to solubilize phosphorus, secrete acetoin and fix nitrogen. Also, ACC deaminase activity and antibiosis were moderately conserved among seed endophytes from different maize genotypes (Johnston-Monje and Raizada, 2011). These conserved endophyte properties could reflect common needs of the host seeds and their spermosphere (Nelson, 2004).

The seed maturation process not only selects bacteria based on their phenotypic properties, also the diversity of bacterial genera, at least of the cultivable ones, seems to be influenced. Mano and colleagues (2006) found that there was a dominance of Gram-negative isolates in the early stages of seed development, but more Gram-positive isolates appeared when the seeds were maturing. In the very early stages, they found mainly *Methylobacterium* spp. and *Sphingomonas* spp., while later on *Bacillus* spp. and *Curtobacterium* spp. were more abundant (Mano *et al.*, 2006).

Until now, 131 bacterial genera from 4 different phyla and 25 different plants or plant species have been reported as naturally occurring seed endophytes (Table 1 and references herein). The most predominant seed endophytes belong to the Proteobacteria (80 genera) and

mainly the γ -Proteobacteria (41 genera). Also Actinobacteria and Firmicutes are well represented with 25 and 20 genera respectively. The phylum Bacteroidetes is represented by only six genera. It seems as if the phylum composition of bacterial seed endophytes is a good reflection of the endophytic phyla found in other plant tissues. In general, Proteobacteria, and especially γ -Proteobacteria, were found to be the dominant endophytic phylum isolated from a wide variety of plant species, while Actinobacteria, Firmicutes and certainly Bacteroidetes were much less represented (Rosenblueth and Martínez-Romero, 2006).

Some studies considered only the cultivable strains, while others investigated the total bacterial population present in seeds. To identify the cultivable strains, the more recent studies used 16S rRNA gene sequencing (e.g. Ferreira *et al.*, 2008; Mastretta *et al.*, 2009), while earlier studies used fatty acid methyl ester profile analysis (Granér *et al.*, 2003; Vega *et al.*, 2005) or biochemical methods (Mundt and Hinkle, 1976; Kremer, 1987; Dunleavy, 1989). To identify the total bacterial population, 16S rRNA gene libraries in combination with 16S rRNA gene sequencing (e.g. Johnston-Monje and Raizada, 2011; Liu *et al.*, 2012) or polymerase chain reaction (PCR)-based differential denaturing gel electrophoresis (PCR-DGGE) whether or not in combination with 16S rRNA gene sequencing were used (e.g. Hardoim *et al.*, 2012).

Although several of the studies listed in Table 1 had the description of the seed bacterial community as a primary objective, others discovered seed endophytes while studying other plant-associated bacterial populations or other seed characteristics. This might explain the low numbers of bacterial genera recovered from some types of seeds as well as the differences in population size. The cultivation of seed endophytes is also challenging because of the specific habitat they are derived from. Seed endophyte population sizes range from 55 colony-forming units (cfu) per gram in bean (Rosenblueth *et al.*, 2010) to as high as 10^7 cfu g⁻¹ in rapeseed (Granér *et al.*, 2003). In general, common bacterial genera reported in seeds of very different plant species are *Bacillus* and *Pseudomonas*. Also *Paenibacillus*, *Micrococcus*, *Staphylococcus*, *Pantoea* and *Acinetobacter* are often found inhabiting the seed.

Seed endophyte dynamics

Seed colonization

The majority of the bacterial taxa detected inside seeds were similar to common soil strains. Plant roots are exposed to bacteria in soil during development and growth, enabling entry of bacteria into the plants and

