Invited Review

ERWINIA AMYLOVORA IN THE GENOMICS ERA: FROM GENOMES TO PATHOGEN VIRULENCE, REGULATION, AND DISEASE CONTROL STRATEGIES

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SUMMARY

The publication of the first *Erwinia amylovora* genome has greatly accelerated and advanced our understanding of the fire blight organism. With the availability of multiple genomes, it quickly became clear that chromosomal diversity is relatively small, and that most of the pan-genome variance is attributable to plasmids. In addition to gaining a more detailed view of the known virulence factors, genomics has enabled new breakthrough studies of virulence regulation mechanisms. Furthermore, several niche adaptation and ecological fitness factors, though not directly influencing virulence, have been studied in greater detail, providing novel insights into the physiology and ecology of the bacterium. Additionally, application of genome data has yielded improved diagnostics and enabled population studies at different geographic scales.

Keywords: intraspecies diversity, niche adaptation, type III secretion system, diagnostics.

INTRODUCTION

Fire blight, the first plant disease to be described to be caused by a bacterium, is able to kill complete pome fruit orchards within a single growing season (Vanneste, 2000). After being detected first in North America, the disease has subsequently spread to New Zealand, Europe, North Africa, the Middle East, Russia, Central Asia and South Korea (Bonn and van der Zwet, 2000; Djaimurzina *et al*., 2014; Myung *et al*., 2016). This rapid and seemingly uncontrollable spread may reflect the relative weakness of its hosts to the disease, but also the strengths of the pathogen (Malnoy *et al*., 2012).

The yearly economic losses to commercial pome fruit orchards caused by fire blight infections are enormous (Duffy *et al*., 2005). A major line of research has thus been to start studying the genetics of its causative agent, *Erwinia amylovora*, with the aim to understand why the organism is this effective as a pathogen. Whereas in the past individual virulence factors were identified that largely or partly explained the pathogenicity (Oh and Beer, 2005), the era of genomics after the introduction of next generation sequencing devices has given us the chance of expanding this research to the genome scale. In contrast, efforts made to study the effect of the pathogen on the apple tree (Baldo *et al*., 2010; Kamber *et al*., 2016; Sarowar *et al*., 2011; Vrancken *et al*., 2013) are still in its early phase of understanding, mainly due to the complexity of the apple tree as a system.

Although another *E. amylovora* genome was sequenced as well, the first genome of *E. amylovora* to be published was the genome of the French isolate CFBP 1430 (Smits *et al*., 2010b). Though of most economical importance, *E. amylovora* was not the first species in the genus to be fully sequenced, as the genomes of *Erwinia tasmaniensis* Et1/99 (Kube *et al*., 2008) and *Erwinia pyrifoliae* DSM 12163^T (Smits *et al*., 2010a) were published before. This review intends to update current knowledge of the biology of *E. amylovora* that has been obtained since its genome was published in 2010. We will particularly discuss genomic insight of this pathogen in the larger context of its ecology, evolution and population genetics.

INTRA- AND INTERSPECIES DIVERSITY OF *E. AMYLOVORA* AT THE GENOMIC LEVEL

***E. amylovora* genomics.** Currently, fifteen genome sequences of *E. amylovora* strains are publically available, of which three isolates are reported to specifically be pathogenic on *Rubus* spp. (Mann *et al*., 2013; McManus and Jones, 1995; Smits *et al*., 2014b; Starr *et al*., 1951). Many further genome sequences are still unpublished, including

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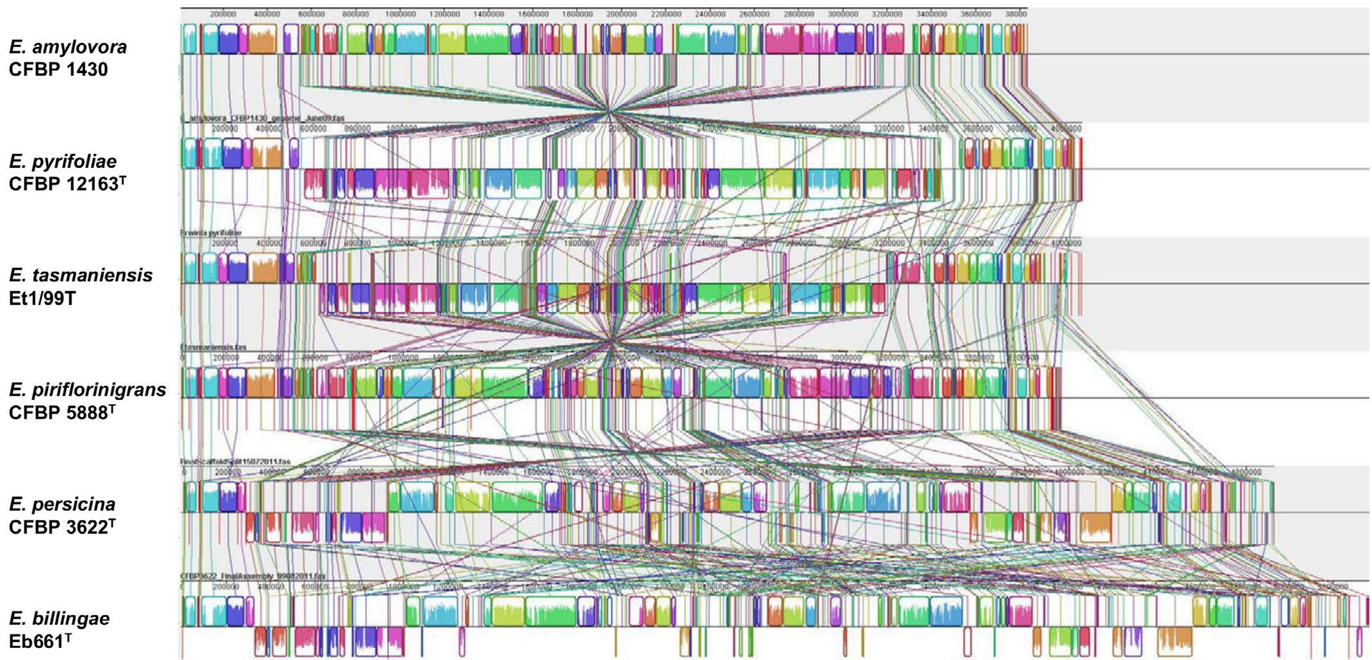

Fig. 1. Mauve alignment of the (draft) genomes of *E. amylovora* CFBP 1430, *E. pyrifoliae* DSM 12163^T, *E. tasmaniensis* Et1/99^T, *E. piriflorinigrans* CFBP 5888^T, *E. persicina* CFBP 3622^T and *E. billingiae* Eb661^T. All strains have plasmids of different sizes.

additional *Rubus* isolates. Though largely biased to Spiraeoideae strains belonging to the economically and more worldwide spread CRISPR group I (Rezzonico *et al.*, 2011), this collection of genomic data allows the conclusion that there is only a limited diversity within this cosmopolitan CRISPR group, with sequence identities between two strains exceeding 99.99% (Smits *et al.*, 2010b) as demonstrated by the presence of less than 2000 SNPs in the complete dataset of CRISPR I strains (T.H.M. Smits, unpublished information). On the other hand, the diversity among the genomes of *Rubus*-infecting strains is substantially higher (Mann *et al.*, 2013), while also more diverse sequences of Spiraeoideae isolates have been obtained (T.H.M. Smits, unpublished information).

A major factor contributing to genomic diversity in Spiraeoideae-infecting isolates of *E. amylovora* is the presence of different plasmids in individual strains (Ismail *et al.*, 2014; Llop *et al.*, 2012). This has an influence on the total pan-genome of *E. amylovora*, leading to the impression that the pan-genome of the species is open. On the other hand, comparing only the chromosomal sequences, the pan-genome of Spiraeoideae-infecting isolates appears nearly closed (Mann *et al.*, 2013).

Intraspecies diversity of *E. amylovora*. Many studies have already observed that it is relatively easy to separate *Rubus*-infecting isolates, currently reported only from USA and Canada (Braun and Hildebrand, 2005; Heimann and Worf, 1985; Ries and Otterbacher, 1977; Starr *et al.*, 1951), from Spiraeoideae-infecting isolates, constituting the only, most certain differentiation at the subspecies level for *E. amylovora*. Initial studies have unequivocally shown that *Rubus*-infecting isolates are unable to infect Spiraeoideae hosts and vice versa (Braun and Hildebrand, 2005; Heimann and Worf, 1985; Ries and Otterbacher, 1977; Starr *et al.*, 1951). Molecular studies based on housekeeping genes (Rezzonico *et al.*, 2012b), rep-PCR, ribotyping (McManus and Jones, 1995), ITS (McGhee *et al.*, 2002b) and CRISPR analysis (Rezzonico *et al.*, 2011) allowed a fast assay to identify new strains. However, despite the long history of *E. amylovora* isolation, only few *Rubus*-infecting isolates are currently described and available (Rezzonico *et al.*, 2012b).

Using the genome sequences, first efforts were undertaken to resolve the genetic base of the differential host range of *Rubus*-infecting isolates. Rezzonico *et al.* (2012b) have observed three major groups of *Rubus*-infecting isolates, each of them currently supported by a genome sequence (Mann *et al.*, 2013). When comparing the genomes to those of Spiraeoideae-infecting isolates, several small-scale differences were observed. The gene cluster encoding lipopolysaccharide biosynthesis genes in *Rubus*-infecting isolates is substantially different from that of Spiraeoideae-infecting isolates (Rezzonico *et al.*, 2012b). Furthermore, *Rubus*-infecting isolates contain a larger IT region (Mann *et al.*, 2012). Analysis of the Hrp pathogenicity cluster showed that there are differences in single genes that exceed the average genome variation. Especially the *eop1* gene, which shares 99% sequence identity within the *Rubus*-infecting isolates, has only 67% sequence identity to that of Spiraeoideae isolates. Deletion of *eop1* from both Spiraeoideae-infecting and *Rubus*-infecting strains does not affect virulence. However, the addition of the *Rubus eop1* to Spiraeoideae-infecting strains reduced their virulence on apple shoots, indicating that *eop1* can function as a host specificity determinant (Asselin *et al.*, 2011).

Table 1. Selected factors analyzed by comparative genomic approaches in a variety of genome-sequenced *Erwinia* spp. Only a single genome sequence is reported for each species. A "+" indicates the presence of the factor, a "–" indicates absence. The exopolysaccharides (EPS) are differentiated as amylovoran-like (AMS) or stewartan-like (CPS), while CRISPR systems are differentiated as *Escherichia coli*-type *cas* genes (Ecoli) or *Yersinia pestis*-type *cas* genes (Ypest).

Species and strain	Hrp T3SS	Inv/Spa T3SS	EPS-type	Desferri-oxamine	CRISPR	pEA29-like plasmid	References
E. amylovora CFBP 1430	+	+	AMS	+	Ecoli	+	(Smits *et al.,* 2010b)
E. pyrifoliae DSM 12163^T	+	+	AMS	+	Ecoli, Ypest	+	(Smits *et al.,* 2010a)
E. tasmaniensis Et1/99^T	+	+	CPS	+	Ypest	–	(Kube *et al.,* 2008)
E. piriflorinigrans CFBP 5888^T	+	+	AMS	+	Ypest	+	(Smits *et al.,* 2013)
E. persicina CFBP 3622^T	–	–	CPS	–	–	+	Smits *et al.*, unpublished
E. billingiae Eb661^T	–	–	CPS	+	–	–	(Kube *et al.,* 2010)
E toletana DAPP-PG735	–	–	CPS	–	–	–	(Passos da Silva *et al.,* 2013)
E. tracheiphila PSU1	+	+	CPS	–	–	–	(Shapiro *et al.,* 2016)
E. gerundensis EM595^T	–	–	CPS	–	–	(+)	(Rezzonico *et al.,* 2016)

The *Rubus*-infecting isolates contain a 20 kb NRPS/PKS gene cluster close to the CRISPR regions (Rezzonico *et al.*, 2011), which has eroded in the genome of Spiraeoideae-infecting isolates (Mann *et al.*, 2013).

Interspecies comparisons. When comparing the genomes of *E. amylovora* to those of the closely related pathoadapted *Erwinia* spp. (*E. pyrifoliae*, *E. tasmaniensis* and *Erwinia piriflorinigrans*) (Kamber *et al.*, 2012; Smits *et al.*, 2013), it was observed that *E. amylovora* has the smallest genome size (3.8 Mb; Fig. 1) (Smits *et al.*, 2010b). This is even more evident when comparing to the genome of the epiphyte *Erwinia billingiae* Eb661 (Kube *et al.*, 2010). Although members of the closely related genus *Pantoea* may also have chromosomes as small as 4 Mb (Kamber *et al.*, 2012), they appear not to have undergone a pathoadaptation process. On the other hand, the inclusion of several large gene clusters encoding proven and putative pathogenicity factors (Table 1), like diverse secretion systems and a second flagellum in the genomes of the pathoadapted species (Kamber *et al.*, 2012; Smits *et al.*, 2010b; Zhao and Qi, 2011), clearly shows that significant genome reduction must have taken place during the pathoadaptation process, as the size of the additional gene clusters sums up to more than 1 Mb, while the overall size of the genomes did not increase. Based on the comparison of the core genomes of *Pantoea* spp. and *E. billingiae* Eb661 (Kamber *et al.*, 2012), it was hypothesized that the latter species has not undergone pathoadaptation, supporting the conclusion of a more generalist behavior for this species (Mergaert *et al.*, 1999). With the addition of more genomes of this genus including the recently described novel species *Erwinia gerundensis* (Rezzonico *et al.*, 2016), it was observed that pathoadaptation might have been induced several times during the shaping of the genus, as other species like *Erwinia tracheiphila* also show a pathoadaptation pattern (Shapiro *et al.*, 2016).

