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REGULAR ARTICLE

Endophytes-assisted biocontrol: novel insights in ecology and the mode of action of *Paenibacillus*

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Abstract
Background Biological control is an environmentally sound and effective means of reducing pathogen-induced damage to agriculture using natural antagonists. *Paenibacillus* is a cosmopolitan and ubiquitously occurring bacterial genus with antagonistic activity against phytopathogens. Many species and strains with promising potential for plant growth promotion and biocontrol of pathogens have been identified since *Paenibacillus* was first described 20 years ago. Nevertheless, important questions regarding the colonization of plants, and the mode of action of *Paenibacillus* remain unanswered.
Scope This review focuses on the occurrence of *Paenibacillus* in microbial metagenomes, the endophytic lifestyle of *Paenibacillus,* and the function of *Paenibacillus*-derived volatile organic compounds (VOCs) combining actual literature with our own results.
Conclusions This review provides new insights into the endophytic lifestyle of *Paenibacillus* and discusses strain-specific and system-dependent growth promotion effects on plants. VOCs, in particular pyrazine derivatives emitted by *Paenibacillus,* showed high activity against other organisms. This suggests that VOCs play an important role in communication and interaction. Overall, *Paenibacillus* strains demonstrate promising potential not only for sustainable agriculture and biological control, but also as a source for novel bioactive volatiles.

Keywords PGPR · Plant growth promotion · Endophyte · Volatiles · Metagenomics

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Introduction

Novel high-throughput sequencing techniques in microbial ecology have opened up an immense treasure chest

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of microbial diversity that has been observed in the vast majority of plant-associated habitats (Berg et al. 2014). While the microbiology of the rhizosphere has been thoroughly studied for more than 100 years (Hartmann et al. 2008; Philippot et al. 2013), it has long been assumed that the endosphere was sterile (Smith 1911; Compant et al. 2010). It is now known to be colonized by a variety of microorganisms including beneficial archaea, bacteria and fungi (Hallmann et al. 1997; Hardoim et al. 2015; Müller et al. 2015). A substantial part of the plant-associated microbiome is known for its antagonistic activity against other microorganisms including pathogens (Berg et al. 2013). This functional group of antagonists is a valuable resource in the ongoing development of biological control agents (BCAs), which are applied in agriculture to suppress pathogens. In addition, they often support microbial diversity, which is reduced in intense agricultural ecosystems (Schmid et al. 2011; Van Elsas et al. 2012; Erlacher et al. 2014). Bacterial and fungal antagonists have been especially well studied; *Pseudomonas* and *Trichoderma* are model organisms important for developing an understanding of their mode of action, their intra-specific diversity, and their effects in the field (Weller 2007; Mukherjee et al. 2012). As early as 1999, Emmert and Handelsman suggested *Bacillus* spp. as alternative BCAs due to their unique type of resting cells called endospores appropriate for long-term formulations. Indeed, most of the BCA and bio stimulant products on the market today are *Bacillus*-based (Calvo et al. 2014). *Paenibacillus*, a phylogenetically related genus, was introduced by Ash et al. (1993) to accommodate the "group 3" of the genus *Bacillus*. However, there are only a few specific phenotypical features of *Paenibacillus* distinguishing them from *Bacillus*, e.g. weak reaction with the Gram's stain and differentiation of the cells into ellipsoidal spores that distinctly swell the mother cell (Ash et al. 1993). One of the most likely potential contributing factors accounting for differences between the behavior of *Bacillus* and *Paenibacillus* spp. relates to their ability to fix nitrogen (Jin et al. 2011; Xie et al. 2014). This is a common and widespread feature of *Paenibacillus* but was also shown for several rhizosphere-associated *Bacillus* strains (Ding et al. 2005). In comparison with other bacterial BCAs like *Pseudomonas* or *Bacillus,* less is known about the properties of plant-associated *Paenibacillus* species.

At present, *Paenibacillus* includes 145 published species of facultative anaerobes, endospore-forming, neutrophilic, periflagellated, heterotrophic, and low G+C Gram-positive bacilli, although the taxonomic classification has been debated for a long time (Tindall 2000; Trüper 2005; Keita et al. 2014). The extent and complexity of the *Paenibacillus* taxon are apparent from a phylogram generated from the 16S rRNA gene sequences of 116 species described for this genus (Supplementary Fig. S1). *Paenibacillus* is a cosmopolitan and ubiquitously occurring genus. Although it occurs naturally in soil and marine sediments, plant-associated habitats like the rhizosphere and roots of crop plants are its preferred environments (McSpadden Gardener 2004). In addition, *Paenibacillus* is well-known for its endophytic lifestyle (Hallmann et al. 1997; Krechel et al. 2004). *Paenibacillus* species can be retrieved as epiphytes and endophytes of animals (Pettersson et al. 1999). Several species are the obligate pathogens of honeybees (Genersch 2010) or scarab beetles (Pettersson et al. 1999). Several *Paenibacillus* species are characterized by a unique behavior, and *P. vortex* and *P. dendritiformis* are the most thoroughly studied examples: when grown under stress inducing conditions, they form colonies that behave much like a multi-cellular organism with cell differentiation and task distribution (Ben-Jacob et al. 2004).

