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MINI-REVIEW

Pantocin A, a peptide-derived antibiotic involved in biological control by plant-associated *Pantoea* species

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Abstract

The genus *Pantoea* contains a broad range of plant-associated bacteria, including some economically important plant pathogens as well as some beneficial members effective as biological control agents of plant pathogens. The most well-characterized representatives of biological control agents from this genus generally produce one or more antimicrobial compounds adding to biocontrol efficacy. Some *Pantoea* species evaluated as biocontrol agents for fire blight disease of apple and pear produce a histidine-reversible antibiotic. Three commonly studied histidine-reversible antibiotics produced by *Pantoea* spp. are herbicolin O, MccEh252, and pantocin A. Pantocin A is a novel ribosomally encoded and post-translationally modified peptide natural product. Here, we review the current knowledge on the chemistry, genetics, biosynthesis, and incidence and environmental relevance of pantocin A and related histidine-reversible antibiotics produced by *Pantoea*.

Keywords *Pantoea agglomerans* · *Pantoea vagans* · Herbicolin O · MccEh252 · Histidine-reversible · Biocontrol · Fire blight · *Erwinia amylovora*

Introduction

The ability of microorganisms to synthesize antibiotic compounds is one of the many traits that can confer a selective advantage to producing bacteria in competition with other microorganisms in specific ecological habitats (Raaijmakers and Mazzola 2012). This principle has been utilized by plant microbiologists for the development of biocontrol agents against plant diseases. A well-studied system of biocontrol agents is the interaction of antagonists with the fire blight pathogen *Erwinia amylovora* (Johnson and Stockwell 1998; Malnoy et al. 2012). Among the agents for biocontrol of fire blight, the genus *Pantoea* includes some of the most effective antagonists (Pusey 2002; Johnson et al. 2004; Stockwell et al. 2010). Three of these *Pantoea* strains (*Pantoea agglomerans* strains E325 and P10c), and *Pantoea vagans* C9-1 [formerly *Pantoea agglomerans* C9-1 (Rezzonico et al. 2009)] have been developed into plant protection products, have gone through the registration processes by national regulatory authorities and are now commercially available alternatives and/or complements to antibiotic use in Canada, New Zealand, and the USA (BlightBan C9-1™, BloomTime Biological™, and BlossomBless™).

Although other mechanisms by which *Pantoea* strains suppress plant diseases like nutritional competition and preemptive exclusion (Wilson and Lindow 1994; Braun et al. 1998; Stockwell et al. 2010; Smits et al. 2011) are known as well, a variety of antibacterial organic acids and peptide antibiotics (Vanneste et al. 1992; Wodzinski et al. 1994; Wright et al. 2001; Stockwell et al. 2002; Pusey et al. 2011; Kamber et al. 2012a) have been described. Many *Pantoea* strains produce their own specific antibiotic: *Erwinia herbicola* strain B247, whose taxonomic identity has not been updated, produces herbicolin A (Kempf et al. 1993), *P. agglomerans* strain Eh1087 produces a phenazine antibiotic

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(Kearns and Mahanty 1998; Giddens et al. 2002), while the biocontrol activity of *P. agglomerans* E325 is correlated positively to production of a unique antibiotic of unknown structure (Pusey et al. 2008, 2011).

When tested on minimal media containing free amino acids, it was noticed that antibiosis by several *P. agglomerans* strains was reduced in the presence of L-histidine (Ishimaru et al. 1988; Wodzinski and Paulin 1994). As these strains were of the most effective strains in biocontrol, the antibiotics were studied further. It was soon discovered that *P. vagans* C9-1 produced three antibiotics, of which one, herbicolin O, was histidinereversible (Ishimaru et al. 1988), and this compound has later been described as pantocin A (Ishimaru et al. 2017). A second antibiotic in this strain has been identified as a dapdiamide antibiotic, called herbicolin I (Ishimaru et al. 1988; Dawlaty et al. 2010; Kamber et al. 2012a), whereas a third antibiotic has not yet been chemically identified. The histidine-reversible antibiotic called MccEh252 of the taxonomically ambiguous *Pantoea* sp. (Rezzonico et al. 2009) strain Eh252 was identified as a microcin (Vanneste et al. 2002b). *P. agglomerans* Eh318 produced two antibiotics, of which one, pantocin B, was arginine-reversible (Brady et al. 1999; Wright et al. 2001), while the second called pantocin A was histidine-reversible and its chemical structure was determined (Wright et al. 2001; Jin et al. 2003a). The genes for biosynthesis of pantocin A, herbicolin O and MccEh252 are virtually identical (Vanneste et al. 2008); consequently, we will use the name pantocin A for these antibiotics throughout this review.