making this an obvious colonization route towards the seeds. Several bacterial traits are important for plant colonization and endophytic capacity. Chemotaxis-induced motility leading to root colonization is probably one of the most important mechanisms determining the endophytic potential of soil bacteria (Bacilio-Jiménez *et al.*, 2003). For example, *cheA* or *pilA* mutants of several *Pseudomonas* strains defective in flagella-driven chemotaxis or twitching motility showed reduced competitive root colonizing abilities (Camacho Carvajal, 2001; de Weert *et al.*, 2002). The next step in the colonization route towards the seeds requires bacteria to enter the root and become endophytes either through passive penetration at the root tip, side root emergence or pathogen entry sites, or through active penetration using cell wall degrading enzymes, such as cellulase and pectinase (Hurek *et al.*, 1994; Ebeltagy *et al.*, 2000; James *et al.*, 2002). Recently, also transport proteins for uptake of plant-synthesized nutrients, secretion and delivery systems involved in switching from a free-living to an endophytic life style, transcriptional regulators for metabolic adaptation and quorum sensing, and detoxification mechanisms used in the protection against oxidative stress induced after host infection have been shown to be determinants of competent endophytes (Sessitsch *et al.*, 2012; Ali *et al.*, 2014). Colonization of the plant interior appears to be very attractive as plant nutrients can be used more efficiently without competition with the very high numbers of other bacteria colonizing the root exterior (Rosenblueth and Martínez-Romero, 2006). Moreover, endophytic bacteria seem to be better protected from abiotic stresses compared with rhizosphere bacteria (Hallmann *et al.*, 1997). Once inside the plant, some endophytes are able to spread systemically and ultimately reach the flowers, fruits and seeds. Some endophytes use the root xylem vessels of their hosts where they are assisted by the movement of their flagella and by the plant transpiration stream (James *et al.*, 2002; Compant *et al.*, 2005), while others use the nutrient-rich intercellular spaces, but this requires the secretion of cell wall degrading enzymes (Dong *et al.*, 1994). The bacteria that become seed endophytes are not exclusively soil derived. Alternative entry points might be the caulosphere, the phyllosphere, the anthosphere as well as the carposphere from where they can colonize plant reproductive organs (James *et al.*, 2002; Berg *et al.*, 2005; Compant *et al.*, 2010; 2011).

Some bacteria can already interact with seeds present in soil, thereby increasing their chances of subsequent plant colonization. The amount and composition of seed exudates released during imbibition and germination influences the bacterial population that can be supported in the spermosphere (Schenck and Stotzky, 1975; Roberts *et al.*, 2009). The *pfkA* gene, encoding a phosphofructokinase, seems to be very important for

colonization of seeds which release only a limited amount of reduced carbon sources (Roberts *et al.*, 1999). Also fimbriae and several adhesion factors, efflux pumps, calcium-binding proteins and transport systems involved in iron acquisition seem to be involved in bacterial seed attachment (Hood *et al.*, 1998; Espinosa-Urgel *et al.*, 2000; Molina *et al.*, 2005; 2006). Several other factors can influence the bacterial communities of seeds. Recently, a contaminant-dependent effect on the cultivable seed endophytic population was reported in *Arabidopsis thaliana* and *Agrostis capillaris*. Both the genera that were found as well as their phenotypic characteristics were different for seeds from Cd-exposed versus non-exposed plants (Truyens *et al.*, 2013; 2014). Also differences in seed endophytic populations were found between different cotton cultivars (Adams and Kloepper, 2002) and between different maize genotypes (Liu *et al.*, 2012). Certain plant genes, such as the receptor-like kinase *shr5* or members of the ethylene signalling pathway, could regulate the plants' interaction with bacteria and determine whether the bacteria are able to endophytically colonize the plant (Vinagre *et al.*, 2006; Cavalcante *et al.*, 2007; Liu *et al.*, 2012).

Vertical transmission, seed location and seed quality

Bacterial endophytes can get in seeds in different ways. They can be transmitted from the vegetative parts of the plant to the seed via vascular connections from the maternal plant through the funiculus and chalaza into the seed endosperm as well as via the micropyle (Ivanoff, 1933; Rand and Cash, 1933; Samish and Etinger-Tulczynska, 1963; Agarwal and Sinclair, 1996). Endophytes can also be transferred through gametes directly, colonizing the resulting embryo and endosperm (Agarwal and Sinclair, 1996; Madmony *et al.*, 2005; Malfanova *et al.*, 2013). Finally, vertical transfer of bacteria might also be possible if shoot meristems, which later become reproductive meristems, are colonized as these give eventually rise to ovules and thus seeds (Pirttilä *et al.*, 2000) (Fig. 1).