Paenibacillus species have been described as promising plant growth promoting bacteria (PGPBs) and/or as BCAs of plant diseases (Berg 2009; Lal and Tabacchioni 2009). Several *Paenibacillus*-based products have been patented and introduced as commercial BCAs (Table 1). The antagonistic potential of *Paenibacillus* spp. against a broad range of phytopathogenic fungi has been well documented *in vitro* as well as *in situ* (Tupinambá et al. 2008; Fürnkranz et al. 2012a, b; Köberl et al. 2013). For example, the biocontrol activity of *P. alvei* K-165 against *Verticillium dahliae* and *Thielaviopsis basicola* was previously demonstrated (Tjamos et al. 2005; Antonopoulos et al. 2008; Schoina et al. 2011). One of the ways how *Paenibacillus* species protect plants from the pathogens is a creation of a biofilm disease shield around the roots (Timmusk et al. 2005). *P. polymyxa* is considered to be the one of the best rhizosphere biofilm formers and is even able to form single species root biofilms under natural conditions (Timmusk et al. 2005, 2011; Timmusk and Nevo 2011). The occurrence of antagonistic *Paenibacillus* in soils and plants of arid zones was reported (Köberl et al. 2011) as well as their potential to compensate plant drought (Timmusk and Wagner 1999; Timmusk et al.

Table 1 Selected patents that are derived from *Paenibacillus* spp. or products thereof applied in plant bioprotection

Patent name (number)	Strain	Target pathogen	Target plant/ disease	Further applications
Paenibacillus terrae biological agent and application thereof in agriculture (CN 103141517 A)	*P. terrae*	*Fusarium oxysporum*	fungal soil-borne diseases, soybean seedling root rot disease	decomposition of mineral phosphorus, potassium and insoluble minerals
Paenibacillus alvei and its applications (CN 103205372 A)	*P. alvei* ZJUB2011-1	*F. oxysporum*	stigma croci bulb rot	–
Biocontrol for plants with *Paenibacillus macerans*, *Pseudomonas putida*, and *Sporobolomyces roseus* (WO 1999005257 A1 and EP 0998554 A1)	*P. macerans*	*Cochliobolus sativus*, *Colletotrichum graminicola*, *F. graminearum*, *F. moniliforme*, *Pyrenophora tritici-repentis*, *Stagonospora nodorum*, *S. avenae* f. sp. *triticea*, *Stenocarpella maydis*	spot blotch/common root rot of cereals, corn, anthracnose, scab of cereals, ear/stalk rot of corn, tan spot of wheat, *Stagonospora nodorum* blotch of wheat, *Stagonospora avenae* blotch of wheat, and stalk/ear rot of corn	PGP; reduces grain contamination by *Fusarium* mycotoxin deoxynivalenol
Paenibacillus alvei strain ts-15 and its use in controlling pathogenic organisms (WO 2012166392 A1)	*P. alvei* TS-15	human foodborne plant pathogens (unspecified)		–
Paenibacillus polymyxa and applications thereof (CN 102851243 A)	*P. polymyxa* JZB120001	broad spectrum pathogenic fungi and pathogenic bacteria, e.g. *Botryosphaeria berengeriana* f. sp. *piricela*, *V. dahliae*, *Monilinia fructicola*, *Rhizoctonia cerealis*, *F. oxysporum* f. sp. *lilii*, *F. oxysporum* f. sp. *conglutinans*; cucumber angular leaf spot fungus, *Agrobacterium tumefaciens*	apple ring rot fungus, peach brown rot, cereal *Rhizoctonia* (*Rhizoctonia cerealis*), Lily base rot pathogen (*Fusarium oxysporum* f. sp. lilii), kale dry outs germs, cucumber angular leaf spot fungus, peach crown thin germs	PGP
Paenibacillus polymyma SHL-1 and application thereof in preventing and controlling stalk rot of *Cymbidium sinense* (CN 102433285 B)	*P. polymyma* SHL-1	Unspecified	*Cymbidium* stem rot	–
Paenibacillus polymyxa for antagonizing *Fusarium oxysporum* in rhizosphere soil of Radix Pseudostellariae (CN 102676435 B)	*P. polymyxa* S960	*F. oxysporum*	*Fusarium oxysporum* in the rhizosphere soil of *Pseudostellaria heterophylla*	–
Anti-microbial agent from *Paenibacillus* sp. and methods and uses thereof (US 20120121543 A1)	*P. polymyxa* JB05-01-1	Gram-negative bacteria	Unspecified	
Novel strains belonging to the genus *Paenibacillus* and method of controlling plant disease by using these strains or culture thereof (EP 1788074 B1)	*P. polymyxa* BS-0105; *Paenibacillus* sp.	Gram-negative bacteria, *F. graminearum*, *F. avenaceum*, *F. oxysporum* f. sp. *cucumerium*, *F. culmorum*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *lycopersici*, *V. dahliae*, *Phytophthora capsici*, *Ralstonia solanacearum*	Scab of barley, wheat, oats and rye, Fusarium wilt of cucumber, Fusarium wilt of melon, Fusarium wilt of tomato, Verticillium wilt, brown rot and bacterial wilt of eggplant	Induces resistance to plant diseases

Table 1 (continued)

Patent name (number)	Strain	Target pathogen	Target plant/ disease	Further applications
Paenibacillus polymyxa for preventing and treating plant fungal diseases and production method thereof (CN 101519639 A)	*P. polymyxa* EBL-06	*Botrytis cinerea*, *Cladosporium cucumerinum*	*Botrytis* mold, cucumber scab	–
Endogenous *Paenibacillus polymyxa* (CN 102250815 A)	*P. polymyxa*	*Phytophora palmivora*	Unspecified	–
Biocontrol agent and pesticide (EP 1079692 A1)	*P. polymyxa* PKB1	*Leptosphaeria maculans*, *Sclerotinia sclerotiorum*, *Marasmius oreades*, *Pythium pythioides*, *Rhizoctonia solani*, *Fusarium avenaceum*, *Alternaria brassicae*	Unspecified	–
Peptide antibiotic against *Leptosphaeria*, *Micrococcus*, *Streptomyces*, *Escherichia*; crops, canola (US 6602500 B1)	*P. polymyxa* ATCC 202127	*Leptosphaeria* spp., *Sclerotinia* spp., *Rhizotonia* spp., *Pythium* spp., *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Sporobolomyces* spp., *Penicillium* spp., *Marasmius* spp.	Unspecified	Produces a peptide antibiotic against fungi