Plasmids. In recent years, the sequencing of multiple genomes has led to the discovery of many new plasmids (Table 2). Whereas the plasmid pEA29 is almost ubiquitous (Bühlmann *et al.*, 2014; Mann *et al.*, 2013), other plasmids appear to be accessory material. Up to now, none of the plasmids has been shown to have an influence on the virulence of *E. amylovora* strains (Ismail *et al.*, 2014; Llop *et al.*, 2011). The novel plasmids are most probably derived from the general environmental plasmid pool, which has also been used as base for plasmids conferring multidrug antibiotic resistance to clinical isolates (Mann *et al.*, 2013; Zhang *et al.*, 2016; Zhu *et al.*, 2009).

Plasmid pEA29 only shows diversity at few loci. It contains the short sequence repeat that was used for early strain typing (Table 1) (Kim and Geider, 1999; Schnabel and Jones, 1998), but also a second repeat, which allows the separation of larger populations (Bühlmann *et al.*, 2014). Within the genome dataset, one isolate was included to have the streptomycin (Sm) resistance cassette in the transposon Tn*5393* on pEA29 (Chiou and Jones, 1993). Additional diversity is present in the pEA29 plasmids from *Rubus*-infecting strains (McGhee *et al.*, 2002a).

New plasmids have been identified within the frame of the genome sequencing, like pEA30 and several small plasmids from *Rubus*-infecting isolates (Mann *et al.*, 2013) (Table 2). The plasmid pEI70, currently only detected in Europe, contains a non-integrative ICE-element (Llop *et al.*, 2011). Plasmid pEA68, isolated from Poland and Belgium (Ismail *et al.*, 2014), is member of a plasmid family that includes the large plasmid pEA72 from strain ATCC 49946 (Sebaihia *et al.*, 2010), but also a 78 kb plasmid found in a Mexican and a Californian strain (Ismail *et al.*, 2014; Smits *et al.*, 2014a, 2014b). Within the set of unpublished sequences, many other plasmids were found, that will be described in the near future (T.H.M. Smits, unpublished information).

NICHE ADAPTATION FACTORS

Bacteria are able to adapt to environmental conditions at a certain location. This niche specialization process can be supported by the loss of functionalities involved in global survival in the environment (Moran, 2002), and

Table 2. Plasmids described in *E. amylovora* strains.

Plasmid name	Original strain(s)	Size (kb)	Description	Replicon type	Mobilization type	Reference(s)
pEA29	Nearly all strains	28	Standard plasmid, *thiOSGF*	IncF	None	(McGhee and Jones 2000; Smits *et al.,* 2010b)
pEA34	NW1/1	35	pEA29 with *Tn5393*, *strAB*	IncF	None	(Chiou and Jones 1993; Smits *et al.*, unpublished)
pEA68	692	68	*mobAB, pil, tra*	IncFIIA	P13	(Ismail *et al.,* 2014)
pEA72	ATCC 49496	71	*mobAB, pil, tra*	IncFIIA	P13	(Sebaihia *et al.,* 2010)
pEA78	LA637	78	*mobAB, pil, tra*	IncFIIA	P13	(Ismail *et al.,* 2014; Smits *et al.,* 2014b)
pEL60	LebB66	60	*mobAB, tra*	IncL/M	P13	(Foster *et al.,* 2004)
pEI70	ACW56400	65	ICE-like plasmid	IncF	C1	(Llop *et al.,* 2011)
pEU30	UTRJ2	30	*virB*	IncL/M	P6	(Foster *et al.,* 2004)
pEA30	Ea495	30	*virB*	IncU	P4	(Mann *et al.,* 2013)
pEA8.7	CA3R	8.7	Identical to RSF1010, *strAB, sul2*	IncQ	Q1	(Palmer *et al.,* 1997)
pEAR5.3	ATCC BAA-2158	5.3		ColE1	P5	(Mann *et al.,* 2013)
pEAR4.2	ATCC BAA-2158	4.2		ColE1	P5	(Mann *et al.,* 2013)
pEA1.7	IH-3	1.7		Unknown	None	(McGhee *et al.,* 2002b)
pEA2.8	IL-5	2.8	Cryptic plasmid, not found with resequencing the strain	ColE1	None	(Mann *et al.,* 2013; McGhee *et al.,* 2002b)

may also include the acquisition of novel genetic material (Casadevall, 2008). Niche adaptation factors may include virulence factors (*i.e.* factors that are involved in the gain of access to and survival in host systems, factors that damage the host or factors that cause dysregulation of the host cellular function) and environmental fitness factors (Hill, 2012). The latter group includes products and strategies involved in attachment, macro and micronutrient acquisition, colonization and microbe-host communication strategies. The available genome sequences of *E. amylovora* have allowed the examination of individual niche adaptation factors, involved in virulence, regulation and adaptation. Below, we summarize recent findings.

The Hrp Type III Secretion System (T3SS). Most Gram-negative bacterial pathogens rely on type III secretion systems (T3SS) for the interaction with their host (Tampakaki *et al.*, 2010). The T3SS forms a needle structure that is able to penetrate the host cell wall and to deliver effector proteins directly into the cytosol of the eukaryotic host cell. The T3SSs in *Yersinia*, *Escherichia*, *Salmonella* and *Shigella* have been extensively studied as they have potentially devastating health effects on human life (Naum *et al.*, 2011). In non-host plants, the T3SSs of plant pathogens cause a hypersensitive response, a collapse of plant tissue several hours after inoculation followed by tissue necrosis as a result of programmed cell death, as a reaction to incompatible pathogens (Tampakaki *et al.*, 2010).

In *E. amylovora*, the Hrp T3SS is one of the most important virulence factors and is absolutely required for pathogenicity (Malnoy *et al.*, 2012; Oh and Beer, 2005). Comparable systems are also present in the other pathoadapted *Erwinia* spp. (Table 1) (Kamber *et al.*, 2012) and the plant pathogen *Pantoea stewartii* subsp. *stewartii* DC283 (Roper, 2011), that might have a common origin (Kirzinger *et al.*, 2015). In contrast to previous reports (Oh and Beer, 2005), novel insights from genomics have shown that the Hrp T3SS was independently introduced from the so-called IT region (Mann *et al.*, 2012). This latter region constitutes a remnant of an Integrative Conjugative Element (ICE), for which both border regions could be identified outside the Hrp T3SS region in the genome of *E. piriflorinigrans* CFBP 5888^T (Smits *et al.*, 2013).

Many plant pathogens have a whole suite of T3SS effectors, that are selected based on the individual host range of the organism (Grant *et al.*, 2006; Hajri *et al.*, 2009). *E. amylovora* has, in contrast, only eight effectors. The genes of four effectors are located within the Hrp T3SS gene cluster (Smits *et al.*, 2010b): harpins HrpN and HrpW, the host-range limiting factor Eop1 and the essential effector DspA/E (Asselin *et al.*, 2011; Malnoy *et al.*, 2012; Oh and Beer, 2005; Smits *et al.*, 2010b). The four remaining effectors are spread over the genome, and are, in contrast to the previous set, only present in *E. amylovora* while not in the other pathoadapted *Erwinia* species (Malnoy *et al.*, 2012; Smits *et al.*, 2011a). One of the latter proteins, the effector AvrRpt2, is a cysteine protease with most similarity to the *Pseudomonas syringae* AvrRpt2 (Zhao *et al.*, 2006). It was shown that a single nucleotide polymorphism in the *E. amylovora avrRpt2* gene is responsible to overcome the *Malus*×*robusta* 5 resistance to fire blight (Vogt *et al.*, 2013), yet it does only affect but not break the resistance of *Malus fusca* (Emeriewen *et al.*, 2015). Genomic sequencing has shown that this mutation is present in a few natural isolates from the USA and Mexico (Smits *et al.*, 2014a, 2014b; Vogt *et al.*, 2013).

Exopolysaccharides – Amylovoran. Bacterial polysaccharides include lipopolysaccharide (LPS), lipooligosaccharide (LOS) and extracellular polysaccharide (EPS). The EPS is described to play a role in the plant-pathogen interactions, but also in maintaining convenient conditions for pathogen growth (de Pinto *et al.*, 2003). The production of the EPS amylovoran is the another most important virulence factor for *E. amylovora* involved in biofilm

formation (Koczan *et al.*, 2009) and defense against antimicrobial compounds (Ordax *et al.*, 2010). Deletion of the biosynthetic *ams* genes or the regulatory *rcs* genes in *E. amylovora* leads to a loss of virulence (Bernhard *et al.*, 1996; Koczan *et al.*, 2009; Wang *et al.*, 2009). The monomer structures of amylovoran and the related EPS stewartan of *P. stewartii* subsp. *stewartii* show some significant differences (Schollmeyer *et al.*, 2012), that are also reflected in the genetic complement of these two strains and their sequenced relatives (Table 1) (Kamber *et al.*, 2012). Recent studies have shown that two additional gene clusters are present in *E. amylovora* that are required for the export of alternative monomers to the periplasm (Langlotz *et al.*, 2011; Wang *et al.*, 2012c). Based on a complementation study, the additional genes were shown to be reduced active in *E. amylovora*, whereas these genes are required for the major structure of stewartan (Wang *et al.*, 2012c).

Regulation of virulence factors. A regulatory cascade is responsible for the regulation of the Hrp cluster of *E. amylovora* (Khan *et al.*, 2012; Zhao, 2014). Molecular genetic studies of *E. amylovora* pathogenesis demonstrated that expression of the *hrp*-T3SS genes is activated by the master regulator HrpL, a member of the exocytoplasmic functions (ECF) subfamily of sigma factors (Wei and Beer, 1995). Subsequently, HrpL binds to the *hrp* box in the promoter regions of structural genes and effectors of the T3SS and allows transcription of these genes (Oh and Beer, 2005; Wei *et al.*, 2000). The regulon of HrpL includes all *hrp* and *hrc* genes and five putative T3SS effectors. As eight genes displayed apparent indirect HrpL regulation, it was suggested that the HrpL regulon of *E. amylovora* encompasses more than just T3SS regulation and that HrpL may communicate with other signaling networks to coordinate gene expression during pathogenesis (McNally *et al.*, 2012). In *E. amylovora*, *hrpL* transcription is in turn controlled by sigma factor 54 (RpoN), which along with its modulation protein YhbH and an integration host factor (IHF), interacts with enhancer binding protein (EBP) HrpS to trigger the onset of T3SS (Ancona *et al.*, 2014; Lee and Zhao, 2016; Lee *et al.*, 2016). Furthermore, it has been recently reported that the linear nucleotide second messengers, guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), are also essential for the expression of the T3SS and virulence by activating the RpoN-HrpL alternative sigma factor cascade (Ancona *et al.*, 2015; Zhao and Sundin, 2017).

Two-component signal transduction systems (TCSTs), consisting of a histidine kinase (HK) and a response regulator (RR), represent a major paradigm for signal transduction in prokaryotes. TCSTs play critical roles in sensing and responding to environmental conditions, and in bacterial pathogenesis. Genome-wide screening of two-component system identified four groups of mutants which exhibited varying levels of amylovoran production *in vitro* (Zhao *et al.*, 2009b). Among them, the Rcs phosphorelay system is an essential regulatory system for pathogenicity and gene expression of virulence factors, especially of amylovoran (Wang *et al.*, 2009, 2011). Microarray studies identified a large set of genes that are regulated by this system. While RcsB acts as a positive regulator, the sensor kinase RcsC positively controls *ams* cluster expression *in planta* but negatively *in vitro* (Wang *et al.*, 2012b). In addition, the EnvZ/OmpR and GrrS/GrrA (GacS/GacA) systems, two widely distributed TCSTs in gammaproteobacteria, negatively co-regulate amylovoran biosynthesis and T3SS in *E. amylovora* (Li *et al.*, 2014). It has recently been demonstrated in *E. amylovora* that negative regulation of virulence by GrrS/GrrA acts through the non-coding small regulatory RNA *csrB/rsmB* sRNA, which binds to the RNA-binding protein CsrA/RsmA and neutralizes its positive effect on T3SS gene expression and amylovoran production (Ancona *et al.*, 2016). Thus, CsrA plays a central role in *E. amylovora* virulence and in positive regulation of T3SS and amylovoran (Ancona *et al.*, 2016).