Transfer of endophytes from mature fruits to seeds was shown by Puente and colleagues (2009b). They found bacteria in the endocarp, mesocarp, exocarp, on the surface and at the embryonic site of cactus seeds. Most bacteria found inside seeds were associated with the seed husk, seed coat or cortex (Mukhopadhyay *et al.*, 1996; Cankar *et al.*, 2005; Puente *et al.*, 2009b). Some endophytes were also found in endosperm and embryonic tissues (Mukhopadhyay *et al.*, 1996; Cankar *et al.*, 2005). Compant and colleagues (2011) used fluorescence *in situ* hybridization (FISH) to demonstrate the presence of *Bacillus* spp. along the cell walls of some cells inside grapevine seeds. FISH was also used by Pirttilä and colleagues (2000) to show the presence of bacteria in

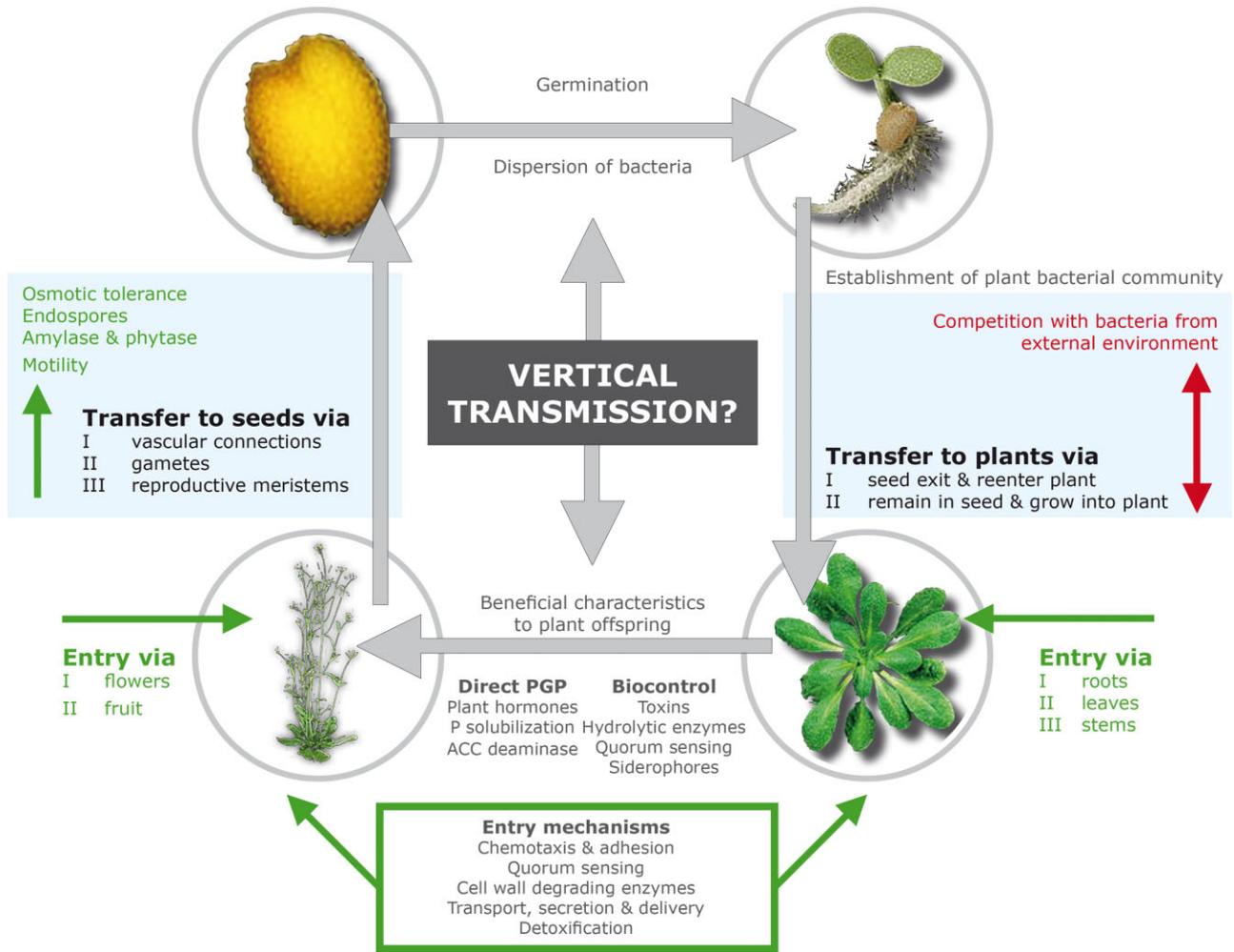


Fig. 1. Schematic summary of colonization routes and determinants, transmission modes and functions of bacterial seed endophytes. After seed germination, the plant bacterial community is established from seed endophytes and from bacteria present in the external environment. Some of these bacteria could be transferred to the next generation of plants via the seeds. This would not only lead to dispersion of bacteria, but also ensures the transfer of beneficial bacterial characteristics to the next generation of plants.

(ovuliferous) pine buds. They detected bacterial RNA particularly in the cells of scale primordia and in the epithelial cells of the resin ducts. Bacterial RNA was less abundant in the apical meristem itself, but some could be found in the outermost cells and in the cells of the developing stem, right below the apical meristem (Pirttilä *et al.*, 2000).

Vertical transmission of bacterial endophytes has already been suggested in several plant species. Gagne-Bourgue and colleagues (2013) described the presence of the same *Bacillus* spp. and *Microbacterium* spp. in seeds harvested in 1 year and switchgrass plants grown from these seeds the next year. Also, Ringelberg and colleagues (2012) suggested that seeds are a major contributor of mature wheatgrass endophytes as they recovered the same genera from seeds and from mature plant tissues. Moreover, the same *Enterobacter* spp. were reported in seeds of consecutive rice generations