2014). While the mechanisms involved in drought tolerance are not well-understood, the production of plant hormones like indole-3-acetic acid (IAA) or cytokinins was often described (Lebuhn et al. 1997; Timmusk et al. 1999; Spaepen et al. 2007; Da Mota et al. 2008). In addition, a long list of *Paenibacillus*-derived antibiotic compounds was identified (reviewed by Raza et al. 2008; Table 2). The antimicrobial potential of *P. polymyxa* and its unique antibiotic spectrum has been known for several decades. The first soluble antibiotic substances showing a remarkable activity against Gram-negative bacteria were isolated as early as 1947 from culture filtrates of a soil isolate of *P. polymyxa* by Stansly and Schlosser. Post 1947, many more peptide antibiotics from various *P. polymyxa* strains were obtained and classified. These strains were primarily isolated from soil and rhizosphere samples (Wilkinson and Lowe 1966; Kimura et al. 1969; Shoji et al. 1977a; Pichard et al. 1995). Currently, polymyxins derived from *Paenibacillus* are again attracting an increasing amount of attention for the treatment of multidrug-resistant Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia* (Landman et al. 2008; Giamarellou and Poulakou 2009). LI-F antibiotics (gatavalin and fusaricidins) with a broad inhibitory effect against fungi and Gram-positive bacteria were also found (Nakajima et al. 1972; Kurusu et al. 1987; Kajimura and Kaneda 1996, 1997; Deng et al. 2011c; Bionda et al. 2013) as well as iturin-like compunds with activity against dermatophytic fungi (Cotta et al. 2012). Recently, volatile organic compounds (VOCs) from *Paenibacillus* spp. were identified and found to induce resistance in host-plants (Lee et al. 2012). Still, their role in plant-microbe interaction and biocontrol is not holistically understood and requires further exploration.

This review addresses several unanswered questions regarding the ecology and physiology of *Paenibacillus*. The first section is an analysis and assessment of metagenomic datasets with the objective of gaining better insights into the ecology and abundance of *Paenibacillus*. The second section discusses the specific properties of the endophytic lifestyle of *Paenibacillus.* Finally, the third section is an analysis of the potential functions of VOCs derived from *Paenibacillus* combining literature research and actual results.

Table 2 Antimicrobial properties of *Paenibacillus* isolates

Paenibacillus species	Isolation source	Antagonistic potential	Mode of action	Reference
P. polymyxa		antibacterial (*Bacillus subtilis*, *Corynebacterium diphtheria*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi, S. typhimurium*, *Shigella flexneri*, *S. sonnei*, *Vibrio cholera*)	soluble cyclic lipopeptide antibiotics polymyxins	Wilkinson and Lowe (1966), Kimura et al. (1969), Shoji et al. (1977a, b, c)
P. polymyxa		antifungal (*Saccharomyces cerevisiae*, *Torulaspora delbrueckii*); antibacterial (*Bacillus subtilis*, *Micrococcus luteus*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *P. vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens*)	soluble cyclic peptide antibiotic jolipeptin	Ito and Koyama (1972)
P. polymyxa L-1129	bulk soil of Odawara City in Kanagawa Prefecture, Japan	antifungal (*Aspergillus flavus*, *A. nidulans*, *A. niger*, *A. oryzae*, *A. versicolor*, *Candida albicans*, *C. guilliermondii*, *C. tropicalis*, *C. parapsilosis*, *C. utilis*, *Cladosporium fulvum*, *C. shaerospermum*, *Crytpcoccus neoformans*, *Fonsecaea pedrosoi*, *Fusarium moniliforme*, *F. oxysporum*, *F. roseum*, *F. solani*, *Geotrichum candidum*, *Gibberella fujikuroi*, *Helminthosporium sesamum*, *Microsporum canis*, *M. gypseum*, *Penicillium expansum*, *Saccharomyces cerevisiae*, *Sporothrix schenckii*, *Trichophyton rubrum*, *T. mentagrophytes*); antibacterial (*Bacillus subtilis*, *Corynebacterium diphtheria*, *Enterococcus faecalis*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *S. epidermidis*)	soluble cyclic lipodepsipeptide antibiotics LI-F03a to LI-F08a and LI-F03b to LI-F08b	Kurusu et al. (1987)
P. polymyxa KT-8	rhizosphere of *Allium sativum* L.	antifungal (*Aspergillus niger*, *A. oryzae*, *Candida albicans*, *Fusarium oxysporum*, *Penicillium thomii*, *Saccharomyces cerevisiae*); antibacterial (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*)	fusaricidin A-D of cyclic lipodepsipeptide antibiotics	Kajimura and Kaneda (1996, 1997)
P. polymyxa PKB1	canola stubble from a field near Edmonton, Canada	antifungal (*Alternaria brassicae*, *Fusarium avenaceum*, *Leptosphaeria maculans*, *Marasmius oreades*, *Neurospora crassa*, *Penicillium roquefortii*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*); antibacterial (*Micrococcus luteus*, *Streptomyces clavuligerus*)	fusaricidins of cyclic lipodepsipeptide antibiotics	Beatty and Jensen (2002)
P. polymyxa SCE2			protease	Alvarez et al. (2006)
P. polymyxa JSa-9	bulk farmland soil of Nanjing in Jiangsu province, China	antifungal (*Aspergillus niger*, *A. oryzae*, *Penicillium expansum*, *P. notatum*, *Rhizopus stolonifer*);	soluble cyclic and linear LI-F type antibiotics, polymyxin B_6	Deng et al. (2011a, b, c)