Properties of pantocin A

Isolation of pantocin A from *P. agglomerans* Eh318 suffered from low yields and its acid, base and thermal lability. Therefore, it was impossible to obtain sufficient starting material for chemical analysis (Vanneste et al. 2002b; Jin et al. 2003a). This problem was solved by the expression of the pantocin A genes on a high-copy number plasmid in *E. coli*. The structure (Fig. 1) was then determined by spectroscopic methods and the synthesis of a hydrogenated derivative. Finally, the molecular formula of the heterologously expressed compound was established as $C_{13}H_{16}N_4O_5$ with a molecular mass of 308.3 g/mol. In contrast to *P. agglomerans* Eh318, it was possible to enrich active pantocin A from *P. vagans* C9-1 from a culture medium using several chromatography steps (Ishimaru et al. 1988, 2017).

Properties associated with a histidine-reversible antibiotic from culture filtrates, partially purified and purified antibiotic preparations from the three *Pantoea* strains

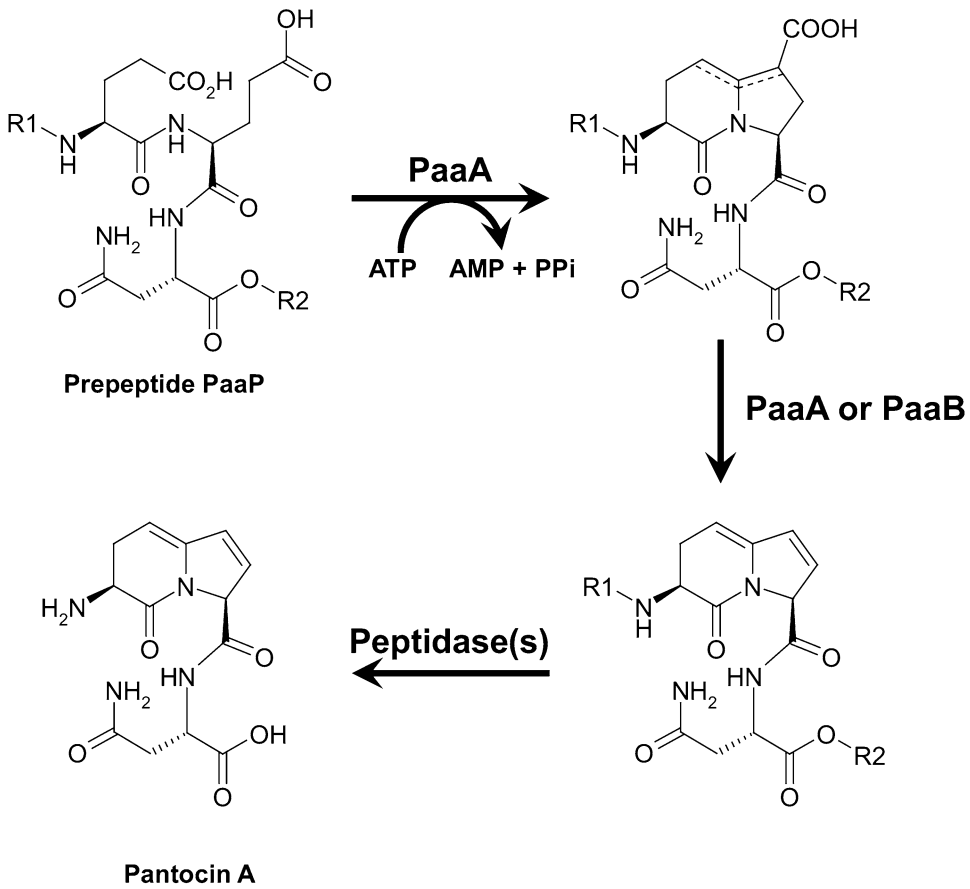

Fig. 1 Potential pathway from the PaaP precursor oligopeptide R-Glu-Glu-Asn-R′ to pantocin A. Picture adapted from Ghodge et al. (2016)

were variable (Table 1), but similarities were observed as well (Vanneste et al. 2008). Pantocin A, herbicolin O and MccEh252 are all histidine-reversible peptide-derived antibiotics and have a molecular mass below 3500 g/mol, as estimated by passage through a dialysis membrane (Ishimaru et al. 1988; Wright et al. 2001; Vanneste et al. 2002b), a reported value far above the calculated molecular mass of 308.3 g/mol (Jin et al. 2003b). More exact measurements of the molecular weight from pantocin A producing strains were not reported in the early descriptions of herbicolin O and MccEh252. It was later confirmed that herbicolin O has the same molecular weight as pantocin A (Ishimaru et al. 2017). Differences were observed in the inactivation of pantocin A by pronase, pH range and sensitivity to high temperatures (Table 1). Inactivation studies characterizing heat, pH and amino acid sensitivities have not been conducted systematically with all three compounds. Thus, some of the reported differences could be explained by experimental differences, including the type of media and the purity of the antibiotic preparations used in the bioassays. Results from experiments to characterize stability are also difficult to interpret because two of the three pantocin A-producing strains produce at least one additional antimicrobial compound. It remains unclear whether MccEh252 and similar histidine-reversible antibiotics from *Pantoea* are identical to pantocin A, because structures have been confirmed for only pantocin A and herbicolin O; however, genetic evidence corroborates that MccEh252 is, if not identical structurally, also a ribosomally encoded and post-translationally modified peptide (Vanneste et al. 2002b).