(Mukhopadhyay *et al.*, 1996). Johnston-Monje and Raizada (2011) demonstrated in maize that a core microbiota, consisting of the same bacterial species, exists which is conserved even across boundaries of evolution, human selection and following cross-continental migration. Also Liu and colleagues (2012) found continuity of endophytic bacterial species from parental to offspring maize seeds. Moreover, the seed endophytic population of genetically related maize hybrids consisted of similar species and genera (Liu *et al.*, 2012). Correspondence analysis of the cultivable endophytic communities of roots of *Brassica napus* grown on a Cd, Zn and Pb-contaminated field and a non-contaminated control field revealed a correlation between endophytic communities of roots between fields, suggesting that plants on the two sites contained similar obligate endophytes possibly derived from a common seed endophytic community

(Croes *et al.*, 2013). The abovementioned findings are all based on 16S rRNA gene sequencing generating only genus and species level information. Confirmation of the vertical transmission of bacterial endophytes can only be obtained if strain information is available. Nevertheless, the possible existence of vertical transmission is very interesting as it enables a plant with an established endophytic community to pass bacteria with beneficial characteristics to their offspring (Ferreira *et al.*, 2008). Conserved bacteria that are vertically transmitted point towards an evolved form of mutualism or benign parasitism with their host plant (Ewald, 1989; Johnston-Monje and Raizada, 2011). For example, Ferreira and colleagues (2008) suggested that vertical transmission of endophytes might maintain the stability of bacterial communities in *Eucalyptus* plants. Also in *Arabidopsis thaliana*, selection of seed endophytes was not completely at random. It is partly based on the bacterial properties and the environmental stressors experienced by the parental plant (Truyens *et al.*, 2013).

On the contrary, it could even be possible that seed endophytic bacteria use seeds for their own dispersion as many of these bacteria are ubiquitous in different environmental niches (Hardoim *et al.*, 2012) (Fig. 1). The same taxa indeed were commonly found in the endosphere of rice (Okunishi *et al.*, 2005; Mano *et al.*, 2006), maize (Johnston-Monje and Raizada, 2011) and leguminous plants such as soybean (Oehrle *et al.*, 2000), and in the soil where these plants were growing.

