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Genome-wide identification of the Sec-dependent secretory protease genes in *Erwinia amylovora* and analysis of their expression during infection of immature pear fruit*#

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Abstract: The general secretory (Sec) pathway represents a common mechanism by which bacteria secrete proteins, including virulence factors, into the extracytoplasmic milieu. However, there is little information about this system, as well as its associated secretory proteins, in relation to the fire blight pathogen *Erwinia amylovora*. In this study, data mining revealed that *E. amylovora* harbors all of the essential components of the Sec system. Based on this information, we identified putative Sec-dependent secretory proteases in *E. amylovora* on a genome-wide scale. Using the programs SignalP, LipoP, and Phobius, a total of 15 putative proteases were predicted to contain the N-terminal signal peptides (SPs) that might link them to the Sec-dependent pathway. The activities of the predicted SPs were further validated using an *Escherichia coli*-based alkaline phosphatase (PhoA) gene fusion system that confirmed their extracytoplasmic property. Transcriptional analyses showed that the expression of 11 of the 15 extracytoplasmic protease genes increased significantly when *E. amylovora* was used to inoculate immature pears, suggesting their potential roles in plant infection. The results of this study support the suggestion that *E. amylovora* might employ the Sec system to secrete a suite of proteases to enable successful infection of plants, and shed new light on the interaction of *E. amylovora* with host plants.

Key words: *Erwinia amylovora*; Sec-dependent pathway; Protease; Gene expression; Plant infection
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1 Introduction

Fire blight is a destructive and highly infectious disease of apple, pear, quince, blackberry, raspberry, and many rosaceous ornamentals (Eastgate, 2000; Malnoy et al., 2012). The disease has spread to more than 50 countries around the world since it was first reported in North America in the late 1700s (Malnoy et al., 2012). The causative agent of fire blight has long been known to be *Erwinia amylovora*, a Gram-negative enterobacterium (Eastgate, 2000; Malnoy

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et al., 2012), although some studies showed that *Erwinia pyrifoliae* (Kim et al., 1999, 2001) and *Erwinia piriflorinigrans* (López et al., 2011), two closely related bacterial species, could also cause fire blight symptoms in pear. In nature, *E. amylovora* cells are disseminated among host plants via wind, rain, and insects (Eastgate, 2000; Malnoy et al., 2012). By using natural openings and wounds, particularly in flowers, as infection routes, *E. amylovora* enters the host and causes the initial symptoms of water soaking, followed by the wilting and necrosis that result in the scorched and blackened appearance of infected tissue. The symptoms are generally localized to the blossom bracts or young shoots. However, in a highly susceptible host, *E. amylovora* can systemically migrate into mature tissues, leading to the death of an entire tree (Eastgate, 2000; Malnoy et al., 2012).

In recent decades there has been remarkable progress toward elucidating the virulence mechanisms of *E. amylovora* in host plants (Eastgate, 2000; Malnoy et al., 2012; Piqué et al., 2015; Castiblanco et al., 2018; Lee et al., 2018, 2019; Emeriewen et al., 2019; Schachterle and Sundin, 2019). Many factors, such as exopolysaccharide (EPS), amylovoran, levan, lipopolysaccharide (LPS), biofilm formation, and motility, have been determined to play roles in the pathogenicity of *E. amylovora* or host colonization (Koczan et al., 2009; Piqué et al., 2015; Kharadi et al., 2018). Moreover, it has been found that *E. amylovora* has evolved diverse secretion systems, including types I, II, III, and VI (T1SS, T2SS, T3SS, and T6SS, respectively), to promote the infection of plants (Bogdanove et al., 1996; Zhang et al., 1999; Oh and Beer, 2005; Cianciotto and White, 2017; Kamber et al., 2017; Tian et al., 2017). Among them, T3SS is one of the most remarkably versatile pathogenic factors used by numerous Gram-negative bacterial pathogens of animals and plants to inject proteins (also called effectors) directly into host cells (Tang et al., 2006; McCann and Guttman, 2008). *E. amylovora* uses T3SS to secrete several effector proteins (Zhao et al., 2006; Nissinen et al., 2007): DspA/E is essential for the multiplication and survival of the bacterium in host plants (Gaudriault et al., 1997) and non-host plants (Oh et al., 2007; Degrave et al., 2013); HrpN contributes to the elicitation of callose (Boureau et al., 2011); Eop1 acts as a host-range limiting factor (Asselin et al., 2011); and, $AvrRpt2_{EA}$ causes symptoms that mimic those of fire blight and induces a salicylic acid-dependent defense response when expressed transgenically in apple (Schröpfer et al., 2018). Both DspA/E and HrpN are required to trigger defense responses and cell death in host and non-host plants (Gaudriault et al., 1997; Boureau et al., 2006; Degrave et al., 2008).