Mechanism of action and inhibition of enteric bacteria

Based on cross-feeding experiments using other tripeptides, the effect of pantocin A on *E. amylovora* could be suppressed by the addition of the tripeptide Ala-Gly-Gly (Jin et al. 2003a). This finding indicates that the compound is entering the cell via tripeptide transporters. To be effective in suppressing the growth of *E. amylovora*, pantocin A requires a nitrogen-poor environment, where import by tripeptide transporters and amino acid biosynthesis is essential.

Once pantocin A enters the cell, it blocks the enzyme L-histidinol phosphate aminotransferase, an enzyme involved in the biosynthetic pathway of L-histidine (Jin et al. 2003a), explaining why the inhibition by pantocin A is histidine-reversible. The deficiency for histidine caused by blocking the conversion of imidazole acetol phosphate to L-histidinol is abrogated by externally supplied L-histidine and L-histidinol (Ishimaru et al. 1988; Jin et al. 2003a).

The best-characterized target organism inhibited by pantocin A is *E. amylovora*, but other *Enterobacteriaceae* are also inhibited and include current and former plant pathogenic *Erwinia* species (*Erwinia rhapontici, Pectobacterium carotovorum, Dickeya dadantii*) and several *Pantoea* spp.

Table 1 Properties of three histidine-reversible antimicrobial compounds identified in culture supernatants and purified or partially purified preparations of pantocin A, herbicolin O and MccEh252

Characteristic[a]	Pantocin A[b]	Herbicolin O[c]	MccEh252[d]
Histidine-reversible	Yes	Yes	Yes
Molecular weight	< 3000 Da	< 3500 Da	< 1200 Da
pH range	Acid and base sensitive	Labile to acid (pH 3.5) and base (pH 10)	Active 3.0 < pH < 9.0
Temperature	Thermal labile	Stable at room temperature 3 days	No loss after boiling for 20 min
IC_{50}	200 nM	ND	ND
Pronase	ND	Resistant (diffusion in agar)	Sensitive (culture supernatant)
Proteinase K	ND	ND	Sensitive
A506 AprX	ND	Sensitive	Sensitive
BSA	ND	ND	Insensitive
Produced by	*P. agglomerans* Eh318	*P. vagans* C9-1	*Pantoea* sp. Eh252
Produced during	ND	ND	Late log/stationary phase
Effect on *E. amylovora*	Inhibits L-histidinol phosphate aminotransferase	Not a bacteriocin	Inhibits growth, does not kill
Gene sequences available	Yes	Yes	No (but sequenced)
Heterologously expressed in *E. coli*	Yes	Yes	Yes

[a]ND: no data available

[b]Data from Wright et al. (2001), Jin et al. (2003a, b) and Walterson et al. (2014)

[c]Data from Ishimaru et al. (1988, 2017), Davis and Ishimaru (1993), Anderson et al. (2004) and Smits et al. (2011)

[d]Data from Vanneste et al. (2002b, 2008) and Anderson et al. (2004)

(Ishimaru et al. 1988; Vanneste et al. 1992; Wright et al. 2001). Here, some differences in the spectrum of activity observed among the three pantocin A producers may be explained by the presence of other antibiotics, or by other growth inhibition mechanisms, like acidification (Pusey et al. 2008). The partially purified histidine-reversible antibiotic of *P. vagans* C9-1 was tested on a broader collection of *Enterobacteriaceae* and shown to inhibit many strains thereof (Ishimaru et al. 1988). This indicates that pantocin A has a broader spectrum of activity compared to herbicolin I from *P. vagans* C9-1 (Ishimaru et al. 1988; Kamber et al. 2012a) or to a base-labile antibiotic from *P. agglomerans* E325 (Pusey et al. 2008).

Genetics and biochemistry of pantocin A biosynthesis

Cloning of the genes from three pantocin A producing bacteria (Fig. 2) (Davis and Ishimaru 1993; Wright et al. 2001; Vanneste et al. 2008) was achieved using a cosmid library generated and introduced in *E. coli*. Resulting clones were tested for their ability to produce histidine-reversible pantocin A.