A possible link exists between the endophyte content of seeds and seed quality. A high quality seed lot is disease-free and shows a high germination percentage and high vigour (Perry, 1978; Dent *et al.*, 2004). Hill and colleagues (2005) demonstrated that fungal endophyte infection rates of tall fescue seedlings were lower when seeds were harvested before physiological maturity. Seeds harvested before maturity showed a lower germination percentage and reduced seedling vigour (Hill *et al.*, 2005). Moreover, certain seed storage conditions such as increased temperature or humidity can reduce fungal endophyte viability in tall fescue seeds (Hill and Roach, 2009). Also the amount of bacterial endophytes that can be isolated decreases with the duration of seed storage (Mundt and Hinkle, 1976; Bacon and Hinton, 1996; Cankar *et al.*, 2005; Mastretta, 2007). The seed sterilization protocol used before sowing was a major determinant of the amount of bacterial endophytes found in seeds of *Arabidopsis thaliana* (Truyens *et al.*, 2013). Using a mild seed sterilization protocol increased the homogeneity of germination and reduced variability in plant growth (Truyens, S., Weyens, N and J. Vangronsveld, unpublished results). Also, Holland and Polacco (1994) suggested that seed endophytes may be beneficial during germination and that germination declines in case bacteria die.

Role and fate of seed endophytes during germination and growth

Seed endophytes are believed to be capable of infecting the next generation of plants through two infection routes or a combination of both: (i) exiting the seed and then entering the plant via other places on the plant surface and (ii) remaining inside the seed to be spread through plant growth or move within the plant tissue (Kaga *et al.*, 2009) (Fig. 1). A germinating seed starts to release organic molecules from the developing root system, which leads to an increase in the bacterial population compared with the low nutrient environment in the soil around the seed (Baker and Cook, 1974). Endophytic bacteria inhabiting the seeds have the advantage of rapid infection of the new generation of plants because they experience less competition with bacteria originating from the external environment for space and nutrients available in the host plant and because they are already adapted to living inside plant tissue (Kaga *et al.*, 2009; Hardoim *et al.*, 2012) (Fig. 1). Nevertheless, bacteria from the external environment will also colonize the host, leading to shifts in bacterial community structure during seedling development (Hallmann *et al.*, 1997; Mahaffee and Kloepper, 1997).

In rice seeds, seed endophytes were important founders of the bacterial community present during the early plant growth stages (Hardoim *et al.*, 2012). They mainly colonize rice shoots, where there is less competition than in the roots, which are surrounded by large bacterial communities in soil. Some strains were even able to spread out into the rhizosphere and soil. In mature rice plants, endophytes originating from the seeds seemed to coexist with bacteria that originated from the external environment (Mano *et al.*, 2006). Also, in bean, it was demonstrated that seed-borne bacteria are able to multiply and persist in the seedlings as almost all bacterial genera isolated from seeds were also recovered from bean roots (López-López *et al.*, 2010).

Bacterial communities hosted by seeds can support the development of their host. Due to their metabolic diversity and depending on the local conditions, different beneficial bacterial functions can be stimulated thereby increasing plant fitness and giving the host a competitive advantage over other plants, which can affect whole-ecosystem functioning (Klironomos, 2002). Puente and colleagues (2009b) found that the endophytic bacteria from cactus seeds contributed to rock weathering, soil formation and plant growth thereby supporting the establishment of cactus seedlings in barren desert areas without the need for acquiring bacteria from the environment. When these endophytic bacteria were removed from the seeds, seedling development was stopped.

Several authors have tried to determine the exact location of seed-borne bacteria inside the seedling using

histological cuttings or green fluorescent protein (gfp)-labelled seed endophytes. Puente and colleagues (2009b) found seed-borne endophytic bacteria mainly in the root cortex and the vascular system of cactus. In rice, some bacterial cells were localized in the root cortex, but the highest concentration was found in the stele (Mukhopadhyay *et al.*, 1996). A gfp-labelled *Pantoea* sp. inoculated on the seeds of *Eucalyptus* was carried into the embryo through breaks in the seed husk and continued to colonize the seedlings: the gfp-labelled strain was present on the rhizoplane, in the intercellular spaces of the root and in the interior of the stem xylem vessels (Ferreira *et al.*, 2008). Johnston-Monje and Raizada (2011) injected a gfp-labelled seed-borne *Pantoea* or *Enterobacter* strain into the stem of maize plants. They demonstrated that both strains were able to move systemically through vascular tissues: the *Pantoea* strain was found in the metaxylem vessels, and the *Enterobacter* strain in phloem cells at the base of the stem. The latter was even able to exit the plant and colonize the rhizosphere.