Protein secretion in bacterial pathogens is one of the most high-profile research topics in relation to the virulence of these organisms. The general protein secretory (Sec) pathway, in combination with the twin-arginine translocation (Tat) pathway, accounts for most bacterial protein transport across cytoplasmic membranes (Holland, 2010; Segers and Anné, 2011; Tsirigotaki et al., 2017). The Sec-dependent pathway is found widely in bacteria and includes three transmembrane proteins SecY, SecE, and SecG that are assembled into channel SecYEG to translocate proteins with the aid of the peripheral membrane ATPase SecA. In addition, the Sec complex has at least three other membrane proteins (SecD, SecF, and YajC) that are involved in efficient protein export (Tsirigotaki et al., 2017; Cranford-Smith and Huber, 2018; Smets et al., 2019). A protein secreted via the Sec system is synthesized at the ribosome as an unfolded preprotein with an N-terminal signal peptide (SP) fused to the mature domain. Once the preprotein is translocated into the periplasm, the SP is cleaved and removed by exocytoplasmic signal peptidase I (LepB) or lipoprotein signal peptidase II (LspA). This results in the release and folding of a mature domain that is either retained in the periplasm or secreted as an outer membrane or extracellular protein via secondary secretion pathways, such as T5SS, the localization of lipoprotein (Lol) pathway, or T2SS (Tsirigotaki et al., 2017; Cranford-Smith and Huber, 2018; Smets et al., 2019).

Many proteases in Gram-negative bacteria have been found to play key roles during the infection process of pathogens. Those that are most well-known include the two ATP-dependent serine proteases, Lon and Clp, which serve as the major proteolytic components in the cytosol and contribute to pathogenesis by their timely degradation of the transcriptional regulators that control expression of T3SS (Frees et al., 2013; Figaj et al., 2019). HtrA/DegP is another highly characterized protease. As a non-ATP-dependent serine protease, HtrA/DegP is localized in

the periplasm and involved in the turnover and export of extracellular virulence factors (Frees et al., 2013; Figaj et al., 2019). Recent studies have shown that HtrA/DegP of two species of human pathogenic bacteria, *Helicobacter pylori* and *Chlamydia trachomatis*, can be secreted into either the extracellular environment or host cell cytosol (Löwer et al., 2008; Hoy et al., 2010; Wu et al., 2011), indicating its involvement in diverse mechanisms of bacterial virulence. In contrast to cytoplasmic and periplasmic proteases, proteases anchored to the bacterial surface, secreted into the extracellular environment, or injected into the host cells interact closely with the host components, and thus may serve directly as virulence factors (Figaj et al., 2019).

In this study, we first mined the annotated genome data of *E. amylovora* in the GenBank public database and confirmed that the bacterium possesses all of the essential components of the Sec system. Based on this information, we identified the Sec-dependent secretory proteases from the whole genome of *E. amylovora* using web-based algorithms coupled with a laboratory-based *Escherichia coli* alkaline phosphatase (PhoA) assay (Liu et al., 2019). Further, we examined gene expression levels of the putative Sec-dependent secretory proteases when *E. amylovora* infected immature pears.