Genetic screening also identified other global regulatory genes for amylovoran biosynthesis, including AmyR, Lon protease, and H-NS (Eastgate *et al.*, 1995; Hildebrand *et al.*, 2006; Wang *et al.*, 2012a). The novel regulator AmyR (EAMY_1304) plays a role of a negative regulator in the regulation of virulence factors including amylovoran biosynthesis (Wang *et al.*, 2012a). Mutation of the *amyR* gene leads to a mucoid phenotype. In a background of amylovoran-overproducing mutants in other two-component signal transduction genes, overexpression of *amyR* leads to a strong reduction of amylovoran production (Wang *et al.*, 2012a), showing that this regulator acts at a different level.

Two other global pathways controlling virulence regulation in *E. amylovora* have recently been discovered. The second messenger compound cyclic di-GMP (c-di-GMP) also extensively regulates the major pathogenesis systems in *E. amylovora* including the T3SS, amylovoran biosynthesis, biofilm formation, and motility (Edmunds *et al.*, 2013). Cyclic di-GMP impacts transcriptional regulation of target genes through binding to protein regulators or to riboswitches; in addition, binding to c-di-GMP can functionally activate proteins (Römling *et al.*, 2013). Intracellular levels of c-di-GMP in *E. amylovora* are controlled through the actions of five diguanylate cyclase enzymes that synthesize c-di-GMP and three phosphodiesterase enzymes that degrade the molecule (Edmunds *et al.*, 2013). High intracellular levels of c-di-GMP in *E. amylovora* promote amylovoran synthesis and biofilm formation while simultaneously inhibiting the T3SS.

The second global regulatory pathway involves non-coding regulatory small RNAs (sRNAs) that require the RNA chaperone Hfq for stability and functional activation. An *E. amylovora hfq* mutant exhibits severely-reduced virulence, most likely due to a reduction in translocation of DspA/E into host cells and other impacts on proteins secreted by the T3SS (Zeng *et al.*, 2013). In addition, amylovoran production is significantly reduced in the *hfq*

mutant, and cells exhibit a hyper-attaching phenotype (Zeng *et al.*, 2013). *E. amylovora* encodes at least 40 Hfq-dependent sRNAs, and deletion of at least three of these (*arcZ* [*ryhA*], *hrs21*, *rprA*) results in significant reductions in virulence in an immature pear model (Zeng *et al.*, 2013). Additional Hfq-dependent sRNAs including *hrs6* and *omrAB* significantly affect motility and amylovoran production (Zeng and Sundin, 2014).

It is clear that the regulatory networks for *E. amylovora* virulence factors are highly complex and may be far more complex than currently known. Understanding how all of the varied regulatory pathways of *E. amylovora* intersect and respond to environmental signals is the next challenge in the development of a complete model of pathogenesis in this organism.

Inv-Spa-type T3SSs. Additionally to the Hrp T3SS, *E. amylovora* also contains two Inv/Spa-type T3SSs (Smits *et al.*, 2010b), a situation also observed in *E. piriflorinigrans* CFBP 5888^T (Table 1) (Smits *et al.*, 2013). Only one of these is present in *E. pyrifoliae*, while the second is also partially present in *E. tasmaniensis* Et1/99 (Kamber *et al.*, 2012). As these T3SS were proven to not play a role in host plant pathogenicity (Zhao *et al.*, 2009a) and resembled the Inv/Spa T3SS of the insect symbiont *Sodalis glossinidius* str. *morsitans* (Dale *et al.*, 2001; Kirzinger *et al.*, 2015), they were hypothesized to play a potential role in the interaction with insects (Smits *et al.*, 2010b). This hypothesis is supported by a recent study describing that the Inv/Spa-type T3SS in *P. stewartii* subsp. *stewartii* DC283 is required for persistence in its flee beetle vector *Chaetocnema pulicaria* (Correa *et al.*, 2012). Although a close vector relationship is not recognized with *E. amylovora*, it would be worth examining the potential roles for Inv/Spa T3SSs in the *E. amylovora* life cycle.

Autoinduction and quorum-sensing. In Gram-negative bacteria, two major quorum-sensing systems are known, that are defined by the chemical structure of the autoinducer (AI) signal molecule (Miller and Bassler, 2001). For both systems, a conclusive support of their presence is lacking for *E. amylovora*. Previous reports on the presence of the gene pair *expRI*, encoding the enzyme responsible for the production of the AI-1 signal *N*-acylhomoserine lactone (AHL) and its cognate receptor in *E. amylovora* (Molina *et al.*, 2005; Venturi *et al.*, 2004), were not confirmed by the annotation of multiple genomes within the species (Mann *et al.*, 2013; Smits *et al.*, 2010b).

Whereas the genomes of *E. tasmaniensis* Et1/99 and *E. piriflorinigrans* CFBP 5888^T encode orthologs of the *Pectobacterium carotovorum expRI* genes (Sabag-Daigle and Ahmer, 2012; Smits *et al.*, 2011a, 2013), the absence of the *expRI* genes in *E. amylovora* can be explained by the replacement of the region between the *smpB* and *tsx* genes, which contains the quorum-sensing genes in *E. tasmaniensis* Et1/99 and *E. piriflorinigrans* CFBP 5888^T, through a flagellar gene cluster in *E. amylovora* CFBP 1430 and *E. pyrifoliae* DSM 12163 (Smits *et al.*, 2011a, 2010b). Indeed, a BlastN search with a previously sequenced DNA fragment (GenBank Accession No. AJ841286) purportedly spanning the *expRI* genes in *E. amylovora* (Venturi *et al.*, 2004) resulted in high sequence identity within the genomes of *Pectobacterium* spp., but no hits to *E. amylovora*. Furthermore, *in silico* analysis on all the sequenced *E. amylovora* genomes did not yield any viable annealing site neither for the EAM1/EAM2 primer pair described in the latter work nor for the AHLea-fw/-rev primer pair designed by (Molina *et al.* (2005), yet both primer sets showed a perfect match with the complete genome sequence of *Pectobacterium carotovorum* SCC3193 (GenBank Accession No. CP003415). This suggests that in both studies, the positive amplification may have been the outcome of a contamination with *Pectobacterium* spp. DNA in the template used for the PCR. In addition to these inconsistencies on a genomic basis, phenotypic tests gave rise to contradictory results. The purported AHL signal was identified to be either *N*-(3-oxo-hexanoyl)-homoserine lactone (3-oxo-C6-HSL) or *N*-(3-hydroxy-hexanoyl)-homoserine lactone (3-OH-C6-HSL) in the earlier paper using thin layer chromatography and reporter strain *E. coli* JM109 (pSB401) (Venturi *et al.*, 2004). Yet neither aforementioned HSLs could be detected by reporter strain *Chromobacterium violaceum* CV026 (Burton *et al.*, 2005), which yielded a positive signal in the experiments performed by Molina *et al.* (2005). To add to the confusion, the above results could not be confirmed by a subsequent work that failed to detect any endogenous AHL production either using *C. violaceum* CV026 or by *in trans* expression of green fluorescent protein after transformation of *E. amylovora* with reporter plasmid pJBA132 (Jakovljevic *et al.*, 2008).

Despite the confusion about the nature of the AHL produced, a gene encoding a LuxR-type transcriptional activator with sequence identity to the cell division control protein SdiA of *E. coli* and *Salmonella typhimurium* (Wang *et al.*, 1991) could be confirmed in the genome of *E. amylovora* CFBP 1430 (Smits *et al.*, 2010b). Directly downstream of *sdiA*, a *luxI* homolog encoding a putative AI-1 synthesis protein that is related to the AHL synthase PhzI of *Pseudomonas chlororaphis* was found (Smits *et al.*, 2010b). Phylogenetic analysis suggests that PhzI may be the long-lost cognate signal synthase for SdiA. The *sdiA/phzI* gene pair is present in all sequenced *Erwinia* and *Pantoea* spp., whereas a deletion event has apparently removed the *luxI* homolog from the remaining members of the Enterobacteriaceae analyzed so far (Sabag-Daigle and Ahmer, 2012). Deletion of both *sdiA* and *phzI* in *E. amylovora* showed that these genes are not involved in virulence, amylovoran production and motility (Zhao *et al.*, 2009b). The identity between *E. amylovora* PhzI and *Pantoea ananatis* RhlI (KKW51348) is 65% at protein level. In the latter species, both C6-HSL and 3-oxo-C6-HSL were shown to be produced and detected by the parallel EanI/EanR

system (Morohoshi *et al.*, 2007), which is not present in *E. amylovora*, and mutation of RhlI was found not to affect the ability of *P. ananatis* to produce short-chain (C4 to C8) HSLs (Sibanda *et al.*, 2016). Altogether, these results may point to the fact that the autoinducer produced by RhlI in *E. amylovora* is not 3-oxo-C6-HSL, but a not yet identified long-chain HSL. In any case, the question remains unsettled given the contradictory results obtained so far.

In several *Vibrio* spp., the LuxS protein was shown to catalyze the production of the AI-2 autoinducer molecule controlling a second quorum sensing system (Schauder *et al.*, 2001). Since the *luxS* gene was found to be widespread among vast subgroups of the bacterial kingdom, it was hypothesized that AI-2 may constitute the basis of a universal microbial chemical language among different species. However, LuxS has also a primary metabolic role in the activated methyl cycle (AMC), which is involved the generation of *S*-adenosyl-L-methionine (SAM), the major methyl donor in the cell, and the recycling of methionine by detoxification of *S*-adenosyl-L-homocysteine (SAH) (Winzer *et al.*, 2003). The direct AI-2 precursor 4,5-dihydroxy-2,3-pentadione (DPD) is formed as a by-product of the AMC and in Vibrionales, this signal is detected by the two-component sensor kinase LuxPQ. In some species belonging to the families of Enterobacteriaceae, Pasteurellaceae and Bacillaceae (Rezzonico and Duffy, 2008; Xavier and Bassler, 2005), the task of detecting the AI-2 signal is carried out by an ABC-transporter, the Lsr-receptor complex. Most of the species carrying the latter are either pathogens or endosymbionts of animals, or live in closed ecosystems in tight association with plants or fungi (Rezzonico *et al.*, 2012a). A thorough approach based on bioinformatics was not able to detect any ortholog of known AI-2 receptors in the genomes of currently sequenced *Erwinia* spp. (Rezzonico and Duffy, 2008; Rezzonico *et al.*, 2012a). This reinforced the results of a previous work (Rezzonico and Duffy, 2007), where no significant AI-2 production was detected in wild-type strains of *E. amylovora* and where mutational analysis could not identify any quorum-sensing related effects in co-culture experiments of wild-type and *luxS* negative strains. The phenotype of the *luxS*-mutant strain showed rather that the primary role of LuxS is limited to the AMC and the methionine metabolism (Rezzonico and Duffy, 2007; Smits *et al.*, 2010b). Additionally, after deletion of the *luxPQ* genes in *E. amylovora*, the deletion mutant was not showing a different phenotype for virulence, amylovoran biosynthesis and motility than the wild-type strain (Zhao *et al.*, 2009b).

Type VI secretion systems. Unlike first reports (Hood *et al.*, 2010; Schell *et al.*, 2007), the type VI secretion systems (T6SS) are now regarded as interaction factors rather than virulence factors (Jani and Cotter, 2010; Schwarz *et al.*, 2010). *E. amylovora* contains a complete (T6SS-1) and a partial (T6SS-2) T6SS gene cluster (Smits *et al.*, 2010b), a situation also found in other genome-sequenced *Erwinia* and *Pantoea* spp. (De Maayer *et al.*, 2011). *E. amylovora* contains a third T6SS gene cluster (T6SS-3), which only has counterparts in *E. billingiae* Eb661 and several individual strains of *Pantoea* species, including *Pantoea agglomerans* E325 (De Maayer *et al.*, 2011; Sarris *et al.*, 2012). In different liquid media, all three T6SS of *E. amylovora* CFBP 1430 were shown to be transcribed (Kamber *et al.*, 2011).

When deleting the serine kinase (EAMY_3011) in the T6SS-1 of *E. amylovora* Ea1189, there was no influence on virulence, but in contrast to the wild-type strain, motility was irregular (Zhao *et al.*, 2009b). Similar results were obtained when deleting two of the T6SS core genes in *E. amylovora* CFBP 1430, independent if the deletion is in T6SS-1 or T6SS-3 (Kamber *et al.*, 2017). Transcriptome analysis showed differential expression of membrane-related functions, including the T3SSs, iron acquisition, chemotaxis, flagellar, and fimbrial genes, but the deletion of either or both T6SS clusters had only a minor effect on the virulence *in planta* (Kamber *et al.*, 2017).

Pathogen self-defense. There is a dynamic interaction between pathogenic microorganism and their competitors (Duffy *et al.*, 2003). Phytopathogens are able to defend themselves against influences by other bacteria and to the reaction of the plant to their presence. Although largely unexploited, self-defense is a common mechanism for phytopathogens. Especially the reaction of the phytopathogen to biological control organisms has received little attention.

On apple flowers, the main entry site for *E. amylovora*, many bacterial species can be found, several of which are close relatives to the pathogen (Pusey *et al.*, 2009). The members of the genus *Pantoea* are known to be effective agents of biocontrol, which is exerted through several mechanisms: competition for space and nutrients (Smits *et al.*, 2011b; Wilson and Lindow, 1994), acidification of the habitat (Pusey *et al.*, 2008, 2011) or antibiotic production (Stockwell *et al.*, 2002; Vanneste *et al.*, 1992). It is not exactly known how *E. amylovora* reacts to these mechanisms.