The *P. agglomerans* Eh318 pantocin A biosynthesis gene cluster was identified from a cosmid library followed by sub-cloning of smaller fragments (Wright et al. 2001). This process yielded a 3.5 kb fragment that contained all sequences required for biosynthesis of pantocin A. Sequence analysis of the insert identified three open reading frames, *paaABC*, plus a small precursor protein-coding gene *paaP* (Fig. 2). For *P. vagans* C9-1, one of around 2000 clones from a cosmid library in pLAFR3 was positive for pantocin A biosynthesis (Davis and Ishimaru 1993; Ishimaru et al. 2017). The genome sequence of C9-1 revealed that the *paaPABC* genes were located on a 28 kb low G+C genomic island on the chromosome of this strain (Smits et al. 2010, 2011). The *Pantoea* sp. Eh252 pantocin A biosynthesis cluster was located on an around 3.2 kb fragment (Vanneste et al. 2008). The genes of *Pantoea* sp. Eh252 were 96–99% identical to the pantocin A genes of *P. agglomerans* Eh318 and *P. vagans* C9-1, but also to the gene clusters from 23 other *P. agglomerans* strains (Vanneste et al. 2008).

The *paaP* gene encoding the precursor of pantocin A is located in the upstream region of the *paaABC* gene cluster. This ribosomally produced 30-amino acid protein belongs to the pantocin A family RiPP [TIGR04310] including members of ribosomally synthesized and post-translationally modified peptide (RiPP) precursors about 30–50 amino acids in length (Ghodge et al. 2016). The small open-reading frame was discovered as the *paaABC* genes expressed from the T7 promoter did not yield in pantocin A biosynthesis (Jin et al. 2003b). In the central region of the gene, where the precursor is encoded, the sequences of *paaP* are absolutely conserved but single nucleotide polymorphisms are in the flanks (Vanneste et al. 2008). The core region, containing the three important residues, is modified by PaaA and PaaB (Fig. 1) and subsequently clipped to pantocin A by the host proteases (Ghodge et al. 2016).

The *paaA* gene encodes for a ThiF family of ubiquitin-like proteins [COG0476]. The protein has a Rossmann-fold NAD(P)H/NAD(P)(+) binding (NADB) domain [NCBI CDD: cd00757]. Its role in the biosynthesis of pantocin A was to catalyze the double dehydration and decarboxylation of the two glutamic acid residues in the 30-amino acid precursor protein PaaP in an ATP and Mg^{2+}-dependent

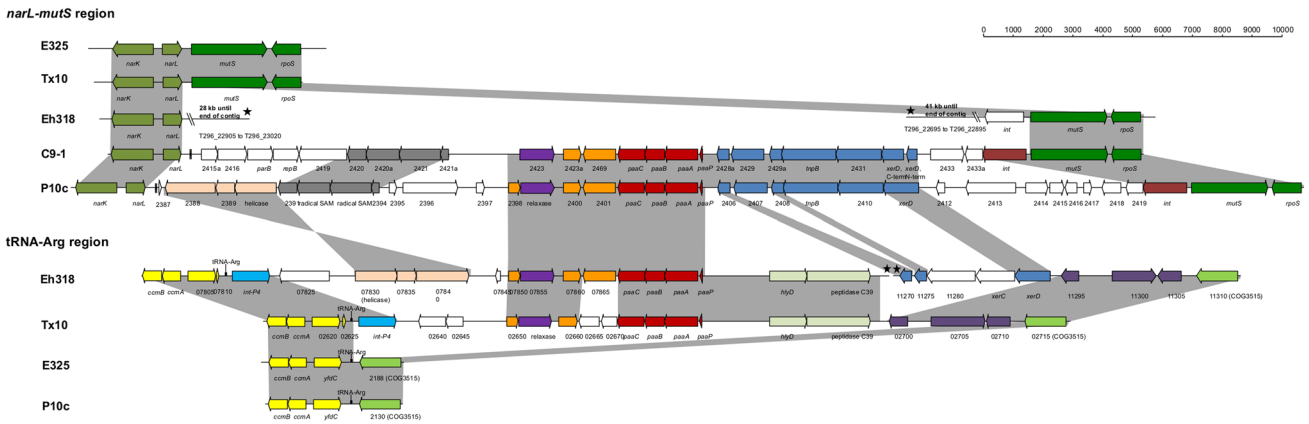

Fig. 2 Genetic mapping of pantocin A islands and their insertion sites in genome sequenced strains of *Pantoea agglomerans* and *Pantoea vagans*. For *P. agglomerans* P10c, Eh318 and Tx10, both potential insertion sites are shown. The corresponding sites in *P. agglomerans* E325 are shown as an example of a strain that lacks a pantocin A biosynthetic gene cluster. The pantocin A biosynthesis gene cluster (red arrows), non-conserved genes (white arrows), conserved blocks (arrows with other colors), conserved regions (grey shading), and contig ends with potential for non-contiguity (stars) are indicated

reaction (Fig. 1) (Ghodge et al. 2016). The protein acts as a homodimer with two domains: an adenylation domain and a smaller globular domain that recognizes the precursor peptide.