2 Materials and methods

2.1 In silico analysis

A total of 3393 putative proteins, including 3368 chromosomal proteins (GenBank No. NC_013961) and 25 proteins encoded by plasmids (GenBank No. NC_013957), were predicted in *E. amylovora* CFBP1430. Using SignalP Version 4.0 with default settings for Gram-negative bacteria (Petersen et al., 2011), the 3393 *E. amylovora* proteins were screened for those bearing N-terminal SPs that could conceivably link them to the Sec pathway. The obtained Sec-dependent secretory proteins were filtered further to select putative proteases according to protein annotations previously designated (Smits et al., 2010). The presence of the N-terminal SPs in putative extracytoplasmic proteases was re-examined with the additional programs LipoP Version 1.0 (Juncker et al., 2003) and Phobius (Käll et al., 2004, 2007).

2.2 PhoA assay

The SP of each putative Sec-dependent secretory protease was tested using an *E. coli*-based PhoA assay as described previously (Liu et al., 2019). Briefly, based on the *E. amylovora* CFBP1430 genomic sequence (GenBank No. NC_013961.1), specific forward and reverse primers (Table S1) were first designed to amplify the DNA sequence of each putative SP. The resulting polymerase chain reaction (PCR) product was then ligated into *Nde*I-*Hin*dIII-digested pET-mphoA, generating a construct with an in-frame gene fusion between the SP and the *mphoA* gene (see the schematic diagram of vectors in Fig. 1a). The construct was subsequently transformed into chemically competent cells of *E. coli* BL21, followed by the detection of PhoA activity of transformants on indicator LB agar containing 90 μg/mL 5-bromo-4-chloro-3-indoyl phosphate (BCIP), 100 mmol/L isopropy-β-D-thiogalactoside (IPTG), and 75 mmol/L Na_2HPO_4. After 12 h of incubation at 37 °C, a transformant that remained white indicated a lack of PhoA, while a colony that turned blue denoted PhoA activity. The BL21 cells that harbored pET-phoA were used as positive controls and those that harbored pET-mphoA as negative controls.

2.3 Immature pear infection and total RNA extraction

Immature pear fruits were inoculated with cells of *E. amylovora* DSM17948 as previously described with some modifications (Pletzer and Weingart, 2014; Pletzer et al., 2015). The cells were grown overnight in LB broth at 28 °C, harvested by centrifugation at 3000*g* for 10 min at 4 °C, and resuspended to an OD_{600} (optical density at 600 nm) of about 2 in sterile distilled water. Four two-month-old pears (*Pyrus bretschneideri* L. cv. Dangshan Su) were surface-sterilized with 0.02 g/mL sodium hypochlorite for 20–30 min. Following six rinses with sterile distilled water, the pears were cut in half, and each cut surface was immediately inoculated with two 100-μL drops of cell suspension and incubated in a humidified chamber at 28 °C. At 8 and 24 h post-inoculation (hpi), the pear tissues at the inoculation site were excised with a sterilized blade and stored at −80 °C. The experiment was carried out three times. The start culture (designated 0 h) used to infect the immature pear fruits, and the tissues collected at 8 and 24 hpi were

individually subjected to total RNA extraction using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.4 Quantitative reverse transcription PCR and data analysis

By using the primers listed in Table S2, quantitative reverse transcription PCR (RT-qPCR) analysis was performed as descried previously (Hong et al., 2017). The *E. amylovora recA* (recombinase A) gene (GeneID: 8914602) was included as an internal reference. Each experiment was performed with three technical replicates. The $2^{-\Delta\Delta C_T}$ method was used to calculate relative gene expression values (Livak and Schmittgen, 2001), which were transformed to fold-change and plotted in figures. Statistical analyses were conducted using Student's *t*-test (SPSS 10.0, Chicago, IL, USA).