Table 2 (continued)

Paenibacillus species	Isolation source	Antagonistic potential	Mode of action	Reference
		antibacterial (*Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *P. fluorescens*)		
P. polymyxa PB71	spermosphere of *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb., Austria	antifungal (*Didymella bryoniae*); antibacterial (*Pectobacterium carotovorum*, *Pseudomonas viridiflava*, *Xanthomonas cucurbitae*)	unknown soluble and volatile antibiotics	Fürnkranz et al. (2012a)
P. polymyxa M-1	endorhiza of wheat, China	antibacterial (*Erwinia amylovora*, *E. carotovora*)	polymyxin P	Niu et al. (2013)
P. polymyxa Wb2-3, Mc5Re-14	bulk soil of the Sinai desert and endorhiza of *Matricaria chamomilla* L., Egypt	antifungal (*Fusarium culmorum*, *Rhizoctonia solani*, *Verticillium dahlia*); antibacterial (*Escherichia coli*); nematicidal (*Meloidogyne incognita*)	β-1,3-glucanase, siderophores, unknown soluble antibiotics	Köberl et al. (2013)

New insights into *Paenibacillus* ecology

Exploring the occurrence of *Paenibacillus* in plant-associated habitats using metagenomics

Plant-associated habitats like the rhizosphere and endosphere of crop plants are often the environments in which *Paenibacillus* is reported to have been found. In order to gain greater insight into the relative abundance of *Paenibacillus* we have analyzed 17 publicly available datasets (Delmotte et al. 2009; Knief et al. 2011; Köberl et al. 2011; Grube et al. 2015; Moissl-Eichinger et al. 2015) including our own datasets from the metagenomes of seven plant habitats, four soils, and three lichens, and compared them with three indoor habitats (Supplementary Table S1). We used sequence-rich datasets based on 16S rRNA gene amplicon sequencing and whole metagenomic shotgun sequencing. The most common preparation technique for amplicon sequencing includes multiplexing of numerous samples which can reduce the sequencing costs and thus facilitate higher replicate numbers for statistically documented results. Metagenomic shotgun sequencing based on next generation high-throughput sequencing is less applicable for high replicate numbers, but delivers functional information in addition to taxonomic assignments based on 16S rRNA and other marker genes. Interpretation of obtained data requires knowledge about the possibilities, but also constraints of the respective approaches. Both techniques are suitable for taxonomic analysis; however they are known to be error-prone to a certain degree (Logares et al. 2014). On the one hand, amplicon-based sequencing relies on PCR-based amplification of predefined targets, and can therefore be affected by amplification-induced errors. On the other hand, metagenomic sequencing of very complex samples often does not result in sufficient coverage of the analyzed sample necessary to completely describe the present diversity (Roh et al. 2010; Zhou et al. 2015). Rarefaction analyses are useful in estimating the species richness and the coverage within specific datasets. The majority of the datasets analyzed were below saturation due to high bacterial diversity.

Within analyzed biomes, 0–4.06 % of the bacterial sequences were assigned to *Paenibacillus* spp. (Supplementary Table S1). In all rhizosphere samples, *Paenibacillus* was abundant and represented 0.7–2.05 % of the microbiome (Knief et al. 2011). We found no difference in the utilized datasets between plants

grown under humid or arid conditions. Timmusk et al. (2009) employed a qPCR-based approach to quantify *P. polymyxa* in the rhizosphere of wild barley sampled from arid soils. The observed abundance was significantly higher than in the adjoining microclimate, suggesting a possible role in adaptive co-evolution of plants. It was also shown that *P. polymyxa* isolates from the arid soil rhizosphere samples were metabolically different from their counterparts in moderate soils (Timmusk et al. 2011). Interestingly, both the highest and the lowest observed percentages of *Paenibacillus* spp. were found in the plant phyllosphere (Delmotte et al. 2009; Knief et al. 2011), which reflects the extremely changeable conditions of this habitat. The plant phyllosphere is directly exposed to external stress factors, hence more susceptible to fluctuations. Furthermore, host specificity is a key factor which shapes the taxonomic composition and spectrum of the bacterial microbiome on various plant species. *Paenibacillus* was also substantially abundant in all soil samples studied (Köberl et al. 2011). Different lichen species were shown to harbor rather small proportions of *Paenibacillus* (0.01–0.04 %; Grube et al. 2015). In contrast, high *Paenibacillus* abundance was found in cleanrooms (0.1–4.0 %; Moissl-Eichinger et al. 2015). These artificial habitats are repeatedly exposed to decontamination-related stress, and therefore spore-forming bacteria may have better chances of survival or at least be more persistent. While metagenomics is a powerful new technique allowing analyzing and quantifying bacterial species present in different environments, we still have to rely on experimental data in order to speculate on the habitat-specific modes of action of bacterial species. For example, some studies suggest that *Paenibacillus* spp. adapted to harsh environments are potentially good candidates for use as BCAs or PGPBs with plants that are growing under severe conditions (Köberl et al. 2013; Timmusk et al. 2014).