The enzyme encoded by *paaB* is a member of the 2-OG-Fe^{II} oxygenase superfamily [pfam13640]. It might be involved in the oxidation of a precursor of pantocin A (Jin et al. 2003b). It was speculated that this protein may perform a two-electron dehydrogenation to yield the conjugated olefin in pantocin A (Fig. 1) (Ghodge et al. 2016).

The gene *paaC* encodes a putative transmembrane protein. When cloned in *E. coli*, this gene conferred resistance to pantocin A (Jin et al. 2003b). It is a member of the EamA-like transporter family [pfam00892], a family that includes many hypothetical membrane proteins of unknown function and was formerly called DUF6. Distantly related orthologs were found to be encoded on many bacterial genomes, sometimes associated with other antibiotic biosynthesis genes (Kamber et al. 2012a).

Functional characterization of the *paaPABC* gene cluster

The function of the pantocin A biosynthesis genes has been shown for all three characterized strains. Direct mutants of the pantocin A biosynthesis genes were generated by marker exchange for *P. agglomerans* Eh318 (Wright et al. 2001) and by transposon mutagenesis for *Pantoea* sp. Eh252 (Vanneste et al. 1992). In the case of *P. agglomerans* Eh318, the mutant still produced pantocin B, but also a double mutant was obtained. The double mutant lacked any antibiosis activity against *E. amylovora* (Wright et al. 2001). The mutants of *Pantoea* sp. Eh252 were significantly reduced in their ability to inhibit *E. amylovora in vitro* and prevent fire blight in orchard trials (Vanneste et al. 1992; Stockwell et al. 2002).

For *P. agglomerans* Eh318, transposon mutagenesis and linker scan analysis were done on the plasmid containing the 3.5 kb fragment (Jin et al. 2003b). Positions for insertions were determined by sequencing. Most insertions in *paaA, paaB* and *paaC* gave inactive clones, while some clones with reduced activity had an insertion in *paaC*. The latter mutants produced pantocin A but grew poorly. In a similar experiment with the cosmid clone containing the pantocin A biosynthetic gene cluster of *P. vagans* C9-1, out of 300 EZ::TN mutants tested, 26 mutants lost the ability to produce pantocin A (Lansdell 2000). Of these 26 mutants, the flanking regions of six mutants were sequenced. Five of them were located within *paaA* or *paaB*, and one was located in the promoter region approximately 150 bp upstream of *paaP* (Ishimaru et al. 2017).

Genomic context and occurrence of the pantocin A gene cluster

The advent of genome sequencing has delivered much information about the genetic environment of the pantocin A biosynthetic gene cluster. Currently, five *Pantoea* spp. are sequenced that contain the complete pantocin A biosynthesis cluster (Fig. 2), P. *agglomerans* strains Eh318 (Walterson et al. 2014), Tx10 (Smith et al. 2013) and P10c (Smits et al. 2015), *P. vagans* C9-1 (Smits et al. 2011) and *Pantoea stewartii* strain S301 (Accession number LIIU00000000; Adam and Tambong, unpublished). However, there are also other closely related *Pantoea* genome sequences available that do not contain the *paaPABC* cluster (Adams et al. 2011; De Maayer et al. 2012; Matsuzawa et al. 2012; Walterson et al. 2014), indicating the rare presence in the total *Pantoea* community (see below).

Analysis of the genomic context in which the pantocin A biosynthetic genes are inserted shows that the operon was acquired (lower G + C content) and that they are inserted in a region that is highly variable containing many, largely different, fingerprints of mobile genetic elements. As described below, two chromosomal insertion positions were observed: either at the 3′ end of the *mutS* gene or directly adjacent to a tRNA-Arg.

The pantocin A genomic island in *P. agglomerans* Eh318 and the clinical isolate Tx-10 is inserted next to a tRNA-Arg (Fig. 2). Directly adjacent to the tRNA, a P4 phage-like integrase was found that could be responsible for the insertion. At the other side of the cluster, the type VI secretion system 3 (T6SS-3) (De Maayer et al. 2011) is located. In *P. agglomerans* Eh318, the 31-kb pantocin A region is non-contiguous (Fig. 2) and located on two contigs. It can thus not be excluded that an additional contig would be located within this gene cluster. The gene content of the clusters in the two strains is largely similar, but there are some larger differences, one of which is located on a contig border in *P. agglomerans* Eh318.