3 Results

3.1 In silico analyses of the Sec system and Sec-dependent secretory proteases in *E. amylovora*

To date, there are three *E. amylovora* strains, CFBP1430 (Smits et al., 2010), ATCC 49946 (Sebaihia et al., 2010), and E-2 (Lagonenko et al., 2008), whose complete genomic sequences are available in the GenBank database. Data mining of their chromosome-encoded proteins (GenBank Nos. NC_013961.1, NC_013971.1, and NZ_CP024970.1) showed that each strain had the essential components of the Sec pathway, including SecA, SecY, SecE, SecG, SecD, SecF, and YajC, as well as LepB and LspA, two exocytoplasmic signal peptidases (Table 1), according to the protein annotations previously designated (Smits et al., 2010). These findings indicate that *E. amylovora* can secrete proteins using the Sec-dependent secretion process. Therefore, the Sec-dependent secretory proteins of *E. amylovora* were predicted on the basis of the CFBP1430 genome using SignalP 4.0 (Petersen et al., 2011). As a result, we identified 168 *E. amylovora* proteins bearing N-terminal SPs that could potentially be secreted via the Sec system (Table S3). Based on the protein annotations previously designated (Smits et al., 2010), these proteins included 15 proteases (Table 2). Given the general significance of bacterial proteases in virulence (Xia, 2004; Figaj et al., 2019), the experiments described below focused on these putative extracytoplasmic proteases.

The presence of N-terminal SPs in the putative proteases was subjected to additional verification using LipoP (Juncker et al., 2003) and Phobius (Käll et al., 2004, 2007). Notably, among the 15 extracytoplasmic proteases, only EaPrt11 contained a lipoprotein SP that was cleaved by LspA, while all the others had SPs that were processed by LepB (Table 2). According to the MEROPS protease database (Rawlings et al., 2018), the 15 putative proteases were members of the serine, cysteine, or metalloprotease classes (Table 2), the three most abundant types of proteolytic enzymes in bacteria (Supuran et al., 2001). A BLAST search of the GenBank database showed that all the putative proteases were highly conserved

Table 1 Essential components of the putative Sec system in *Erwinia amylovora*

Gene	Locus_tag		
	CFBP1430	ATCC 49946	E-2
SecA	EAMY_RS30645	EAM_RS03465	AD997_RS03640
SecD	EAMY_RS21905	EAM_RS04720	AD997_RS04940
SecF	EAMY_RS21910	EAM_RS04725	AD997_RS04945
SecE	EAMY_RS18480	EAM_RS01205	AD997_RS01265
YajC	EAMY_RS21900	EAM_RS04715	AD997_RS04935
SecG	EAMY_RS19030	EAM_RS15075	AD997_RS15760
SecY	EAMY_RS32865	EAM_RS15600	AD997_RS16320
LepB	EAMY_RS29460	EAM_RS12265	AD997_RS12890
LspA	EAMY_RS30890	EAM_RS03220	AD997_RS03390

The genes referred to in this study were identified from three *E. amylovora* strains, CFBP1430 (GenBank No. NC_013961.1), ATCC 49946 (GenBank No. NC_013971.1), and E-2 (GenBank No. NZ_CP024970.1), whose complete genomic sequences are available

Table 2 Putative Sec-dependent secretory proteases identified from *Erwinia amylovora*

Protein	GenBank No.	Signal peptidase	Cleavage site	Protease type
EaPrt1	WP_004155063.1	LepB	24–25	Serine protease
EaPrt2	WP_004155111.1	LepB	20–21	Serine protease
EaPrt3	WP_004155489.1	LepB	22–23	Cysteine protease
EaPrt4	WP_004155561.1	LepB	22–23	Metalloprotease
EaPrt5	WP_013035939.1	LepB	29–30	Serine protease
EaPrt6	WP_004156768.1	LepB	25–26	Serine protease
EaPrt7	WP_004156843.1	LepB	30–31	Serine protease
EaPrt8	WP_004157647.1	LepB	20–21	Serine protease
EaPrt9	WP_004157888.1	LepB	21–22	Serine protease
EaPrt10	WP_004158423.1	LepB	19–20	Serine protease
EaPrt11	WP_004158446.1	LspA	21–22	Cysteine protease
EaPrt12	WP_004159031.1	LepB	27–28	Metalloprotease
EaPrt13	WP_004159492.1	LepB	26–27	Serine protease
EaPrt14	WP_004161039.1	LepB	23–24	Metalloprotease
EaPrt15	WP_004158249.1	LepB	24–25	Serine protease