Paenibacillus as a facultative endophyte

Endophytes as microorganisms that live in the internal tissues of plants without altering the normal functioning of the tissues are very promising and popular as PGPBs or BCAs (Bacon and Hinton 1997; Berg et al. 2005; Hardoim et al. 2015). Endophytic *Paenibacillus* isolates obtained from medicinal plants cultivated on a desert farm (*Matricaria chamomilla* L., and *Solanum distichum* Schumach. and Thonn) were identified as being amongst the most efficient broad-spectrum antagonists against the soil-borne pathogens present (Köberl et al. 2013). Endophytic *Paenibacillus* spp. have been found in association with various plants like *Arabidopsis thaliana*, *Pinus* sp., *Coffea arabica* or *Curcuma longa* (Sakiyama et al. 2001; Bent and Chanway 2002; Timmusk et al. 2005; Aswathy et al. 2013). *P. polymyxa* PB71 isolated from the spermosphere of the Styrian oil pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.) was able to reduce disease severity of the Styrian oil pumpkin caused by the phytopathogenic fungus *Didymella bryoniae* under greenhouse conditions (Fürnkranz et al. 2012a) and led to an increase in harvest yield and a suppression of powdery mildew under field conditions (Fürnkranz et al. 2012b, Table 2). *Paenibacillus* spp. were found in all parts of plants, even in grape berries (Verginer et al. 2010). Although endophytes are generally harmless, the interaction between endophytic *Paenibacillus* spp. and their hosts can be very diverse. For example, it was shown that beneficial *Paenibacillus* spp. can overgrow other endophytic bacteria in plant cell cultures (Ulrich et al. 2008a). While *Paenibacillus* spp. comprised 1.4 % of the total endophytic bacteria isolates colonizing the aerial parts of trees (Ulrich et al. 2008b), they became the predominant endophytic bacteria in poplar tissue cultures when cultured *in vitro* (Ulrich et al. 2008a). Inoculation of poplar seedlings with the enriched *Paenibacillus* isolate resulted in a significantly higher number of roots per cutting and in increased root length when compared with the control plants after 3 weeks (Ulrich et al. 2008b). It was also shown that an endophytic *Paenibacillus* strain strongly affected the composition of the plant metabolites of *in vitro*-grown poplars free from other culturable endophytic bacteria (Scherling et al. 2009). The shifts in the primary metabolism of the poplar plants indicated a mutualistic interaction between the *Paenibacillus* strain, which was capable of fixing nitrogen, and the host plant with altered nitrogen assimilation patterns.

The main difference between endophytic and non-endophytic bacteria is their ability to enter into the plant tissues. *Paenibacillus* spp. are known to produce high amounts of different hydrolyzing enzymes that facilitate plant tissue colonization (Sakiyama et al. 2001; El-Deeb et al. 2013). Even though *P. polymyxa* is generally considered a free-living rhizobacterium, Timmusk et al. (2005) could detect GFP-labelled *P. polymyxa* cells for the first time inside the root tissue by using confocal

laser scanning microscopy (CLSM). However, not all *P. polymyxa* strains are capable of invading the plant tissue. Two PGP *P. polymyxa* strains Pw-2 and L6-16R isolated from lodgepole pine were studied in depth for their ability to live inside plant tissues (Holl and Chanway 1992; Shishido et al. 1996; Anand et al. 2006). While *P. polymyxa* Pw-2 has been identified as an endophyte of the lodgepole pine, the *P. polymyxa* strain L6-16R was not able to enter into the plant tissue even when co-inoculated with an endophytic organism (Shishido et al. 1995; Bent and Chanway 1998). Both strains possessed similar metabolic capabilities with several potentially important exceptions (Shishido et al. 1995). In contrast to the non-endophytic L6-16R isolate, the endophytic Pw-2 strain showed the capacity to metabolize sorbitol, D-melezitose and D-galacturonic acid. Sorbitol is associated with the bacteria's ability to grow anaerobically on highly reduced, scarce substrates; D-melezitose is a sugar detected in the sap of conifers (Lehninger 1975), and D-galacturonic acid is the primary component of pectin, a major component of the middle lamellae of plant cell walls. The ability of the Pw-2 strain to degrade pectin is especially interesting as it may explain how endophytic bacteria avoid cell defense mechanisms. Endophytic bacteria, in particular those that are found in the plants intracellularly, must destroy plant cell walls in order to enter into plant cells. The breakdown products of cell wall components are known to induce systemic disease responses in plants (Heil and Bostock 2002). It was therefore suggested that endophytic bacteria may be able to avoid plant defense mechanisms by metabolizing degradation products of cell wall components like pectin (Anand et al. 2006).

While the bioprotection and plant growth promotion qualities of *Paenibacillus* spp. are at present undisputed, the opposite effect of endophytic *Paenibacillus* spp. on the plant growth has been reported in several cases. For example, *P. polymyxa* was shown to induce mild biotic stress in *A. thaliana* grown under gnotobiotic conditions (Timmusk and Wagner 1999). The bacterial cells invaded the intracellular space of the plant root causing degradation of the root cup and severe root damage resulting in a 30 % reduction of the growth of the plant and a stunting of root systems. A similar phenotype was observed in oilseed rape and cauliflower seedlings when the respective seeds were bioprimed with *Paenibacillus* strains (Rybakova et al. 2015). The *Paenibacillus* cells were found mainly in the intercellular space of oilseed rape roots. Large colonies were observed in the cavities remaining from destroyed plant cells (Fig. 1). Interestingly, this was only observed for plants grown under gnotobiotic conditions in germination pouches. When seeds treated in the same manner were sown in unsterile soil, they did not impair plant growth significantly, and were even shown to promote enhanced plant growth under sterile soil conditions (Rybakova et al. 2015). This observation substantiates the hypothesis that while defined and functioning as a PGP bacterium, *P. polymyxa* can also act as a deleterious bacterium (Timmusk et al. 2005). Root cell damage of *A. thaliana* seedlings by *P. polymyxa* was observed by Timmusk et al. (2005) not only in a gnotobiotic system, but also in sterile and non-sterile soil. The differences between inoculation methods (biopriming of the seeds versus root dipping of the seedlings) as well as the choice of different plant cultivars (oilseed rape and cauliflower versus *A. thaliana*) used in the two studies may in part explain the variations between observations. Because no data are available on the PGP effect of *P. polymyxa* on *A. thaliana* seedlings, the results of both studies cannot be compared in detail.