Coexistence on flowers is possible when the available nutrient sources are shared. Organisms living in a similar niche also will have a similar nutrient utilization pattern. Competition for nutrient sources by biocontrol strains as a mechanism has been hypothesized to depend upon a high niche overlap index (NOI) (Wilson and Lindow, 1994). There is a large overlap in substrate utilization patterns between *E. amylovora* and biocontrol strain *Pantoea vagans* C9-1, with a NOI of 0.96 (Smits *et al.*, 2011b). Compared to the *Pseudomonas fluorescens* A506, the NOI was only 0.71 (Stockwell *et al.*, 2010). In fact, *E. amylovora* is able to compete efficiently with some *Pantoea* strains when it is present on the flower before the biocontrol strain is applied (Giddens *et al.*, 2003).

Multidrug resistance transporters. Bacterial multidrug efflux pumps, primarily mediated by secondary transporters that typically utilize the proton motive force,

are common protection tools as a reaction to foreign compounds (Nikaido and Pagès, 2012). In *E. amylovora*, several multidrug efflux transporters have been characterized up to now. The AcrAB transport system (EAMY_1008 – EAMY_1009) is a protein complex that protects the pathogen from naturally occurring phytoalexins (Burse *et al.*, 2004a). Mutation of *acrB* in *E. amylovora* dramatically reduced tolerance to apple phytoalexins as well as virulence on apple plants. AcrAB confers ecological benefits during colonization of the host plant and, thus, influences the virulence of *E. amylovora* on apple. The transporter NorM (EAMY_1684) confers resistance to several hydrophobic cationic antibiotics (Burse *et al.*, 2004b). Mutation of *norM* in *E. amylovora* significantly reduced tolerance to toxins produced by epiphytic *P. agglomerans* strains isolated from apple and quince blossoms. Therefore, NorM is involved in the counteraction of biocontrol mechanisms on the host plant. The RND family efflux pump AcrD (EAMY_2508) displays only a limited range of amphiphilic compounds that are exported, and there is no effect on virulence (Pletzer and Weingart, 2014a). Two three-component RND transporters, MdtABC (EAMY_2262 – EAMY2264) and MdtUVW (EAMY_0683 – EAMY_0685), are involved in resistance to plant antimicrobials like tannin or flavonoids (Pletzer and Weingart, 2014b). Mutants are impaired in their virulence, as their ability to multiply in apple root stocks was reduced. The different multidrug resistance genes are under the regulation of different sets of global stress regulators, including BaeR and CpxR (Pletzer *et al.*, 2014, 2015), indicating that the ecological role is coupled to environmental signals.

Streptomycin resistance. The use of the aminoglycoside streptomycin has long been the only alternative to effectively reduce fire blight (Stockwell and Duffy, 2012). *E. amylovora* wild type strains are highly sensitive to this antibiotic, and are unable to grow on media amended with concentrations of streptomycin as low as 8 µg ml^{-1} (Chiou and Jones, 1995). Nevertheless, two different mechanisms of resistance have developed in areas that allowed a relatively uncontrolled use of the antibiotic (McManus and Jones, 1994).

Chromosomal resistance is acquired due to spontaneous single nucleotide mutations in the *rpsL* gene, which encodes the S12 protein included in the 30S small ribosomal subunit, and result in bacteria that are capable to grow at very high streptomycin concentration exceeding 16,000 µg ml^{-1} (M. Escursell, unpublished information). These mutations cause a substitution of the existing lysine either at position 43 or 88 of the protein that prevents inhibitory binding of streptomycin while preserving the functionality of the ribosome (Chiou and Jones, 1995). *In vitro* competition assays have demonstrated that among all the possible mutations examined, only the substitution of lysine by arginine at position 43 (K43R) results in a resistant variant with fitness comparable to that of the wild-type under conditions mimicking the flower environment when the selective pressure is relieved, which explains its prevalence in the field (M. Escursell, unpublished information).

The other resistance mechanism consists in the transposable element Tn*5393* containing the gene cluster *strA-strB*, which encodes a phosphotransferase that enzymatically inactivates streptomycin. This transposon is common to several Gram-negative bacteria and in *E. amylovora* was found to be incorporated either in plasmid pEa29 or pEa34 (Chiou and Jones, 1993). These strains display a lower resistance to streptomycin (approximately 1000 µg ml^{-1}) with respect to the one carrying the chromosomal mutation and are thus termed medium resistant (Chiou and Jones, 1995). Tn*5393*-mediated streptomycin resistance has now been reported in three geographically distinct locations in the United States (Förster *et al.*, 2015; McGhee *et al.*, 2011; Tancos and Cox, 2016; Tancos *et al.*, 2016). Concerns that this type of resistance may be horizontally transferred to the target bacteria via formulations contaminated by the resistance genes of the antibiotic producer organism used to prepare them have been cleared by showing their absence in a range of commercial products (Rezzonico *et al.*, 2008).

Biosynthesis of sulfur-containing compounds. In recent years, several reports on the biosynthesis of sulfur-containing compounds have been published. Whereas the function of glutathione is well-known as a redox compound, the newly identified compound ovothiol A could very well have a similar function in *E. amylovora* (Seebeck, 2013). The compound has a very acidic thiol group that can serve as a one-electron donor and has a redox potential in the range of that of protein disulfide isomerases. Therefore, it might be an efficient scavenger for radicals and peroxides (Braunshausen and Seebeck, 2011), which are the result of the primary defense of apple after challenge with the pathogen. The gene encoding the first step in ovothiol A biosynthesis has been identified as *ovoA* (EAMY_0003), and encodes an iron(II)- and oxygen-dependent sulfoxide synthase (Mashabela and Seebeck, 2013).

The biosynthesis of the cytotoxic compound 6-thioguanine has been reported already long ago for *E. amylovora* (Feistner and Staub, 1986), but it is only recently that the biosynthetic cluster (EAMY_1020 – EAMY_1024) has been characterized (Coyne *et al.*, 2013; Wensing *et al.*, 2014). Its role in virulence is contradictory: Coyne *et al.* have defined a key role for 6-thioguanine in pathogenicity (Coyne *et al.*, 2013), whereas Wensing *et al.* reported that the compound was not affecting virulence (Wensing *et al.*, 2014). The exact biochemical pathway of 6-thioguanine biosynthesis still remains to be elucidated.

Siderophores. Although an essential nutrient for living organisms, iron is only limited available in plants. To obtain sufficient iron for microbial growth, many bacteria

have the ability to produce low-molecular compounds that are able to chelate iron at high affinity (Expert *et al.*, 1996), while other bacteria have the ability to capture these iron siderophores for their own iron metabolism (Deiss *et al.*, 1998). As competition for iron influences the virulence both in human and plant pathogens, siderophores are regarded as a virulence factor (Franza and Expert, 2013).

E. amylovora produces the siderophore desferrioxamine E (Feistner *et al.*, 1993) as a reaction to low-iron conditions. Initially also interpreted as a virulence factor (Expert *et al.*, 1996), it soon showed out that desferrioxamine uptake does not affect virulence towards apple, but that desferrioxamine uptake mutants are less able to colonize floral tissues and to cause necrosis (Dellagi *et al.*, 1998). This indicates that desferrioxamine biosynthesis and uptake rather play a general role in colonization, and are as such rather to be regarded as niche adaptation factors in *E. amylovora*.

With the description of the *E. amylovora* genome, the biosynthetic pathway for desferrioxamine E could be fully elucidated (Smits *et al.*, 2010b). Comparative genomic studies have shown that several genome-sequenced species of *Erwinia* and *Pantoea* have the *dfoJAC* gene cluster for biosynthesis of desferrioxamine E (Smits and Duffy, 2011). As the iron ferrioxamine uptake is also present in these organisms, they would be able to compete for iron-ferrioxamine with *E. amylovora*. With the sequencing of more genomes, it has become evident that not all *Erwinia* species have this potential (Table 1).

CRISPR and phage defense. The CRISPR system confers acquired heritable immunity against mobile nucleic acid elements in prokaryotes, limiting phage infection and horizontal gene transfer of plasmids (van der Oost *et al.*, 2009). CRISPR regions are composed by highly conserved direct DNA repeats that are interspersed by unique, similarly sized spacers acquired when the cell is challenged with foreign nucleic acids. Together with the associated Cas proteins, the system confers immunity against infection by foreign DNA or RNA by a mechanism based on the strict identity between the incorporated CRISPR spacer and the nucleic acid target (Brouns *et al.*, 2008). Several spacers sequences pointing to known plasmids or phages of Enterobacteriaceae were found in the CRISPR regions of a series of worldwide isolates of *E. amylovora* (McGhee and Sundin, 2012; Rezzonico *et al.*, 2011). Nevertheless, several novel phages isolated from fruit production environments in Switzerland displayed variable host ranges with respect to the different *E. amylovora* strains analyzed (Born *et al.*, 2011), even if the latter carried identical CRISPR profiles (Rezzonico *et al.*, 2011). This suggests that there is at least an additional mechanism that regulates phages resistance in *E. amylovora*. Given the high genetic homogeneity of the isolates analyzed, this mechanism could reside more in the regulation of certain genes rather than in a specific interaction with the phages, as demonstrated by the preference of certain viruses for either high or low exopolysaccharide producing bacterial hosts (Roach *et al.*, 2013), which can be mediated for example through the production of a phage depolymerase that enables enzymatic bacterial capsule removal (Born *et al.*, 2014).

APPLIED GENOMICS FOR PHYTOSANITARY CONTROL STRATEGIES

Detection and diagnostics. The current standard for detection of *E. amylovora* in Europe is described in the EPPO protocol, a collection of established and validated protocols (European and Mediterranean Plant Protection Organization – EPPO, 2004). Since then, a large variety of methods improving the detection was developed. A lateral-flow immunostrip assay was developed for specific detection of the fire blight bacterium in symptomatic plant material that can be applied directly on the field (Braun-Kiewnick *et al.*, 2011). Other methods use molecular techniques. Optimization of standard PCR protocols [reviewed in Powney *et al.* (2011)] and use of quantitative PCR methods [reviewed in Dreo *et al.* (2012) and Pirc *et al.* (2009)] are now available and validated. A recent addition to this is the use of loop-mediated isothermal amplification (LAMP), for which three different primer sets based on different targets are developed (Bühlmann *et al.*, 2013; Moradi *et al.*, 2012; Temple and Johnson, 2011).

An important factor is the design of primers for such method. With the availability of genome sequences of a broader range of *Erwinia* spp. and other genera, it is now possible to design assays that are highly specific for *E. amylovora* (Bühlmann *et al.*, 2013). This avoids the detection of closely related species yielding false positive reactions. A good example is the widely used primer set for detection of the SSR on plasmid pEA29 of *E. amylovora* (Llop *et al.*, 2000), the target of which is also present in the plasmid pEP36 of *E. pyrifoliae* (Jock *et al.*, 2003). Even the highly specific LAMP primer set designed by Moradi *et al.* (2012) using six primers is able to detect *E. pyrifoliae* (Bühlmann *et al.*, 2013); a fact that could easily be avoided by checking the primer set against the genome of the latter species (Smits *et al.*, 2010a). It is thus important to use the maximum of information contained in a genome and to combine it with the maximum number of closely related species to validate the specificity of a primer set.

Monitoring and epidemiology. Efficient phytosanitary and control measures must be built upon epidemiological understanding of the fire blight disease cycle. Measures aimed at the protection of commercial pome fruit objects entail identification and containment of inoculum reservoirs, both from existing orchards as well as from alternate host plants from the environment. Recently, new genomics-insights have presented an opportunity to confirm identity of otherwise indistinguishable pathogen isolates (Smits *et al.*, 2010b). The development of this knowledge into

source-tracking methods (Bühlmann *et al.*, 2013; Rezzonico *et al.*, 2011) has the potential to improve the successful implementation of phytosanitary strategies by focusing on removal of only true inoculum sources, while preventing unneeded clearing of old-growth landscape trees, native forest species or unrelated orchards (Gusberti *et al.*, 2015).

Fire blight infection during the blossoming period is a main driver of epidemic development and spread. Application of control products to flowers reduces infection, limits pathogen inoculum build-up, and reduces available inoculum for vector dissemination (Johnson and Stockwell, 1998). Correct timing is critical for efficacy and can be implemented by performing the applications in accordance with established fire blight forecasting models such as Maryblyt or Cougarblight (Smith and Pusey, 2010; van der Zwet *et al.*, 1994). Through the input of weather and phenological variables, it is possible to predict pathogen population growth and inform when potential infection days are likely to occur. However, in current models the presence of the pathogen in a given location is only presumed, so that the use of detection methods for routine flower monitoring to quantify the actual level of inoculum has the potential to reduce unneeded treatments thereby improving the efficacy of control applications (V. Stockwell, unpublished information).