The protein annotations referred to in this study are based on *E. amylovora* CFBP1430 (GenBank No. NC_013961.1). The putative proteases were defined as serine, cysteine, and metalloproteases based on the MEROPS protease database (Rawlings et al., 2018), and their cleavage sites were predicted using SignalP 4.0 (Petersen et al., 2011), LipoP 1.0 (Juncker et al., 2003), and Phobius (Käll et al., 2004, 2007)

among strains of the genus *Erwinia* (data not shown). However, most have no orthologs in the well-studied bacterial plant pathogens of the genera *Xanthomonas*, *Pseudomonas*, or *Agrobacterium*, with the exception of EaPrt1, EaPrt9, EaPrt14, and EaPrt15, which share 40%–50% similarity with the Do family serine endopeptidase (GenBank No. WP_169707362) of *Xanthomonas campestris*, the carboxy terminal-processing peptidase (GenBank No. WP_170826269), M20 family metallopeptidase (GenBank No. WP_055001508) of *Pseudomonas syringae*, and the α/β fold hydrolase (GenBank No. WP_174031637) of *Agrobacterium rhizogenes*, respectively.

3.2 Validation of the Sec-dependent signal peptides via the PhoA assay

Next, we used the *phoA* gene fusion assay (Liu et al., 2019) to determine whether the putative extracytoplasmic proteases could be exported via the Sec translocon. The DNA sequence that encoded the putative SP of each protein was cloned and fused in-frame with *mphoA* of pET-mphoA (Fig. 1a), resulting in 15 constructs that were transformed individually into *E. coli* BL21. After 12 h of incubation on the indicator LB agar, the transformants that expressed Prt4SP-mphoA and Prt11SP-mphoA turned pale blue, and those that harbored the other 13 constructs turned dark blue, while the negative control (BL21 cells with pET-mphoA) remained white (Fig. 1b). These results indicated that although the SPs tested displayed different activities in the *E. coli*-based PhoA assay, each could translocate the mphoA moiety from the cytoplasm to periplasm, suggesting that the 15 putative proteases that contained the cognate SPs would also be secreted via the *E. amylovora* Sec pathway.

3.3 Gene expression analysis of the Sec-dependent secretory proteases during plant infection

To determine the potential involvement of the 15 extracytoplasmic proteases in host plant infection, we examined their levels of gene expression during *E. amylovora* infection of the immature pear fruit using two-step RT-qPCR. Of the 15 proteases, the expression of *EaPrt4*, *5*, *8*, *14*, and *15* steadily increased during the course of a 24-h infection (Fig. 2a), while the expression of *EaPrt6*, *11*, and *13* continuously decreased (Fig. 2b). In addition, the messenger RNAs