It has been reported that morphological changes of the root have been associated with auxin production and excretion by PGP bacteria (Xie et al. 1996; Dobbelaere et al. 1999, 2003; Da Mota et al. 2008). Auxin is a class of plant hormones that play a crucial role in the coordination of many growth and behavioral processes in the plant's life cycle and are essential for plant body development (Davies 2010). On the other hand auxin is essential for bacterial phytopathogenesis (reviewed by Hayat et al. 2010). A variety of studies has shown that auxin promotes the sensitization of the host towards the bacterial pathogen and results in the development of disease symptoms (reviewed by Ludwig-Müller 2014). Because many bacterial pathogens can produce auxin, it can be hypothesized that this feature is a part of the strategy used by the pathogen to bypass the plant defense system. It is therefore speculated that this strategy may be utilized by auxin producing *P. polymyxa,* resulting in a deleterious effect on the host plant grown in gnotobiotic conditions. On the other hand, the very recent study of Timmusk (2015) showed that NRP/PK origin compounds produced by *P. polymyxa* may be the primary reason for its mild deleterious influence. The authors constructed a *P. polymyxa* A26 mutant A26Δ*sfp* with inactivated A26 Sfp-type phosphopantetheinyl transferase. This

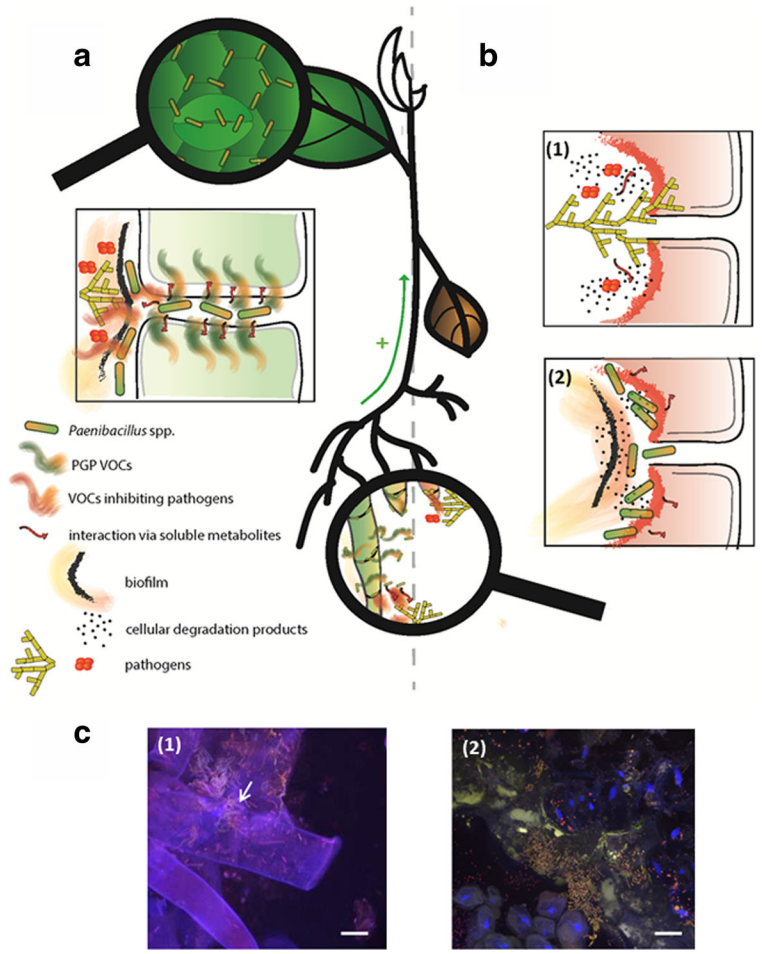

Fig. 1 *Paenibacillus*-plant interactions. *Paenibacillus* spp. are typical soil bacteria that are able to interact with plants and pathogenic microorganisms. They can also colonize plant tissues and thus adopt an endophytic lifestyle (lower magnified image in the illustration (**a**)). The image in **a** illustrates how the interaction with *Paenibacillus* spp. improves plant health. *Paenibacillus* forms a biofilm around the roots to protect root tissues from the pathogens, inhibits the growth of pathogens via soluble and volatile metabolites (indicated as *red arrows* and *orange* volatiles symbols, respectively), and induces systemic resistance (ISR). It also promotes plant growth by solubilizing inorganic elements like phosphorus or nitrogen and produces soluble and volatile metabolites promoting plant growth. The last three factors are summarized in the image as a *green arrow* with a "+" sign. All these factors lead to the development of a plant that is more resistant to pathogens. The image **b** shows how a plant can be exposed to attacks by pathogens in the absence of *Paenibacillus* spp. and/or other beneficial endophytic bacteria (1). Stunting of the root system and inhibition of plant growth may result from degradation of the plant root cells by *Paenibacillus* spp. in the absence of other competing microorganisms and a low nutrient environment (2). The image **c** shows a visualization of *Paenibacillus* colonization patterns by FISH-CLSM using an equimolar ratio of the *Firmicutes*-specific FISH probes LGC354A, LGC354B and LGC354C labeled with the fluorescent dye FITC. *P. polymyxa* Mc2-9 colonies are denoted with arrows. *P. polymyxa* Mc2-9 macrocolonies were detected in the root tissue (1) and cavities of the damaged oilseed rape leaves (2). Bar represents 25 μm. The images are taken from Rybakova et al. (2015)

mutant was characterized by increased biofilm secretion, no lipopeptide antibiotics production and the ability to promote growth and enhance drought tolerance of its host plant *A. thaliana* better than the wild type A26 strain. Of interest, A26 and its extracts caused damage and reduced root growth of the host plant compared to A26Δ*sfp* bacteria and their metabolite extracts suggesting that the presence of NRP/PK polypeptides in A26 metabolites was responsible for the deleterious effect on the plant.