Seed endophyte functions

Until now, only limited research is conducted to unravel the exact role and possible applications of seed endophytes. The existing studies mainly focus on the use of seed endophytes as plant growth-promoting agents, while some also discuss their biocontrol potential. In general, the beneficial functions found in seed endophytes do not seem to be different from those found in endophytes isolated from other plant tissues.

Direct plant growth promotion

Inoculating plants with bacteria isolated from seeds can increase plant growth, especially in suboptimal or harsh environmental conditions. Gagne-Bourgue and colleagues 2013 showed that the inoculation of switchgrass seedlings with seed-borne *Bacillus* or *Microbacterium* strains improved plant growth compared with the non-inoculated controls. Both strains produced indole-3-acetic acid (IAA), cytokinins and volatiles such as butanediol and acetoin, and the *Bacillus* strain was able to solubilize phosphorus. Also, Xu and colleagues (2014) found a seed-borne *Bacillus* strain that improved the root and shoot growth of tomato, probably through the production of ACC deaminase and nitrogen fixation. Johnston-Monje and Raizada (2011) used a potato bioassay to test the plant growth-promoting ability of maize seed endophytes. They found a *Burkholderia* strain, producing ACC deaminase and solubilizing phosphorus, that was able to improve potato shoot growth and a *Hafnia* strain, producing acetoin, that increased potato root growth. Seed endophytes of cactus allowed the growth of

cactus seedlings in extreme conditions for at least a year without fertilization and without showing distress (Puente *et al.*, 2009a). These seed endophytes were able to release inorganic nutrients, such as phosphorus, from pulverized rock through the production of organic acids and possessed the capacity to fix nitrogen (Puente *et al.*, 2009b). Several other studies have assumed that the seed endophytes they have isolated are beneficial for the plant based on plant growth-promoting effects described in literature, but did not verify this experimentally (Cankar *et al.*, 2005; López-López *et al.*, 2010).

The plant growth-promoting effect of seed endophytes in suboptimal/harsh environmental conditions can be exploited in practical applications such as phytoremediation. Mastretta and colleagues (2009) demonstrated that inoculation of tobacco with its seed endophytes improved biomass production under Cd exposure and increased plant Cd content compared to the non-inoculated plants probably due to increased uptake of essential nutrients from the growth medium. Also the inoculation of the grass *Agrostis capillaris* with its seed endophytes, which were capable of producing ACC deaminase, IAA, siderophores and acetoin, able to solubilize phosphorus, and tolerant to Cd might be beneficial for its establishment during phytoextraction and phytostabilization of Cd-contaminated areas (Truyens *et al.*, 2014). In case of non-exposed plants, inoculation resulted in a significantly improved plant growth, while after inoculation of Cd-exposed plants an increased Cd uptake was achieved without affecting plant growth. Also Cd-tolerant seed endophytes of *Arabidopsis thaliana*, producing ACC deaminase, IAA, siderophores and organic acids, were able to increase primary root length and root growth rate upon Cd exposure (Truyens *et al.*, 2013).