Closely related pathogens that cause symptoms similar to fire blight but are not included in current phytosanitary regulations have recently been described from East Asia (*E. pyrifoliae* and *Erwinia uzenensis*) (Kim *et al.*, 1999; Matsuura *et al.*, 2012) and Spain (*E. piriflorinigrans*) (Palacio-Bielsa *et al.*, 2012). Whether these occur more widely in Europe or pose an emerging phytosanitary threat is largely unstudied, a lack due to of efficient methods for detecting these bacteria in plant samples.

Source-tracking and regulatory strategies. *E. amylovora* is a relatively genetically homogenous bacterial species: only limited genotype groupings could be obtained across large geographic areas using the most currently available molecular methods like AFLP (Rico *et al.*, 2004), rep-PCR (Rico *et al.*, 2008), PFGE (Jock *et al.*, 2002) or ribotyping (Donat *et al.*, 2007; McManus and Jones, 1995). These methods are thus inadequate for discrimination among isolates within local populations or for source-tracking of inoculum reservoirs and outbreak origins. Recent diversity studies based on the genome sequence of *E. amylovora* CFBP 1430 (Smits *et al.*, 2010b) have exploited the local diversity in clustered regularly interspaced short palindromic repeats (CRISPR). Sequence analysis of CRISPR repeat regions (CRR) showed that there is a larger diversity in *Rubus*-infecting isolates, but that also diversity exists (in the form of three major CRISPR groups) among Spiraeoideae-infecting isolates from North America (McGhee and Sundin, 2012; Rezzonico *et al.*, 2011), which is the geographic center of origin of the disease. However, there are hardly differences within European or Mediterranean isolates, leading to the conclusion that there was an evolutionary bottleneck in the spreading of *E. amylovora* from North America, with only strains belonging to CRISPR group I (typical of the U.S. East coast) having spread to Europe and New Zealand in the 20th century (Rezzonico *et al.*, 2011). A major disadvantage of this technique is the extensive amount of labor needed for sequencing the complete CRRs, which can individually be as long as 6 kb and that may not contain any related repeat, thus requiring the design of ever-new primers to carry out a time-consuming spacer crawling strategy.

A possible alternative to CRISPR is the use of variable number tandem repeats (VNTRs), which are locations in the genome that are composed from short sequences that show variations in length between individuals. In *E. amylovora*, comparison between the genomes of strains CFBP 1430 and ATCC 49946 (Ea273) (Dreo *et al.*, 2011; Smits *et al.*, 2010b) revealed six suitable VNTRs that potentially show enough variability to be employed to analyze the diversity within local isolates of *E. amylovora*. With respect to CRISPRs, automation of multiple locus VNTR analysis (MLVA) is quite straightforward, e.g. in form of labeled fragment analysis, and can be applied to larger sets of isolates providing insights into diversity, biogeography and phylogeny of the pathogen (Bühlmann *et al.*, 2014).

Single nucleotide polymorphisms (SNPs) have the potential to offer the most detailed fingerprint for each strain and are thus the most promising features that can be used as unequivocal phylogenetic markers to pinpoint diversity in genetically monomorphic bacteria (Achtman, 2008; Comas *et al.*, 2009; Holt *et al.*, 2010). As the costs of whole-genome sequencing and re-sequencing techniques is constantly decreasing (Logares *et al.*, 2012), it is foreseeable that the complete variome of hundreds of bacterial isolates will become readily accessible soon, thus making the mapping of SNPs the method of choice to study diversity within a species. In *E. amylovora*, a point mutation in the *galE* gene was able to distinguish isolates of North American origin from those of European/Mediterranean origin, but the analysis was based on a single SNP position and the number of strains considered was very limited (Sauer *et al.*, 2008). The first data available from sequencing 140 isolates of *E. amylovora* are promising, and will be published in due time (T.H.M. Smits, unpublished information), setting the stage for diversity studies through extensive SNP analysis.

CHALLENGES GOING FORWARD

The entry into the genomics era has facilitated major advancement on the knowledge of *E. amylovora* and related *Erwinia* spp. This has not only led to an improved understanding of the virulence factors (Kamber *et al.*, 2012; Smits *et al.*, 2011a), but also now allows for complete genome-wide regulation and proteomic studies

(Holtappels *et al.*, 2016; McNally *et al.*, 2012; Sarowar *et al.*, 2011; Wang *et al.*, 2012b; Wu *et al.*, 2013). The development of highly specific assays to detect *E. amylovora* in field samples (Bühlmann *et al.*, 2013) and of source tracking assays (Bühlmann *et al.*, 2014; McGhee and Sundin, 2012; Rezzonico *et al.*, 2011) was only possible due to the knowledge gathered from the first fully sequenced genomes. Still, there is a lack of information on the epidemiology of *E. amylovora*, for which the newly developed methods may deliver the molecular fundamentals. This increased knowledge will, together with improved understanding of the pathogen's Achilles heel, ultimately lead to novel control strategies.

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REFERENCES

Achtman M., 2008. Evolution, population structure, and phylogeography of genetically monomorphic bacterial pathogens. *Annual Review of Microbiology* **62**: 53-70.

Ancona V., Li W., Zhao Y., 2014. Alternative sigma factor RpoN and its modulation protein YhbH are indispensable for *Erwinia amylovora* virulence. *Molecular Plant Pathology* **15**: 58-66.

Ancona V., Lee J.H., Chatnaparat T., Oh J., Hong J.-I., Zhao Y., 2015. The bacterial alarmone (p)ppGpp activates the type III secretion system in *Erwinia amylovora*. *Journal of Bacteriology* **197**: 1433-1443.

Ancona V., Lee J.H., Zhao Y.F., 2016. The RNA-binding protein CsrA plays a central role in positively regulating virulence factors in *Erwinia amylovora*. *Scientific Reports* **6**: 37195.

Asselin J.A., Bonasera J., Kim J., Oh C.-S., Beer S.V., 2011. Eop1 from a *Rubus* strain of *Erwinia amylovora* functions as a host-range limiting factor. *Phytopathology* **101**: 935-944.

Baldo A., Norelli J.L., Farrell Jr R.E., Bassett C.L., Aldwinckle H.S., Malnoy M., 2010. Identification of genes differentially expressed during interaction of resistant and susceptible apple cultivars (*Malus × domestica*) with *Erwinia amylovora*. *BMC Plant Biology* **10**: 1.

Bernhard F., Schullerus D., Bellemann P., Geider K., Nimtz M., Majerczak D.R., Coplin D.L., 1996. Genetics and complementation of DNA regions involved in amylovoran synthesis of *Erwinia amylovora* and stewartan synthesis of *Erwinia stewartii*. *Acta Horticulturae* **411**: 269-274.

Bonn W.G., Van Der Zwet T., 2000. Distribution and economic importance of fire blight. In: Vanneste J.L. (ed.). Fire Blight: the Disease and its Causative Agent, *Erwinia amylovora*, pp. 37-53. CAB International, Wallingford, UK.

Born Y., Fieseler L., Marazzi J., Lurz R., Duffy B., Loessner M.J., 2011. Novel virulent and broad-host-range *Erwinia amylovora* bacteriophages reveal a high degree of mosaicism and a relationship to *Enterobacteriaceae* phages. *Applied and Environmental Microbiology* **77**: 5945-5954.

Born Y., Fieseler L., Klummp J., Eugster M.R., Zurfluh K., Duffy B., Loessner M.J., 2014. The tail-associated depolymerase of *Erwinia amylovora* phage L1 mediates host cell adsorption and enzymatic capsule removal, which can enhance infection by other phage. *Environmental Microbiology* **16**: 2168-2180.

Braun-Kiewnick A., Altenbach D., Oberhänsli T., Bitterlin W., Duffy B., 2011. A rapid lateral-flow immunoassay for phytosanitary detection of *Erwinia amylovora* and on-site fire blight diagnosis. *Journal of Microbiological Methods* **87**: 1-9.

Braun P.G., Hildebrand P.D., 2005. Infection, carbohydrate utiliation, and protein profiles of apple, pear, and raspberry isolates of *Erwinia amylovora*. *Canadian Journal of Plant Pathology* **27**: 338-346.

Braunshausen A., Seebeck F.P., 2011. Identification and characterization of the first ovothiol biosynthetic enzyme. *Journal of the American Chemical Society* **133**: 1757-1759.

Brouns S.J.J., Jore M.M., Lundgren M., Westra E.R., Slijkhuis R.J.H., Snijders A.P.L., Dickman M.J., Makarova K.S., Koonin E.V., Van Der Oost J., 2008. Small CRISPR RNAs guide antiviral defence in prokaryotes. *Science* **321**: 960-964.

Bühlmann A., Dreo T., Rezzonico F., Pothier J.F., Smits T.H.M., Ravnikar M., Frey J.E., Duffy B., 2014. Phylogeography and population structure of the biologically invasive phytopathogen *Erwinia amylovora* inferred using minisatellites. *Environmental Microbiology* **16**: 2112-2125.

Bühlmann A., Pothier J.F., Rezzonico F., Smits T.H.M., Andreou M., Boonham N., Duffy B., Frey J.E., 2013. *Erwinia amylovora* loop-mediated isothermal amplification (LAMP) assay for rapid pathogen detection and on-site diagnosis of fire blight. *Journal of Microbiological Methods* **92**: 332-339.

Burse A., Weingart H., Ullrich M.S., 2004a. NorM, an *Erwinia amylovora* multidrug efflux pump involved in in vitro competition with other epiphytic bacteria. *Applied and Environmental Microbiology* **70**: 693-703.

Burse A., Weingart H., Ullrich M.S., 2004b. The phytoalexin-inducible multidrug efflux pump AcrAB contributes to virulence in the fire blight pathogen, *Erwinia amylovora*. *Molecular Plant-Microbe Interactions* **17**: 43-54.

Burton E.O., Read H.W., Pellitteri M.C., Hickey W.J., 2005. Identification of acyl-homoserine lactone signal molecules produced by *Nitrosomonas europaea* strain Schmidt. *Applied and Environmental Microbiology* **71**: 4906-4909.

Casadevall A., 2008. Evolution of intracellular pathogens. *Annual Review of Microbiology* **62**: 19-33.

Chiou C.-S., Jones A.L., 1993. Nucleotide sequence analysis of a transposon (Tn*5393*) carrying streptomycin resistance

genes in *Erwinia amylovora* and other Gram-negative bacteria. *Journal of Bacteriology* **175**: 732-740.

Chiou C.-S., Jones A.L., 1995. Molecular analysis of high-level streptomycin resistance in *Erwinia amylovora*. *Phytopathology* **95**: 324-328.

Comas I., Homolka S., Niemann S., Gagneux S., 2009. Genotyping of genetically monomorphic bacteria: DNA sequencing in *Mycobacterium tuberculosis* highlights the limitations of current methodologies. *PLoS One* **4**: e7815.

Correa V.R., Majerczak D.R., Ammar E.-D., Merighi M., Pratt R.C., Hogenhout S.A., Coplin D.L., Redinbaugh M.G., 2012. The bacterium *Pantoea stewartii* uses two different type III secretion systems for colonizing its plant host and insect vector. *Applied and Environmental Microbiology* **78**: 6327-6336.

Coyne S., Chizzali C., Khalil M.N.A., Litomska A., Richter K., Beerhues L., Hertweck C., 2013. Biosynthesis of the antimetabolite 6-thioguanine in *Erwinia amylovora* plays a key role in fire blight pathogenesis. *Angewandte Chemie International Edition* **52**: 10564-10568.

Dale C., Young S.A., Haydon D.T., Welburn S.C., 2001. The insect endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion. *Proceedings of the National Academy of Sciences USA* **98**: 1883-1888.

De Maayer P., Venter S.N., Kamber T., Duffy B., Coutinho T.A., Smits T.H.M., 2011. Comparative genomics of the type VI secretion systems of *Pantoea* and *Erwinia* species reveals the presence of putative effector islands that may be translocated by the VgrG and Hcp proteins. *BMC Genomics* **12**: 576.

De Pinto M.C., Lavermicocca P., Evidente A., Corsaro M.M., Lazzaroni S., De Gara L., 2003. Exopolysaccharides produced by plant pathogenic bacteria affect ascorbate metabolism in *Nicotiana tabacum*. *Plant Cell Physiology* **44**: 803-810.

Deiss K., Hantke K., Winkelmann G., 1998. Molecular recognition of siderophores: a study with cloned ferrioxamine receptors (FoxA) from *Erwinia herbicola* and *Yersinia enterocolitica*. *Biometals* **11**: 131-137.

Dellagi A., Brisset M.-N., Paulin J.-P., Expert D., 1998. Dual role of desferrioxamine in *Erwinia amylovora* pathogenicity. *Molecular Plant-Microbe Interactions* **11**: 734-742.

Djaimurzina A., Umiralieva Z., Zharmukhamedova G., Born Y., Bühlmann A., Rezzonico F., 2014. Detection of the causative agent of fire blight - *Erwinia amylovora* (Burrill) Winslow et al. - in the southeast of Kazakhstan. *Acta Horticulturae* **1056**: 129-132.

Donat V., Biosca E.G., Peñalver J., López M.M., 2007. Exploring diversity among Spanish strains of Erwinia amylovora and possible infection sources. *Journal of Applied Microbiology* **103**: 1639-1649.