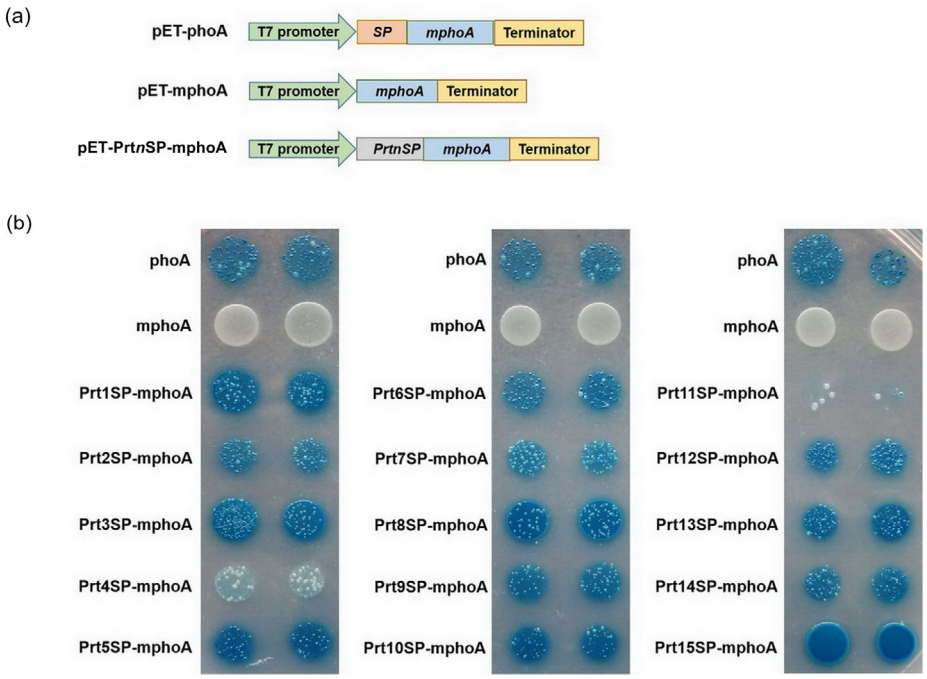

Fig. 1 Extracytoplasmic secretion of the 15 putative *Erwinia amylovora* proteases validated using the PhoA assay
(a) Diagrams of the expression cassettes for the *phoA* gene. pET-phoA harboring the full length *Escherichia coli phoA* gene was used as a positive control, while pET-mphoA harboring *mphoA* lacking its native *SP* was used as a negative control. The encoding sequences of the putative signal peptides in the 15 proteases were designated Prt*n*SP (where "*n*" represents the numbers ranging from 1 to 15) and fused with the *mphoA* gene, producing pET-Prt*n*SP-mphoA. (b) The putative signal peptides of the 15 Sec-dependent secretory proteases directed the extracytoplasmic export of mphoA moiety. After 12 h of incubation on LB media containing isopropy-β-D-thiogalactoside (IPTG, 100 mmol/L), 5-bromo-4-chloro-3-indoyl phosphate (BCIP, 90 μg/mL), and Na_2HPO_4 (75 mmol/L) at 37 °C, the *E. coli* BL21 cells that harbored pET-Prt*n*SP-mphoA turned blue, while the cells that harbored pET-mphoA remained white

(mRNAs) of *EaPrt1*, *2*, *3*, *7*, *9*, *10*, and *12* peaked at 8 hpi, but decreased at 24 hpi, displaying an "up-down" expression pattern (Fig. 2c). Only *EaPrt2* showed levels of expression at both 8 and 24 hpi that were not significantly different from that at 0 h. Note that compared with its level of expression at 0 h, the expression of *EaPrt7* showed about 37-fold increase at 8 hpi, but declined sharply to about 6-fold increase at 24 hpi. This was the most significantly altered expression pattern among all the genes tested.

4 Discussion

Bacterial pathogens have evolved multiple sophisticated systems to secrete proteins that help enable them to use an environmental niche or act as pathogens (Stavrinides et al., 2008; Green and Mecsas, 2016). Most secretory proteins in bacteria are translocated across the cytoplasmic membrane via the Sec pathway (Holland, 2010; Segers and Anné, 2011; Tsirigotaki et al., 2017). However, there is little information about the Sec system in *E. amylovora*. In this study, data mining of the *E. amylovora*-annotated proteins showed that the bacterium possesses the full components of the Sec pathway, which could thereby enable the *E. amylovora* proteins, including potential virulence factors, to be secreted into the extracytoplasmic environment. Thus, we combined the bioinformatics screen and the *E. coli*-based alkaline PhoA assay to further define 15 Sec-dependent secretory proteases from the whole genome of *E. amylovora*.