In the genome of *P. vagans* C9-1 (Smits et al. 2010), the pantocin A biosynthetic gene cluster was found on a 28 kb genomic island (Fig. 2). The analysis of its flanking regions showed that a genomic island is inserted in the N-terminus of the *mutS* gene (Smits et al. 2011). The cognate XerD family integrase is present and intact. For the insertion, a 77 bp repeat is present that is part of the *mutS* N-terminus (57 bp overlap), but this repeat also was found directly downstream of *narL*, at the other flank of the genomic island. In the biocontrol strain *P. agglomerans* P10c (Vanneste et al. 2002a), a 35-kb genomic island containing the pantocin A biosynthesis genes was found, with a conserved core around the *paaPABC* cluster but with variable flanking regions (Fig. 2).

Comparing both types of genomic islands, some conserved regions are present, but there are also large variations. The four genes immediately downstream of *paaC* appear to be conserved in all strains (except in *P. agglomerans* Tx10). Some genes upstream of the pantocin A biosynthesis clusters of *P. vagans* C9-1 and *P. agglomerans* P10c have high sequence identity to genes found in the genomic island of *P. agglomerans* Eh318, but the region in between varies in its gene content. Furthermore, a cluster of genes is shared only between *P. agglomerans* P10c and Eh318, although inverted in these strains. This all shows that these pantocin A genomic islands have quite a mosaic structure and may be prone to continuous modifications. Nevertheless, it cannot be excluded that the pantocin A operons in the genomic islands have a common ancestry.

In early publications, many *P. agglomerans* strains were reported to produce antibiotics that lost their activity in presence of L-histidine (El-Goorani and Beer 1991; Wodzinski and Paulin 1994). A later study showed that of 88 antibiotic-producing *P. agglomerans* strains, the DNA of 61 strains hybridized on Southern blots using a *paaB* probe (Wright et al. 2001; Jin et al. 2003b), indicating that the majority of these strains may have contained the pantocin A gene cluster.

In contrast, the pantocin A cluster was rarely found in a collection of *Pantoea* strains of different origins (i.e., clinical, environmental, diseased plants, and biocontrol isolates). In the diverse collection of *Pantoea* strains, the *paaABC* gene cluster was observed only in the strains already known to produce pantocin A (Rezzonico et al. 2009; Kamber et al. 2012a), a result confirmed by quantitative real-time PCR (qPCR) (Braun-Kiewnick et al. 2012). We speculate that the high prevalence of the *paaABC* cluster in the group of 88 antibiotic-producing *P. agglomerans* strains was because they were preselected for their ability to inhibit *E. amylovora* and for histidine to reverse the inhibition (Wodzinski and Paulin 1994; Wright et al. 2001), whereas the *Pantoea* collection consisted of strains representing the broad (taxonomic?) diversity within the genus (Rezzonico et al. 2009, 2010).

Clusters containing orthologs of PaaPABC are also found in the genomes of other organisms (Ghodge et al. 2016), but these are more distantly related (Fig. 3). Unlike the clusters in *D. chrysanthemi* Ech1591 and *E. hoshinae* NBRC 105699, these clusters encode a PaaP ortholog with, in many cases, a different amino acid (lysine, isoleucine or aspartic acid) instead of the asparagine at the related precursor peptide position (Fig. 3), indicating that these clusters are involved in the production of a different antibiotic with a relatively conserved structure, but a different side chain. Up to now, these products have neither been isolated nor tested for their potential antibiotic activity.