Dreo T., Ravnikar M., Frey J.E., Smits T.H.M., Duffy B., 2011. In silico analysis of variable number of tandem repeats in *Erwinia amylovora* genome. *Acta Horticulturae* **896**: 115-118.

Dreo T., Pirc M., Ravnikar M., 2012. Real-time PCR, a method fit for detection and quantification of *Erwinia amylovora*. *Trees-Structure and Function* **26**: 165-178.

Duffy B., Schouten A., Raaijmakers J.M., 2003. Pathogen self-defense: mechanisms to counteract microbial antagonism. *Annual Review of Phytopathology* **41**: 501-538.

Duffy B., Schärer H.-J., Bünter M., Klay A., Holliger E., 2005. Regulatory measures against *Erwinia amylovora* in Switzerland. *EPPO Bulettinl* **35**: 239-244.

Eastgate J.A., Taylor N., Coleman M.J., Healy B., Thompson L., Roberts I.S., 1995. Cloning, expression, and characterization of the *lon* gene of *Erwinia amylovora*: evidence for a heat shock response. *Journal of Bacteriology* **177**: 932-937.

Edmunds A.C., Castiblanco L.F., Sundin G.W., Waters C.M., 2013. Cyclic di-GMP modulates the disease progression of *Erwinia amylovora*. *Journal of Bacteriology* **195**: 2155-2165.

Emeriewen O.F., Richter K., Hanke M.-V., Malnoy M., Peil A., 2015. The fire blight resistance QTL of *Malus fusca* (*Mfu10*) is affected but not broken down by the highly virulent Canadian *Erwinia amylovora* strain E2002A. *European Journal of Plant Pathology* **141**: 631-635.

EPPO (European and Mediterranean Plant Protection Organization), 2004. Diagnostic protocols for regulated pests PM 7/20, *Erwinia amylovora*. *EPPO Bullettin* **34**: 159-171.

Expert D., Enard C., Masclaux C., 1996. The role of iron in plant host-pathogen interactions. *Trends in Microbiology* **4**: 232-237.

Feistner G.J., Staub C.M., 1986. 6-Thioguanine from *Erwinia amylovora*. *Current Microbiology* **13**: 95-101.

Feistner G.J., Stahl D.C., Gabrik A.H., 1993. Proferrioxamine siderophores of *Erwinia amylovora*. A capillary liquid chromatographic/electrospray tandem mass spectrometry study. *Organic Mass Spectrometry* **28**: 163-175.

Förster H., McGhee G.C., Sundin G.W., Adaskaveg J.E., 2015. Characterization of streptomycin resistance in isolates of *Erwinia amylovora* in California. *Phytopathology* **105**: 1302-1310.

Foster G.C., McGhee G.C., Jones A.L., Sundin G.W., 2004. Nucleotide sequences, genetic organization, and distribution of pEU30 and pEL60 from *Erwinia amylovora*. *Applied and Environmental Microbiology* **70**: 7539-7544.

Franza T., Expert D., 2013. Role of iron homeostasis in the virulence of phytopathogenic bacteria: an ´à la carte´ menu. *Molecular Plant Pathology* **14**: 429-438.

Giddens S.R., Houliston G.J., Mahanty H.K., 2003. The influence of antibiotic production and pre-emptive colonization on the population dynamics of *Pantoea agglomerans* (*Erwinia herbicola*) Eh1087 and *Erwinia amylovora in planta*. *Environmental Microbiology* **5**: 1016-1021.

Grant S.R., Fisher E.J., Chang J.H., Mole B.M., Dangl J.L., 2006. Subterfuge and manipulation: type III effector proteins of phytopathogenic bacteria. *Annual Review of Phytopathology* **60**: 425-449.

Gusberti M., Klemm U., Meier M.S., Maurhofer M., Hunger-Glaser I., 2015. Fire blight control: the struggle goes on. A comparison of different fire blight control methods in Switzerland with respect to biosafety, efficacy and durability. *International Journal of Environmental Research and Public Health* **12**: 11422-11447.

Hajri A., Brin C., Hunault G., Lardeux F., Lemaire C., Manceau C., Boureau T., Poussier S., 2009. A «repertoire for repertoire» hypothesis: repertoires of type three effectors are candidate determinants of host specificity in *Xanthomonas*. *PLoS One* **4**: e6632.

Heimann M.F., Worf G.L., 1985. Fire blight of raspberry caused by *Erwinia amylovora* in Wisconsin. *Plant Disease* **69**: 360.

Hildebrand M., Aldridge P., Geider K., 2006. Characterization of *hns* genes from *Erwinia amylovora*. *Molecular Genetics and Genomics* **275**: 310-319.

Hill C., 2012. Virulence or niche factors; what's in a name? *Journal of Bacteriology* **194**: 5725-5727.

Holt K.E., Baker S., Dongol S., Basnyat B., Adhikari N., Thorson S., Pulickal A.S., Song Y., Parkhill J., Farrar J.J., Murdoch D.R., Kelly D.F., Pollard A.J., Dougan G., 2010. High-troughput bacterial SNP typing identifies distinct clusters of *Salmonella* Typhi causing typhoid in Nepalese children. *BMC Infectious Diseases* **10**: 144.

Holtappels M., Vrancken K., Noben J.P., Remans T., Schoofs H., Deckers T., Valcke R., 2016. The *in planta* proteome of wild type strains of the fire blight pathogen, *Erwinia amylovora*. *Journal of Proteomics* **139**: 1-12.

Hood R.D., Singh P., Hsu F., Güvener T., Carl M.A., Trinidad R.R.S., Silverman J.M., Ohlson B.B., Hicks K.G., Plemel R.L., Li M., Schwarz S., Wang W.Y., Merz A.J., Goodlett D.R., Mougous J.D., 2010. A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe* **7**: 25-37.

Ismail E., Blom J., Bultreys A., Ivanovic M., Obradovic A., Van Doorn J., Bergsma-Vlami M., Maes M., Willems A., Duffy B., Stockwell V.O., Smits T.H.M., Puławska J., 2014. A novel plasmid pEA68 of *Erwinia amylovora* and the description of a new family of plasmids. *Archives of Microbiology* **196**: 891-899.

Jakovljevic V., Jock S., Du Z., Geider K., 2008. Hypersensitive response and acyl-homoserine lactone production of the fire blight antagonists *Erwinia tasmaniensis* and *Erwinia billingiae*. *Microbial Biotechnology* **1**: 416-424.

Jani A.J., Cotter P.A., 2010. Type VI secretion: not just for pathogenesis anymore. *Cell Host Microbe* **8**: 2-6.

Jock S., Donat V., López M.M., Bazzi C., Geider K., 2002. Following spread of fire blight in Western, Central and Southern Europe by molecular differentiation of *Erwinia amylovora* strains with PFGE analysis. *Environmental Microbiology* **4**: 106-114.

Jock S., Kim W.-S., Barny M.-A., Geider K., 2003. Molecular characterization of natural *Erwinia pyrifoliae* strains deficient in hypersensitive response. *Applied and Environmental Microbiology* **69**: 679-682.

Johnson K.B., Stockwell V.O., 1998. Management of fire blight: a case study in microbial ecology. *Annual Review of Phytopathology* **36**: 227-248.

Kamber T., Smits T.H.M., Duffy B., 2011. Type VI secretion systems in *Erwinia amylovora* CFBP 1430. *Acta Horticulturae* **896**: 211-213.

Kamber T., Smits T.H.M., Rezzonico F., Duffy B., 2012. Genomics and current genetic understanding of *Erwinia amylovora* and the fire blight antagonist *Pantoea vagans*. *Trees-Structure and Function* **26**: 227-238.

Kamber T., Buchmann J.P., Pothier J.F., Smits T.H.M., Wicker T., Duffy B., 2016. Fire blight disease reactome: RNA-seq transcriptional profile of apple host plant defence responses to *Erwinia amylovora* pathogen infection. *Scientific Report* **6**: 21600.

Kamber T., Pothier J.F., Pelludat C., Rezzonico F., Duffy B., Smits T.H.M., 2017. Role of the type VI secretion systems during disease interactions of *Erwinia amylovora* with its plant host. *BMC Genomics* **18**: 628.

Khan M.A., Zhao Y., Korban S.S., 2012. Molecular mechanisms of pathogenesis and resistance to the bacterial pathogen *Erwinia amylovora*, causal agent of fire blight disease in Rosaceae. *Plant Molecular Biology Reporter* **30**: 247-260.

Kim W.-S., Gardan L., Rhim S.-L., Geider K., 1999. *Erwinia pyrifoliae* sp. nov., a novel pathogen that affects Asian pear trees (*Pyrus pyrifolia* Nakai). *International Journal of Systematic Bacteriology* **49**: 899-906.

Kim W.-S., Geider K., 1999. Analysis of variable short-sequence DNA repeats on the 29 kb plasmid of *Erwinia amylovora* strains. *European Journal of Plant Pathology* **105**: 703-713.

Kirzinger M.W.B., Butz C.J., Stavrinides J., 2015. Inheritance of *Pantoea* type III secretion systems through both vertical and horizontal transfer. *Molecular Genetics and Genomics* **290**: 2075-2088.

Koczan J.M., McGrath M.J., Zhao Y., Sundin G.W., 2009. Contribution of *Erwinia amylovora* exopolysaccharides amylovoran and levan to biofilm formation: implications to pathogenicity. *Phytopathology* **99**: 1237-1244.

Kube M., Migdoll A.M., Gehring I., Heitmann K., Mayer Y., Kuhl H., Knaust F., Geider K., Reinhardt R., 2010. Genome comparison of the epiphytic bacteria *Erwinia billingiae* and *E. tasmaniensis* with the pear pathogen *E. pyrifoliae*. *BMC Genomics* **11**: 393.

Kube M., Migdoll A.M., Müller I., Kuhl H., Beck A., Reinhardt R., Geider K., 2008. The genome of *Erwinia tasmaniensis* strain Et1/99, a non-pathogenic bacterium in the genus *Erwinia*. *Environmental Microbiology* **10**: 2211-2222.

Langlotz C., Schollmeyer M., Coplin D.L., Nimtz M., Geider K., 2011. Biosynthesis of the repeating units of the exopolysaccharides amylovoran from *Erwinia amylovora* and stewartan from *Pantoea stewartii*. *Physiological and Molecular Plant Pathology* **75**: 163-169.

Lee J.H., Zhao Y., 2016. Integration host factor is required for RpoN-dependent *hrpL* gene expression and controls motility by positively regulating *rsmB* sRNA in *Erwinia amylovora*. *Phytopathology* **106**: 29-36.

Lee J.H., Sundin G.W., Zhao Y., 2016. Identification of the HrpS binding site in the *hrpL* promoter and effect of the RpoN binding site of HrpS on the regulation of the type III secretion system in *Erwinia amylovora*. *Molecular Plant Pathology* **17**: 691-702.

Li W., Ancona V., Zhao Y., 2014. Co-regulation of polysaccharide production, motility, and expression of type III secretion genes by EnvZ/OmpR and GrrS/GrrA systems in *Erwinia amylovora*. *Molecular Genetics and Genomics* **289**: 63-75.

Llop P., Bonaterra A., Peñalver J., López M.M., 2000. Development of a highly sensitive nested-PCR procedure using a single closed tube for detection of *Erwinia amylovora* in asymptomatic plant material. *Applied and Environmental Microbiology* **66**: 2071-2078.

Llop P., Cabrefiga J., Smits T.H.M., Dreo T., Barbé S., Pulawska J., Bultreys A., Blom J., Duffy B., Montesinos E., López M.M., 2011. *Erwinia amylovora* novel plasmid pEI70: complete sequence, biogeography, and role in aggressiveness in the fire blight phytopathogen. *PLoS One* **6**: e28651.

Llop P., Barbé S., López M.M., 2012. Functions and origin of plasmids in *Erwinia* species that are pathogenic to or epiphytically associated with pome fruit trees. *Trees-Structure and Function* **26**: 31-46.

Logares R., Haverkamp T.H.A., Kumar S., Lanzén A., Nederbragt A.J., Quince C., Kauserud H., 2012. Environmental microbiology through the lens of high-throughput DNA sequencing: synopsis of current platforms and bioinformatics approaches. *Journal of Microbiological Methods* **91**: 106-113.

Malnoy M., Martens S., Norelli J.L., Barny M.-A., Sundin G.W., Smits T.H.M., Duffy B., 2012. Fire blight: applied genomic insights of the pathogen and the host. *Annual Review of Phytopathology* **50**: 475-494.

Mann R.A., Blom J., Bühlmann A., Plummer K.M., Beer S.V., Luck J.E., Goesmann A., Frey J.E., Rodoni B.C., Duffy B., Smits T.H.M., 2012. Comparative analysis of the Hrp pathogenicity island of *Rubus*- and Spiraeoideae-infecting *Erwinia amylovora* strains identifies the IT region as a remnant of an integrative conjugative element. *Gene* **504**: 6-12.

Mann R.A., Smits T.H.M., Bühlmann A., Blom J., Goesmann A., Frey J.E., Plummer K.M., Beer S.V., Luck J., Duffy B., Rodoni B., 2013. Comparative genomics of 12 strains of *Erwinia amylovora* identifies a pan-genome with a large conserved core. *PLoS One* **8**: e55644.