Biocontrol

Several of the endophytes isolated from seeds were found to possess anti-fungal properties. *Bacillus* and *Microbacterium* strains isolated from seeds of switchgrass inhibited the mycelial growth of fungal plant pathogens due to the production of several toxins such as surfactins and the lipopeptides iturin and mycobacillin (Gagne-Bourgue *et al.*, 2013). Among rice seed endophytes, the strongest anti-fungal properties against *Rhizoctonia solani*, *Pythium myriotyrum*, *Guamanomyces graminis* and *Heterobasidium annosum* were found in two *Enterobacter* strains because of the production of a volatile anti-fungal compound, probably ammonia, and/or the chitinolytic enzyme N-acetyl- β -D-glucosaminidase (Mukhopadhyay *et al.*, 1996). Also Cottyn and colleagues (2001) detected anti-fungal activity among rice seed endophytes: nearly half of the antagonists of *Rhizoctonia solani* and *Pyricularia grisea* were *Bacillus* strains, but

also in some of the *Pantoea*, *Enterobacter*, *Stenotrophomonas*, *Xanthomonas*, *Acinetobacter*, *Paenibacillus* and *Cellulomonas* strains antagonistic activity against one or both of these fungi was detected. In addition, Ruiz and colleagues (2011) reported anti-fungal activity against *Curvularia* sp., *Fusarium oxysporum* and *Phytium ultimum* in several strains of *Pantoea*, *Microbacterium*, *Pseudomonas*, *Paenibacillus* and *Curtobacterium* isolated from rice seeds. Besides the production of anti-fungal compounds and hydrolytic enzymes, also quorum sensing and the release of siderophores, properties often found in seed endophytes, could contribute to their anti-fungal activity (Ruiz *et al.*, 2011; Sessitsch *et al.*, 2012). The production of siderophores leads to competition with pathogens for iron (Loper and Henkels, 1999), while quorum-sensing molecules may participate in the interaction of plants with pathogens and symbionts by induction of plant gene expression (Mathesius *et al.*, 2003).

Concluding remarks

Bacteria inhabiting seeds form an important group among the plant-associated bacteria. They are presumably present in seeds of all plant species and numerous bacterial genera of different phyla were reported as seed endophytes. They can play important roles during germination, seedling development and plant growth. As they possess plant growth-promoting and biocontrol properties, the study of their application in diverse processes such as biofertilization, bioenergy production and bioremediation should be encouraged.

Most seed endophytes isolated until now are cultivable bacteria and several of them are accidentally discovered during a sidetrack of the main research issue. A large fraction of the bacterial endophytic population in seeds probably has unknown cultivation conditions or is in a viable but non-cultivable state. Metagenomic studies will shed more light on this part of the seed endophyte populations and provide a better understanding of the total bacterial populations present in seeds, both concerning the genera that are present as well as their phenotypic characteristics and possible role(s) in germination and plant development.

Until now, the studies suggesting vertical transmission of bacterial endophytes have used 16S rRNA gene sequencing, leading to identification at genus or species level. To confirm the existence of vertical transmission, techniques allowing to generate strain level identification, such as repetitive element palindromic PCR, single nucleotide polymorphism analysis or pulsed-field gel electrophoresis, should be applied in order to detect the presence of the same bacterial strains in consecutive plant generations (Lopez-Velasco *et al.*, 2013). Moreover, more

research is needed focusing on the (genetic) determinants allowing seed colonization, seed endophyte dispersal and vertical transmission.

Due to the unique seed environment and the selection process required for possible transfer to the next generation, seed endophytes often possess attractive characteristics that can be exploited in several biotechnological applications. To use seed endophytes in agricultural, industrial or environmental applications, several questions still need to be addressed. The fate of seed endophytes during germination and plant development has to be elucidated: are they important founders of the newly established endophytic community or will they get (at least partly) outcompeted by soil bacteria? Moreover, are seed endophytes truly selected by the host plant to benefit the next generation or do these bacteria use seeds as a vector for their own dispersion and colonization of new environments? Also, the evolutionary significance of these associations is not sufficiently clear yet: how can seed endophytes contribute to plant growth and development in ways that the plant cannot accomplish by itself? More fundamental knowledge is needed concerning their interactions with each other, with other plant-associated microorganisms (e.g. mycorrhiza) as well as with their host plant after inoculation. Especially seed endophytes from economically important crops, such as maize, rice or rapeseed, are worthwhile studying as they can be applied in biocontrol, biofertilization, bioenergy production and bioremediation. Finally, thorough research should be performed to unravel the changes that occur in the seed-associated bacterial population because of seed maturation, storage time and storage conditions to guarantee the production of high quality seeds.

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