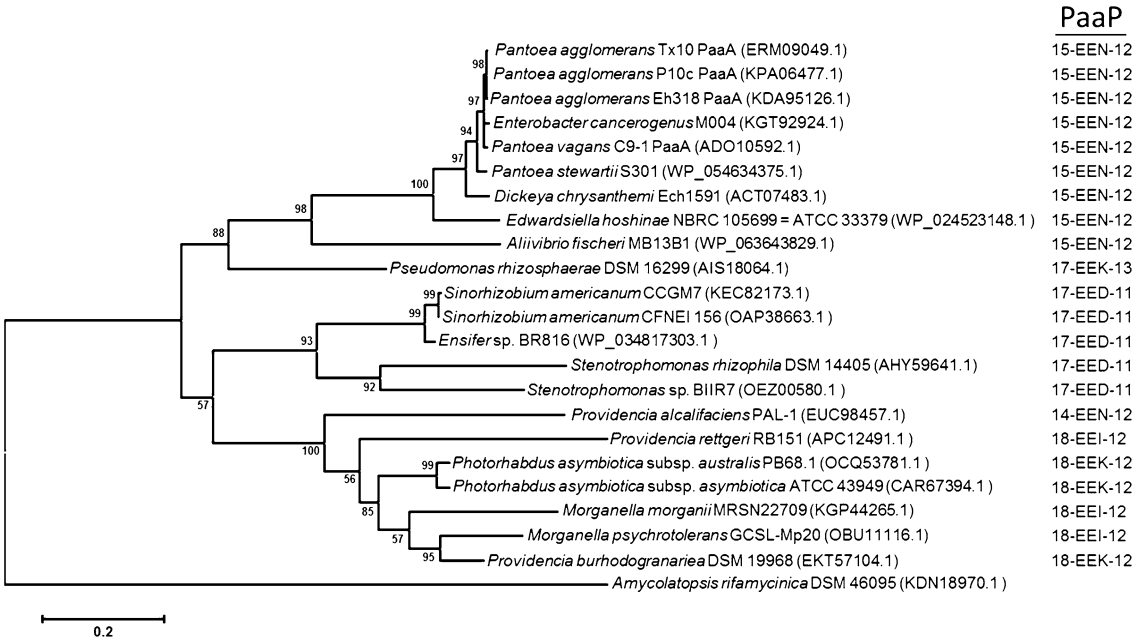

Fig. 3 Maximum likelihood tree of the *Pantoea vagans* C9-1 PaaA protein and its direct orthologs found in genome sequences (in parentheses: NCBI accession numbers). Redundant proteins outside *Pantoea* were omitted. As outgroup, the PaaA ortholog of *Amycolatopsis rifampicinica* DSM 46095 was used. The three-amino acid core sequence of the PaaP protein of each gene cluster is indicated in the column at the right side, flanked by the number of amino acids upstream and downstream

Regulation of the pantocin A biosynthesis genes

The pantocin A biosynthesis gene clusters that are characterized were expressed in *E. coli* as part of the selection procedure for the cloning and sequencing (Jin et al. 2003b; Vanneste et al. 2008). For this, the native promoter was used, as it also contains the *paaP* gene in this region, required for the biosynthesis. The 3.5 kb region cloned for *P. agglomerans* Eh318 does not contain any genes with homology to regulatory elements and the genes were active in the heterologous host (Jin et al. 2003b). This finding indicates that this cluster might be constitutively expressed in *E. coli*.

A recent study (Klein et al. 2017) describes the effect of different nutrients on the transcription from the *paaA* promoter in *P. vagans* C9-1 using a GFP fusion. In principle, the use of a defined minimal medium with gluconate as a carbon source allows an efficient transcription from the *paaA* promoter, while the components present in rich media like LB inhibit transcription. Addition of tryptone or yeast extract to the defined minimal medium increased growth, but also reduced transcription. Whereas growth on minimal medium with gluconate allowed good transcription, the addition of several amino acids did not improve production and in some cases even may be inhibitory (Klein et al. 2017). It shows thus that the promoter is active in media that have low protein content.

Other *Pantoea* species containing the pantocin A biosynthesis cluster

At the time of the first discovery of pantocin A biosynthesis, all isolates were included in the species *Erwinia herbicola* (Ishimaru et al. 1988; Wodzinski and Paulin 1994), later separated to *Enterobacter agglomerans* and *P. agglomerans* (Gavini et al. 1989; Rezzonico et al. 2009). Afterwards, numerous taxonomic reorganizations reallocated many of the *E. agglomerans* strains into new genera and species (Brady et al. 2009, 2010, 2012; Rezzonico et al. 2009, 2010; Pillonetto et al. 2018). One of the most prominent pantocin A producers, strain C9-1 (Ishimaru et al. 1988), was identified as *P. vagans* C9-1 during this process (Rezzonico et al. 2010; Smits et al. 2011). *Pantoea* sp. Eh252 is more distantly related to the type species of *P. agglomerans*, but was excluded from the species *P. agglomerans sensu stricto* (Rezzonico et al. 2010) and its exact taxonomic position remains to be solved.

While using this ecologically diverse *Pantoea* collection for detection of the pantocin A gene cluster (Rezzonico et al. 2009; Braun-Kiewnick et al. 2012), it was observed that strain LMG 5343, now recognized as the type strain of the species *Pantoea brenneri* (Brady et al. 2010), also was positive for the pantocin A cluster with the tested primer sets. This strain is a clinical isolate (Lindh et al. 1991; Brady et al. 2010) and it has not been tested for its ability to inhibit growth of *E. amylovora* or other members of the *Enterobacteriaceae*. Similarly, the gene cluster was detected in the clinical *Pantoea conspicua* strain EM17cb using qPCR (Braun-Kiewnick et al. 2012), but this positive result was not observed with the *paaABC* primer set (Rezzonico et al. 2009). As a genome is not yet available, it is still unknown whether this strain contains a similar pantocin A cluster to other *Pantoea* isolates. Nevertheless, these results indicate that the pantocin A biosynthetic cluster can be mobilized in diverse *Pantoea* spp.