Mashabela G.T.M., Seebeck F.P., 2013. Substrate specificity of an oxygen dependent sulfoxide synthase in ovothiol biosynthesis. *Chemical Communications* **49**: 7714-7716.

Matsuura T., Mizuno A., Tsukamoto T., Shimizu Y., Saito N., Sato S., Kikuchi S., Uzuki T., Azegami K., Sawada H., 2012. *Erwinia uzenensis* sp. nov., a novel pathogen that affects European pear trees (*Pyrus communis* L.). *International Journal of Systematic and Evolutionary Microbiology* **62**: 1799-1803.

McGhee G.C., Jones A.L., 2000. Complete nucleotide sequence of ubiquitous plasmid pEA29 from *Erwinia amylovora* strain Ea88: gene organization and intraspecies variation. *Applied and Environmental Microbiology* **66**: 4897-4907.

McGhee G.C., Foster G.C., Jones A.L., 2002a. Genetic diversity among *Erwinia amylovora*'s ubiquitous plasmid pEA29. *Acta Horticulturae* **590**: 413-421.

McGhee G.C., Schnabel E.L., Maxson-Stein K., Jones B., Stromberg V.K., Lacy G.H., Jones A.L., 2002b. Relatedness of chromosomal and plasmid DNAs of *Erwinia pyrifoliae* and *Erwinia amylovora*. *Applied and Environmental Microbiology* **68**: 6182-6192.

McGhee G.C., Guasco J., Bellomo L.M., Blumer-Schuette S.E., Shane W.W., Irish-Brown A., Sundin G.W., 2011. Genetic analysis of streptomycin-resistant (Sm^R) strains of *Erwinia amylovora* suggests that dissemination of two genotypes is responsible for the current distribution of Sm^R *E. amylovora* in Michigan. *Phytopathology* **101**: 182-191.

McGhee G.C., Sundin G.W., 2012. *Erwinia amylovora* CRISPR elements provide new tools for evaluating strain diversity and for microbial source tracking. *PLoS One* **7**: e41706.

McManus P.S., Jones A.L., 1994. Epidemiology and genetic analysis of streptomycin-resistant *Erwinia amylovora* from Michigan and evaluation of oxytetracycline for control. *Phytopathology* **84**: 627-633.

McManus P.S., Jones A.L., 1995. Genetic fingerprinting of *Erwinia amylovora* strains isolated from tree-fruit crops and *Rubus* spp. *Phytopathology* **85**: 1547-1553.

McNally R.R., Toth I.K., Cock P.J.A., Pritchard L., Hedley P.E., Morris J.A., Zhao Y., Sundin G.W., 2012. Genetic characterization of the HrpL regulon of the fire blight pathogen *Erwinia amylovora* reveals novel virulence factors. *Molecular Plant Pathology* **13**: 160-173.

Mergaert J., Hauben L., Cnockaert M.C., Swings J., 1999. Reclassification of non-pigmented *Erwinia herbicola* strains from trees as *Erwinia billingiae* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **49**: 377-383.

Miller M.B., Bassler B.L., 2001. Quorum sensing in bacteria. *Annual Review of Microbiology* **55**: 165-199.

Molina L., Rezzonico F., Défago G., Duffy B., 2005. Autoinduction in *Erwinia amylovora*: evidence of an acyl-homoserine lactone signal in the fire blight pathogen. *Journal of Bacteriology* **187**: 3206-3213.

Moradi A., Nasiri J., Abdollahi H., Almasi M., 2012. Development and evaluation of a loop-mediated isothermal amplification assay for detection of *Erwinia amylovora* based on chromosomal DNA. *European Journal of Plant Pathology* **133**: 609-620.

Moran N.A., 2002. Microbial minimalism: genome reduction in bacterial pathogens. *Cell* **108**: 583-586.

Morohoshi T., Nakamura Y., Yamazaki G., Ishida A., Kato N., Ikeda T., 2007. The plant pathogen *Pantoea ananatis* produces *N*-acylhomoserine lactone and causes center rot disease of onion by quorum sensing. *Journal of Bacteriology* **189**: 8333-8338.

Myung I.-S., Lee J.-Y., Yun M.-J., Lee Y.-H., Lee Y.-K., Park D.-H., Oh C.-S., 2016. Fire blight of apple, caused by *Erwinia amylovora*, a new disease in Korea. *Plant Disease* **100**: 1774.

Naum M., Brown E.W., Mason-Gamer R.J., 2011. Is a robust phylogeny of the enterobacterial plant pathogens attainable? *Cladistics* **27**: 80-93.

Nikaido H., Pagès J.-M., 2012. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. *FEMS Microbiological Reviews* **36**: 340-363.

Oh C.-S., Beer S.V., 2005. Molecular genetics of *Erwinia amylovora* involved in the development of fire blight. *FEMS Microbiological Letters* **253**: 185-192.

Ordax M., Marco-Noales E., López M.M., Biosca E.G., 2010. Exopolysaccharides favor the survival of *Erwinia amylovora* under copper stress through different strategies. *Research in Microbiology* **161**: 549-555.

Palacio-Bielsa A., Roselló M., Llop P., López M.M., 2012. *Erwinia* spp. from pome fruit trees: similarities and differences among pathogenic and non-pathogenic species. *Trees-Structure and Function* **26**: 13-29.

Palmer E.L., Teviotdale B.L., Jones A.L., 1997. A relative of the broad-host-range plasmid RSF1010 detected in *Erwinia amylovora*. *Applied and Environmental Microbiology* **63**: 4604-4607.

Passos Da Silva D., Devescovi G., Paszkiewicz K., Moretti C., Buonaurio R., Studholme D.J., Venturi V., 2013. Draft genome sequence of *Erwinia toletana*, a bacterium associated with olive knots caused by *Pseudomonas savastanoi* pv. *savastanoi*. *Genome Announcements* **1**: e00205-13.

Pirc M., Ravnikar M., Tomlinson J., Dreo T., 2009. Improved fireblight diagnostics using quantitative real-time PCR detection of *Erwinia amylovora* chromosomal DNA. *Plant Pathology* **58**: 872-881.

Pletzer D., Weingart H., 2014a. Characterization and regulation of the resistance-nodulation-cell division-type multidrug efflux pumps MdtABC and MdtUVW from the fire blight pathogen *Erwinia amylovora*. *BMC Microbiology* **14**: 185.

Pletzer D., Weingart H., 2014b. Characterization of AcrD, a resistance-nodulation-cell division-type multidrug efflux pump from the fire blight pathogen *Erwinia amylovora*. *BMC Microbiology* **14**: 13.

Pletzer D., Schweizer G., Weingart H., 2014. AraC/XylS family stress response regulators Rob, SoxS, PliA and OpiA in the fire blight pathogen *Erwinia amylovora*. *Journal of Bacteriology* **196**: 3098-3110.

Pletzer D., Stahl A., Oja A.E., Weingart H., 2015. Role of the cell envelope stress regulators BaeR and CpxR in control of RND-type multidrug efflux pumps and transcriptional cross talk with exopolysaccharide synthesis in *Erwinia amylovora*. *Archives of Microbiology* **197**: 761-772.

Powney R., Beer S.V., Plummer K.M., Luck J., Rodoni B., 2011. The specificity of PCR-based protocols for detection of *Erwinia amylovora*. *Australasian Plant Pathology* **40**: 87-97.

Pusey P.L., Stockwell V.O., Rudell D.R., 2008. Antibiosis and acidification by *Pantoea agglomerans* strain E325 may contribute to suppression of *Erwinia amylovora*. *Phytopathology* **98**: 1136-1143.

Pusey P.L., Stockwell V.O., Mazzola M., 2009. Epiphytic bacteria and yeasts on apple blossoms and their potential as antagonists of *Erwinia amylovora*. *Phytopathology* **99**: 571-581.

Pusey P.L., Stockwell V.O., Reardon C., Smits T.H.M., Duffy B., 2011. Antibiosis by *Pantoea agglomerans* biocontrol strain E325 against *Erwinia amylovora* on apple blossom stigmas. *Phytopathology* **101**: 1234-1241.

Rezzonico F., Duffy B., 2007. The role of *luxS* in the fire blight pathogen *Erwinia amylovora* is limited to metabolism and does not involve quorum sensing. *Molecular Plant-Microbe Interactions* **20**: 1284-1297.

Rezzonico F., Duffy B., 2008. Lack of genomic evidence of AI-2 receptors suggests a non-quorum sensing role for *luxS* in most bacteria. *BMC Microbiology* **8**: 154.

Rezzonico F., Stockwell V.O., Duffy B., 2008. Plant agricultural streptomycin formulations do not carry antibiotic resistance genes. *Antimicrobial Agents and Chemotherapy* **53**: 3173-3177.

Rezzonico F., Smits T.H.M., Duffy B., 2011. Diversity, evolution and functionality of clustered regularly interspaced short palindromic repeat (CRISPR) regions in fire blight pathogen *Erwinia amylovora*. *Applied and Environmental Microbiology* **77**: 3819-3829.

Rezzonico F., Braun-Kiewnick A., Mann R.A., Goesmann A., Rodoni B., Duffy B., Smits T.H.M., 2012a. Lipopolysaccharide biosynthesis genes discriminate between *Rubus*- and Spiraeoideae-infective genotypes of *Erwinia amylovora*. *Molecular Plant Pathology* **13**: 975-984.

Rezzonico F., Smits T.H.M., Duffy B., 2012b. Detection of AI-2 receptors in genomes of *Enterobacteriaceae* suggests a role of type-2 quorum sensing in closed ecosystems. *Sensors* **12**: 6645-6665.

Rezzonico F., Smits T.H.M., Born Y., Blom J., Frey J.E., Goesmann A., Cleenwerck I., De Vos P., Bonaterra A., Duffy B., Montesinos E., 2016. *Erwinia gerundensis* sp. nov., a cosmopolitan epiphyte originally isolated from pome fruit trees. *Journal of Systematic and Evolutionary Microbiology* **66**: 1583-1592.

Rico A., Ortiz-Barredo A., Ritter E., Murillo J., 2004. Genetic characterization of *Erwinia amylovora* strains by amplified fragment length polymorphism. *Journal of Applied Microbiology* **96**: 302-310.

Rico A., Führer M.E., Ortiz-Barredo A., Murillo J., 2008. Polymerase chain reaction fingerprinting of *Erwinia amylovora* has a limited phylogenetic value but allows the design of highly specific molecular markers. *Phytopathology* **98**: 260-269.

Ries S.M., Otterbacher A.G., 1977. Occurrence of fire blight on thornless blackberry in Illinois. *Plant Disease Reporter* **61**: 232-235.

Roach D.R., Sjaarda D.R., Castle A.J., Svircev A.M., 2013. Host exopolysaccharide quantity and composition impacts bacteriophage pathogenesis of *Erwinia amylovora*. *Applied and Environmental Microbiology* **79**: 3249-3256.

Römling U., Galperin M.Y., Gomelsky M., 2013. Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. *Microbiology and Molecular Biology Reviews* **77**: 1-52.

Roper M.C., 2011. *Pantoea stewartii* subsp. *stewartii*: lessons learned from a xylem-dwelling pathogen of sweet corn. *Molecular Plant Pathology* **12**: 628-637.

Sabag-Daigle A., Ahmer B.M.M., 2012. ExpI and PhzI are descendants of the long lost cognate signal synthase for SdiA. *PLoS One* **7**: e47720.

Sarowar S., Zhao Y., Soria-Guerra R.E., Ali S., Zheng D., Wang D., Korban S.S., 2011. Expression profiles of differentially regulated genes during the early stages of apple flower infection with *Erwinia amylovora*. *Journal of Experimental Botany* **62**: 4851-4861.

Sarris P.F., Trantas E.A., Skandalis N., Tampakaki A.P., Kapanidou M., Kokkinidis M., Panopoulos N.J., 2012. Phytobacterial type VI secretion systems - gene distribution, phylogeny, structure and biological functions. In: Cumagun C.J.R. (ed.). Plant Pathology, pp. 53-84. InTech.

Sauer S., Freiwald A., Maier T., Kube M., Reinhardt R., Kostrzewa M., Geider K., 2008. Classification and identification of bacteria by mass spectrometry and computational analysis. *PLoS One* **3**: e2843.

Schauder S., Shokat K., Surette M.G., Bassler B.L., 2001. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Molecular Microbiology* **41**: 463-476.

Schell M.A., Ullrich R.L., Ribot W.J., Brueggemann E.E., Hines H.B., Chen D., Lipscomb L., Kim H.S., Mrázek J., Nierman W.C., Deshazer D., 2007. Type VI secretion is a major virulence determinant in *Burkholderia mallei*. *Molecular Microbiology* **64**: 1466-1485.

Schnabel E.L., Jones A.L., 1998. Instability of a pEA29 marker in *Erwinia amylovora* previously used for strain characterization. *Plant Disease* **82**: 1334-1336.

Schollmeyer M., Langlotz C., Huber A., Coplin D.L., Geider K., 2012. Variations in the molecular masses of the capsular exopolysaccharides amylovoran, pyrifolan and stewartan. *International Journal of Biological Macromolecules* **50**: 518-522.