In summary, we propose the following model for the interaction of endophytic *Paenibacillus* spp. with the host plant and pathogens as illustrated in Fig. 1. *Paenibacillus* is a typical soil bacterium that is able to

invade plant roots and live inside plant tissue (Fig. 1a). It builds a biofilm around the roots that functions as a protective layer to prevent access by pathogens (Timmusk et al. 2005, Fig. 1a). Additionally, *Paenibacillus* spp. produce soluble and volatile metabolites that inhibit the growth of pathogens. They also induce plants' defense mechanisms resulting in changes in plant gene expression (Timmusk and Wagner 1999; Fig. 1a). Pathogens may invade plant tissue causing plant diseases in the absence of either *Paenibacillus* spp. or other biocontrol agents (Fig. 1b). Under certain conditions, *Paenibacillus* spp. are capable of degrading plant cells as shown by CLSM (Timmusk et al. 2005; Rybakova et al. 2015, Fig. 1b). This results in stunted root systems and eventually reduced plant growth or even death. We speculate that the aforementioned paradox of the *Paenibacillus*-plant relationship may occur when the balance between *Paenibacillus* spp. and the soil microbiome in gnotobiotic conditions is upset. Under those circumstances *Paenibacillus* spp. are able to overpopulate roots as revealed by CLSM (Fig. 1c). This overpopulation results in nutrient depletion inducing a possible switch in bacterial metabolism that may in turn be mediated by *Paenibacillus* spp. production of auxin and/or in local oversaturation of *Paenibacillus*-derived secondary metabolites that are harmful to the plant. As a result, *Paenibacillus* spp. damage root cells instead of protecting plants from pathogens.

Volatile metabolites mediate extended antagonistic potential

Our studies on volatile organic compounds (VOCs) emitted by cultured *Paenibacillus* spp. have demonstrated that specific metabolites are widely distributed and are furthermore involved in various bacteria-host and pathogen interactions. Moreover, different experimental setups have shown that VOCs-mediated effects not only target other microbes, they also target eukaryotic hosts e.g. higher plants. *Paenibacillus* emitted VOCs were shown to induce systemic resistance in *Arabidopsis* plants and to enhance plant growth at the same time (Lee et al. 2012). For example, *P. polymyxa* strain E681 produced a long chain C13 compound that was found to be partially involved in the observed effects. Additional VOCs were also proposed to be present, but could not be detected within the study. A multitude of interactions of *Paenibacillus* spp. with other organisms can be predicted based on the evidence of the natural occurrence of *Paenibacillus* spp. in different soil types and plant habitats, as well as in the built environment. In a multidisciplinary study, different species were shown to colonize the surface of ripe grapes prior to harvesting. Verginer et al. (2010) demonstrated that *Paenibacillus* spp. were amongst the most abundant bacterial colonizers, and that they have a certain influence on fruit aroma. Different isolates were tested for emitted VOCs in sensory evaluations and solid phase microextraction (SPME) headspace analysis. Not only was a mixture of primarily short-chain alcohols, ketones and aldehydes found in the headspace of living cultures, sulphur-containing and cyclic molecules were also found (Table 3; Verginer et al. 2010; Cernava 2012). In addition to the positive contribution of *Paenibacillus* spp. volatiles to the sensory properties of grapes (and the wine produced from them), these molecules are most likely emitted in order to fulfil vital metabolic functions, and interact with the surrounding species.

While antifungal activities of VOCs were reported in different studies (Liu et al. 2008; Zhao et al. 2011), less is known about volatile *Paenibacillus* metabolites with antibacterial properties. Beck et al. (2003) were able to show that in the polymyxin biosynthesis pathway of a *P. polymyxa* strain, a complex mixture of methyl-branched alkyl-substituted pyrazines was produced. However, the function of these volatiles is not fully resolved, although it is known that pyrazines are involved in bactericidal and chemoprotective activities (Beck et al. 2003). We applied the headspace SPME method developed by Verginer et al. (2010) to specifically screen for alkyl-substituted pyrazines in the headspace of different *Paenibacillus* spp. cultures that were shown to express antagonism against pathogenic bacteria and fungi. The investigated *Paenibacillus* cultures included three plant endophytic bacteria isolated from the Styrian oil pumpkin (Fürnkranz et al. 2012a). In addition, five isolates were obtained from medicinal plants grown in desert farming soils, one isolate from desert farming soils, and one isolate from the adjacent desert soils (Köberl et al. 2013). All of the isolates utilized were shown to emit three distinct pyrazine derivatives into the headspace (Cernava 2012). While 2-methyl-5-(1-methylethyl)-pyrazine and 2-(2-methylpropyl)-3-(1-methylethyl)-pyrazine were emitted in species-specific proportions, 2,3,5-trimethyl-6-propyl-pyrazine was the most prominent molecule in all samples. Bacterial pyrazine emission was shown to be both species-specific and strain-specific. *P. polymyxa*

Table 3 Gas chromatography – mass spectrometry headspace SPME identification of volatile metabolites from *Paenibacillus* spp. cultures grown on slope agar in headspace vials