Using a qPCR assay (Braun-Kiewnick et al. 2012), it was possible to evaluate the presence of the *paaB* gene in field samples. Of 108 samples, eight samples were positive using the pantocin A primer-probe set, but samples had a low number of pantocin A producers per flower. Of the eight samples, four were positive for the *P. agglomerans pagR* primer-probe set, indicating that the other samples must have contained pantocin A producing bacteria not belonging to *P. agglomerans* (Braun-Kiewnick et al. 2012). This result correlates with the detection of pantocin A biosynthesis genes in other *Pantoea* spp. (Rezzonico et al. 2009; Kamber et al. 2012b). However, it also shows the presence of the pantocin A biosynthesis genes in untreated orchards at quantifiable amounts.

The role of pantocin A in biocontrol

The pantocin A gene containing strains *Pantoea* sp. Eh252 and *P. vagans* C9-1 has been tested in orchards for their ability to suppress growth of *E. amylovora* and reduce the incidence of fire blight. Both strains established well on apple and pear flowers in orchards and significantly decreased the incidence of detectable *E. amylovora* populations, thereby reducing the disease incidence (Johnson et al. 2000; Stockwell et al. 2011). The ability to produce pantocin A contributes to the biological control efficacy and was demonstrated in experiments comparing the biocontrol ability of wild-type *Pantoea* sp. Eh252 and Tn5 transposon mutants of *Pantoea* sp. Eh252 that were deficient in biosynthesis of pantocin A. The mutants were not as effective as *Pantoea* sp. Eh252 in reducing the severity of fire blight symptoms in an immature pear bioassay (Vanneste et al. 1992). Complementation of the mutant with a cosmid containing the pantocin A genes restored the ability to suppress *E. amylovora* in assays (Vanneste et al. 1992). In orchard experiments, population sizes of the mutant and wild-type *Pantoea* sp. Eh252 were not

different on flowers, but the effectiveness of the mutant to reduce the incidence of fire blight was significantly lower than the wild-type *Pantoea* sp. Eh252 (Stockwell et al. 2002). The mutant, however, still reduced the incidence of fire blight slightly compared to water-treated controls, indicating that other mechanisms, such as competition, also contribute to suppression of fire blight by *Pantoea* sp. Eh252 (Stockwell et al. 2002).

The stability of pantocin A on flowers, as influenced by other bacteria, can also affect the efficacy of biological control of fire blight by *Pantoea* sp. Eh252 and *P. vagans* C9-1. It was demonstrated *in vitro* that the extracellular metalloprotease secreted by the fire blight biocontrol agent *P. fluorescens* A506 degrades pantocin A produced by *Pantoea* sp. Eh252 and *P. vagans* C9-1 (Anderson et al. 2004). Treating flowers on trees in orchards with a combination of *P. fluorescens* A506 with *Pantoea* sp. Eh252 or *P. vagans* C9-1 reduced efficacy of biocontrol of fire blight compared to treatment with single strains of *Pantoea* (Stockwell et al. 1996, 2010). In orchard trials, *Pantoea* sp. Eh252 or *P. vagans* C9-1 combined with an extracellular protease-negative mutant of *P. fluorescens* A506 AprX^{-} (Anderson et al. 2004) provided better control of fire blight than when the biocontrols were applied as single strains (Stockwell et al. 2011).

Conclusions

Pantocin A is a peptidic antibiotic that is integral to microbial interactions in the fire blight pathosystem and efficacy of biological control of fire blight by some strains of *P. agglomerans, Pantoea* sp. and *P. vagans*. In the past years, much was learned about this antibiotic and its bacterial producers. Ecological studies on the establishment, multiplication, and spread of *Pantoea* sp. in orchards have been conducted (Johnson et al. 2000) and pantocin A can be regarded as a model antibiotic involved in biocontrol of *E. amylovora*. The genetics and function of pantocin A in the environment also are well described. The chemistry of its biosynthesis is less so, but what has been described is providing new insights into this and other ribosomally encoded and post-translationally modified peptide natural products. Additionally, although pantocin A-producing *Pantoea* strains do not reduce growth of *P. fluorescens* on flowers (Stockwell et al. 2011), little is known about the influence of pantocin A and growth of pantocin A-producing *Pantoea* strains on the microbiome of apple and pear flowers. By allowing risk assessment of potential non-target impacts on plants or indigenous bacterial communities in orchards, information from microbiome studies will prove vital for future registration of pantocin A producing *Pantoea* strains for the biological control of fire blight in Europe and elsewhere (Berg et al. 2007).

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