Schwarz S., Hood R.D., Mougous J.D., 2010. What is type VI secretion doing in all those bugs? *Trends in Microbiology* **18**: 531-537.

Sebaihia M., Bocsanczy A.M., Biehl B.S., Quail M.A., Perna N.T., Glasner J.D., Declerck G.A., Cartinhour S., Schneider D.J., Bentley S.D., Parkhill J., Beer S.V., 2010. Complete genome sequence of the plant pathogen *Erwinia amylovora* strain ATCC 49946. *Journal of Bacteriology* **192**: 2020-2021.

Seebeck F.P., 2013. Thiohistidine biosynthesis. *Chimia* **67**: 333-336.

Shapiro L.R., Scully E.D., Straub T.J., Park J., Stephenson A.G., Beattie G.A., Gleason M.L., Kolter R., Coelho M.C., De Moraes C.M., Mescher M.C., Zhaxybayeva O., 2016. Horizontal gene acquisitions, mobile element proliferation, and genome decay in the host-restricted plant pathogen *Erwinia tracheiphila*. *Genome Biology and Evolution* **8**: 649-664.

Sibanda S., Theron J., Shyntum D.Y., Moleleki L.N., Coutinho T.A., 2016. Characterization of two LuxI/R homologs in *Pantoea ananatis* LMG 2665^T. *Canadian Journal of Microbiology* **62**: 893-903.

Smith T.J., Pusey P.L., 2010. CougarBlight 2010, a significant update of the CougarBlight fire blight infection risk model. *Acta Hortulturae* **896**; 331-336.

Smits T.H.M., Jaenicke S., Rezzonico F., Kamber T., Goesmann A., Frey J.E., Duffy B., 2010a. Complete genome sequence of the fire blight pathogen *Erwinia pyrifoliae* DSM 12163^T and comparative genomic insights into plant pathogenicity. *BMC Genomics* **11**: 2.

Smits T.H.M., Rezzonico F., Kamber T., Blom J., Goesmann A., Frey J.E., Duffy B., 2010b. Complete genome sequence of the fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* spp. *Molecular Plant-Microbe Interactions* **23**: 384-393.

Smits T.H.M., Duffy B., 2011. Genomics of iron-acquisition in the plant pathogen *Erwinia amylovora*: insights in the biosynthetic pathway of the siderophore desferrioxamine E. *Archives of Microbiology* **193**: 693-699.

Smits T.H.M., Rezzonico F., Duffy B., 2011a. Evolutionary insights from *Erwinia amylovora* genomics. *Journal of Biotechnology* **155**: 34-39.

Smits T.H.M., Rezzonico F., Kamber T., Goesmann A., Ishimaru C.A., Frey J.E., Stockwell V.O., Duffy B., 2011b. Metabolic versatility and antibacterial metabolite biosynthesis are distinguishing genomic features of the fire blight antagonist *Pantoea vagans* C9-1. *PLoS One* **6**: e22247.

Smits T.H.M., Rezzonico F., López M.M., Blom J., Goesmann A., Frey J.E., Duffy B., 2013. Phylogenetic position and virulence apparatus of the pear flower necrosis pathogen *Erwinia piriflorinigrans* CFBP 5888^T as assessed by comparative genomics. *Systematic and Applied Microbiology* **36**: 449-456.

Smits T.H.M., Guerrero-Prieto V.M., Hernández-Escarcega G., Blom J., Goesmann A., Rezzonico F., Duffy B., Stockwell V.O., 2014a. Whole-genome sequencing of *Erwinia amylovora* strains from Mexico detects SNPs in *rpsL* conferring streptomycin resistance and in the *avrRpt2* effector altering host interactions. *Genome Announcements* **2**: e01229-13.

Smits T.H.M., Guerrero-Prieto V.M., Hernández-Escarcega G., Rezzonico F., Blom J., Goesmann A., Duffy B., Stockwell V.O., 2014b. Comparative genomics of *Erwinia amylovora* isolates from México. *Acta Horticulturae* **1056**: 173-177.

Starr M.P., Cardona C., Folsom D., 1951. Bacterial fire blight of raspberry. *Phytopathology* **41**: 915-919.

Stockwell V.O., Duffy B., 2012. Use of antibiotics in plant agriculture. In: Moulin G., Acar J.F. (eds). Antibiotic Resistance in Animal and Public Health. *Scientific and Technical Review of the Office International des Epizooties (Paris)*:199-210.

Stockwell V.O., Johnson K.B., Sugar D., Loper J.E., 2002. Antibiosis contributes to biological control of fire blight by *Pantoea agglomerans* strain Eh252 in orchards. *Phytopathology* **92**: 1202-1209.

Stockwell V.O., Johnson K.B., Sugar D., Loper J.E., 2010. Control of fire blight by *Pseudomonas fluorescens* A506 and *Pantoea vagans* C9-1 applied as single strains and mixed inocula. *Phytopathology* **100**: 1330-1339.

Tampakaki A.P., Skandalis N., Gazi A.D., Bastaki M.N., Sarris P.F., Charova S.N., Kokkinidis M., Panopoulos N.J., 2010. Playing the "Harp": evolution of our understanding of *hrp/hrc* genes. *Annual Review of Phytopathology* **48**: 347-370.

Tancos K.A., Cox K.D., 2016. Exploring diversity and origins of streptomycin resistant *Erwinia amylovora* isolates in New York through CRISPR spacer arrays. *Plant Disease* **100**: 1307-1313.

Tancos K.A., Villani S., Kuehne S., Borejsza-Wysocka E., Breth D., Carol J., Aldwinckle H.S., Cox K.D., 2016. Prevalence of streptomycin-resistant *Erwinia amylovora* in New York apple orchards. *Plant Disease* **100**: 802-809.

Temple T.N., Johnson K.B., 2011. Evaluation of loop-mediated isothermal amplification for rapid detection of *Erwinia amylovora* on pear and apple fruit flowers. *Plant Disease* **95**: 423-430.

Van Der Oost J., Jore M.M., Westra E.R., Lundgren M., Brouns S.J.J., 2009. CRISPR-based adaptive and heritable immunity in prokaryotes. *Trends in Biochemical Sciences* **34**: 401-407.

Van Der Zwet T., Biggs A.R., Heflebower R., Lightner G.W., 1994. Evaluation of the MARYBLIGHT computer model for predicting blossom blight on apple in West Virginia and Maryland. *Plant Disease* **78**: 225-230.

Vanneste J.L., 2000. Fire Blight: the Disease and its Causative Agent, Erwinia amylovora. CABI, Wallingford, UK.

Vanneste J.L., Yu J., Beer S.V., 1992. Role of antibiotic production by *Erwinia herbicola* Eh252 in biological control of *Erwinia amylovora*. *Journal of Bacteriology* **174**: 2785-2796.

Venturi V., Venuti C., Devescovi G., Lucchese C., Friscina A., Degrassi G., Aguilar C., Mazzucchi U., 2004. The plant pathogen *Erwinia amylovora* produces acyl-homoserine lactone signal molecules in vitro and in planta. *FEMS Microbiology Letters* **241**: 179-183.

Vogt I., Wöhner T., Richter K., Flachowsky H., Sundin G.W., Wensing A., Savory E.A., Geider K., Day B., Hanke M.-V., Peil A., 2013. Gene-for-gene relationship in the host-pathogen system *Malus × robusta* 5-*Erwinia amylovora*. *New Phytologist* **197**: 1262-1275.

Vrancken K., Holtappels M., Schoofs H., Deckers T., Valcke R., 2013. Pathogenicity and infection strategies of the fire blight pathogen *Erwinia amylovora* in *Rosaceae*: state of the art. *Microbiology* **159**: 823-832.

Wang D., Korban S.S., Zhao Y., 2009. The Rcs phosphorelay system is essential for pathogenicity in *Erwinia amylovora*. *Molecular Plant Pathology* **10**: 277-290.

Wang D., Korban S.S., Pusey P.L., Zhao Y., 2011. Characterization of the RcsC sensor kinase from *Erwinia amylovora* and other Enterobacteria. *Phytopathology* **101**: 710-717.

Wang D., Korban S.S., Pusey P.L., Zhao Y., 2012a. AmyR is a novel negative regulator of amylovoran production in *Erwinia amylovora*. *PLoS One* **7**: e45038.

Wang D., Qi M., Calla B., Korban S.S., Clough S.J., Cock P.J.A., Sundin G.W., Toth I., Zhao Y., 2012b. Genome-wide identification of genes regulated by the Rcs phosphorelay system in *Erwinia amylovora*. *Molecular Plant-Microbe Interactions* **25**: 6-17.

Wang X., De Boer P.a.J., Rothfield L.I., 1991. A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. *EMBO Journal* **10**: 3363-3372.

Wang X.G., Yang F., Von Bodman S.B., 2012c. The genetic and structural basis of two distinct terminal side branch residues in stewartan and amylovoran exopolysaccharides and their potential role in host adaptation. *Molecular Microbiology* **83**: 195-207.

Wei Z.-M., Beer S.V., 1995. *hrpL* activates *Erwinia amylovora hrp* gene transcription and is member of the ECF subfamily of s factors. *Journal of Bacteriology* **177**: 6201-6210.

Wei Z., Kim J.F., Beer S.V., 2000. Regulation of *hrp* genes and type III protein secretion in *Erwinia amylovora* by HrpX/HrpY, a novel two-component system, and HrpS. *Molecular Plant-Microbe Interactions* **13**: 1251-1262.

Wensing A., Gernold M., Jock S., Jansen R., Geider K., 2014. Identification and genetics of 6-thioguanine secreted by *Erwinia* species and its interference with the growth of other bacteria. *Molecular Genetics and Genomics* **289**: 215-223.

Wilson M., Lindow S.E., 1994. Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. *Applied and Environmental Microbiology* **60**: 4468-4477.

Winzer K., Hardie K.R., Williams P., 2003. LuxS and autoinducer-2: their contribution to quorum sensing and metabolism in bacteria. *Advances in Applied Microbiology* **53**: 291-396.

Wu X., Vellaichamy A., Wang D., Zamdborg L., Kelleher N.L., Huber S.C., Zhao Y., 2013. Differential lysine acetylation profiles of *Erwinia amylovora* strains revealed by proteomics. *Journal of Proteomics* **79**: 60-71.

Xavier K.B., Bassler B.L., 2005. Interference with AI-2-mediated bacterial cell-cell communication. *Nature* **437**: 750-753.

Zeng Q., McNally R.R., Sundin G.W., 2013. Global small RNA chaperone Hfq and regulatory small RNAs control virulence in the fire blight pathogen *Erwinia amylovora*. *Journal of Bacteriology* **195**: 1706-1717.

Zeng Q., Sundin G.W., 2014. Genome-wide identification of Hfq-regulated small RNAs in the fire blight pathogen *Erwinia amylovora* discovered small RNAs with virulence regulatory function. *BMC Genomics* **15**: 414.

Zhang R., Sun B., Wang Y., Lei L., Schwarz S., Wu C., 2016. Characterization of a *cfr*-1 carrying plasmid from porcine *Escherichia coli* that closely resembles plasmid pEA3 from the plant pathogen *Erwinia amylovora*. *Antimicrobial Agents and Chemotherapy* **60**: 658-661.

Zhao Y., He S.Y., Sundin G.W., 2006. The *Erwinia amylovora* *avrRpt2*$_{EA}$ gene contributes to virulence on pear and AvrRpt2$_{EA}$ is recognized by *Arabidopsis* RPS2 when expressed in *Pseudomonas syringae*. *Molecular Plant-Microbe Interactions* **19**: 644-654.

Zhao Y., Sundin G.W., Wang D., 2009a. Construction and analysis of pathogenicity island deletion mutants of *Erwinia amylovora*. *Canadian Journal of Microbiology* **55**: 457-464.

Zhao Y., Wang D., Nakka S., Sundin G.W., Korban S.S., 2009b. Systems level analysis of two-component signal transduction systems in *Erwinia amylovora*: role in virulence, regulation of amylovoran biosynthesis and swarming motility. *BMC Genomics* **10**: 245.

Zhao Y., Qi M., 2011. Comparative genomics of *Erwinia amylovora* and related *Erwinia* species - What do we learn? *Genes* **2**: 627-639.

Zhao Y.F., 2014. Genomics of *Erwinia amylovora* and related species associated with pome fruit trees. In: Gross D., Lichens-Park A., Kole C. (eds). Genomics of Plant-Associated Bacteria, pp. 1-36. Berlin Heidelberg: Springer-Verlag.

Zhao Y.F., Sundin G.W., 2017. Exploring linear and cyclic (di)-nucleotides as messengers for regulation of T3SS and biofilm formation in *Erwinia amylovora*. *Journal of Plant Pathology* **99** (Special Issue): 25-35.

Zhu W., Luo L., Wang J., Zhuang X., Zhong L., Liao K., Zeng Y., Lu Y., 2009. Complete nucleotide sequence of plasmid pCTX-M360, an intermediate plasmid between pEL60 and pCTX-M3, from a multidrug-resistant *Klebsiella pneumoniae* strain isolated in China. *Antimicrobial Agents and Chemotherapy* **53**: 5292-5293.

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