Substance in headspace	Method of identification
Acetaldehyde[2]	spectral database
Ethanole[2]	spectral database
Hydroxyurea[2]	spectral database
Cycloserine[2]	spectral database
Butanal[2]	spectral database
Ethoxyethene[2]	spectral database
2-butanone[1]	reference substance
1-butanol[2]	spectral database
2-methyl-1-propanol[1,2]	reference substance
Methyl-3-methylbutanoate[1]	reference substance
2-pentanone[1]	reference substance
3-hydroxy-2-butanone[2]	spectral database
2-ethyl-1-butanol[2]	spectral database
3-methyl-1-butanol[1]	reference substance
2-methylbutan-1-ol[1,2]	reference substance
Methoxy-phenyl-oxime[2]	spectral database
Benzaldehyde[2]	spectral database
Dimethyl disulfide[1]	reference substance
2-heptanone[1]	reference substance
6-methyl-5-hepten-2-one[1]	reference substance
Dimethyl trisulfide[1]	reference substance
2-methyl-5-(1-methylethyl)-pyrazine[2]	spectral database
Trimethylpyrazine[1]	reference substance
2,3,4-trimethyl-5-propyl-pyrazine[2]	spectral database
2-(2-Methylpropyl)-3-(1-methylethyl)-pyrazine[2]	spectral database
2-ethyl-1-hexanol[1]	reference substance
Phenylacetaldehyde[1]	reference substance
3-methylbutanoic acid[1]	reference substance
2-phenylethanol[1]	reference substance

[1] Substances identified in the headspace above *Paenibacillus* sp. T2B1c.1-B (Verginer et al. 2010)

[2] Substances identified in the headspace above *Paenibacillus* spp. from desert soils and Styrian oil pumpkin-associated isolates (Cernava 2012)

strains that were isolated from different habitats were shown to produce specific proportions of volatile pyrazines. We have studied the antibiotic effects of *Paenibacillus*-derived pyrazines extensively, and have demonstrated that they suppress the growth of bacteria, fungi and yeast under laboratory conditions (Cernava 2012). Antibiotic effects of the detected metabolites were further demonstrated by the subsequent utilization of synthetic alkyl-substituted pyrazines. Concentrations as low as 0.20 pmol cm^{-3} of synthetic compounds were shown to decrease the viability of the same pathogens that are targeted by *Paenibacillus* spp. in dual-culture experiments (Cernava 2012). More interestingly, all synthetic VOCs were administered through the headspace in order to mimic the natural mode of action, although substances applied directly to the growth media were also shown to be highly effective (Cernava 2012).

Conclusion and outlook

1. In our survey on *Paenibacillus* we found that the global mode of action against pathogens or other microorganisms is very similar to those reported for *Bacillus*, however *Paenibacillus* spp. appear to be generally less studied than its closely related genus *Bacillus*. The main reason for this lack of knowledge on *Paenibacillus* spp. is that *Paenibacillus* spp. and especially *P. polymyxa* are particularly hard to manipulate: only recently techniques were discovered that allowed genetic manipulation of some *Paenibacillus* strains (Kim and Timmusk 2013). Although the extent of research is far greater for *Bacillus*, for both genera the mode of action includes antibiosis, lysis, competition and induced resistance (Emmert and Handelsman 1999; Govindasamy et al. 2011). Nitrogen fixation is reported as being a unique feature of *Paenibacillus* with one exception published by Ding et al. (2005). The detailed comparison of the metabolites produced by *Bacillus* and *Paenibacillus* spp. revealed that the spectrum of soluble and especially volatile metabolites produced by both genera is highly diverse and is rather species- or strain- than genus-specific (Liu et al. 2008; Govindasamy et al. 2011; Lee et al. 2012 and this study). Strains of both genera produce powerful weapons, e.g. lipopetides against plant pathogens (Ongena and Jacques 2008). Therefore, *Paenibacillus* strains have an enormous potential in biotechnology as a source of novel bioactive compounds, and to date this potential has only partially been exploited. The antibacterial effect of pyrazine derivatives (and VOCs in general) opens a new door to developing techniques for the suppression of multi-resistant bacterial

pathogens—one of the most important future challenges facing mankind (Woolhouse and Farrar 2014).

2. Our review demonstrates the huge potential of *Paenibacillus* spp. as PGPBs and/or BCAs. Those strains with an endophytic lifestyle show especially interesting capacities. The easy formulation and high shelf life of *Paenibacillus* strains due to their spore formation abilities is an additionally positive quality. In general there is only a low risk of *Paenibacillus* infection of higher animals or humans. Although several cases of infection by *Paenibacillus* have been reported, this interaction was found to be influenced by the susceptibility of the host. Only hosts with a predisposition were reported to have been infected, and the infection was restricted to several *Paenibacillus* species. The genus *Paenibacillus* was characterized as having high intraspecific diversity, as well as strain-specific modes of action and effects on plants. We also observed system-dependent growth effects, and noticed a much more positive effect on the host plant under natural rather than under gnotobiotic conditions in one particular case study (Rybakova et al. 2015). Therefore, the evaluation and risk assessment should be done at strain level and under natural conditions.
3. In future, we need a much deeper insight into the *Paenibacillus* ecology and physiology. For example, *Bacillus subtilis* was shown to act as a multicellular organism (Aguilar et al. 2007) responsible for biofilm formation on the root surface as well as for biocontrol (Vlamakis et al. 2013). Moreover, this biofilm formation is induced by plant polysaccharides (Beauregard et al. 2013) but also by cannibalism of the *Bacillus* strains themselves (López et al. 2009). In addition, we have no insight into the epigenom of plant-associated *Paenibacillus* strains, which can have an enormous impact on the effect. Such knowledge on *Paenibacillus* can improve their biotechnological applications.

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