Secretory proteins previously identified from *E. amylovora* have almost all been Type III effectors (Nissinen et al., 2007; Schröpfer et al., 2018), which are directly injected into the host cells and thus closely interact with the host components. Based on the properties of Sec-dependent secretory proteins

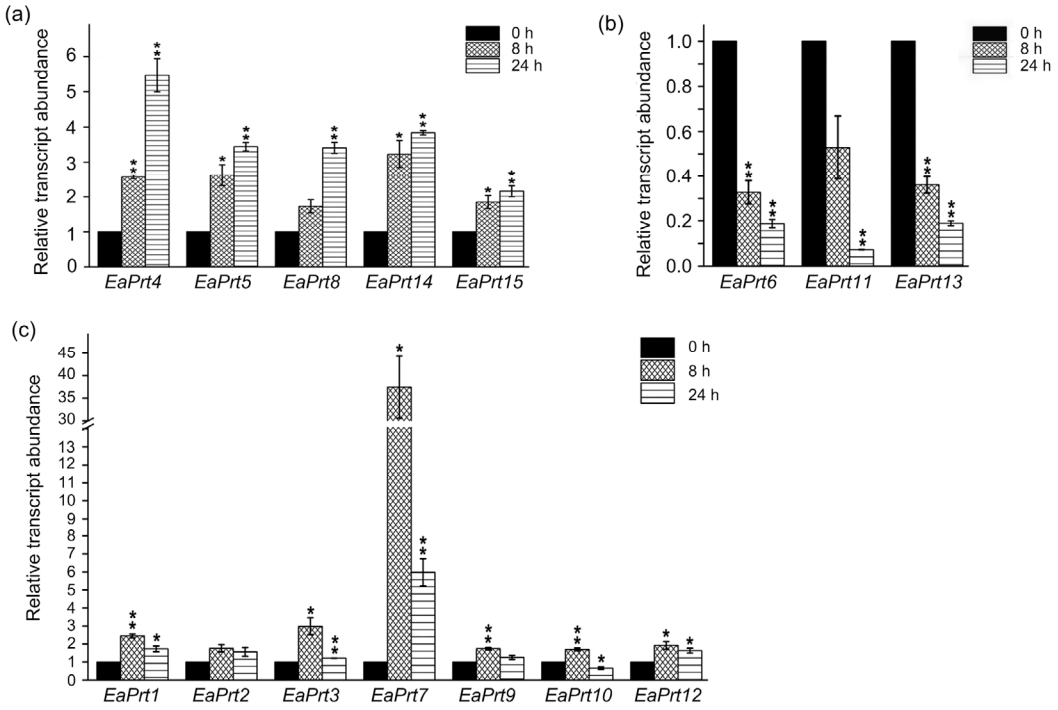

Fig. 2 Gene expression of the 15 *Erwinia amylovora* extracytoplasmic proteases on immature pear tissues

Total RNA was prepared from the immature pears infected by *E. amylovora* at 8 and 24 h post inoculation (hpi) and from the start culture used to infect the immature pear fruits (0 h). Two-step RT-qPCR was used to assess the gene expression of the putative proteases relative to the *recA* gene. Over the course of a 24-h infection, the levels of expression of *EaPrt4*, *5*, *8*, *14*, and *15* steadily increased (a), while the expression of *EaPrt6*, *11*, and *13* continuously decreased (b), and *EaPrt1*, *2*, *3*, *7*, *9*, *10*, and *12* exhibited an "up-down" expression pattern (c). The experiment was performed with three technical replicates. The mean relative expression levels and the standard errors are indicated for each gene. The asterisks denote significant differences according to a Student's *t*-test (** P<0.01 and * P<0.05, vs. 0 h)

(Tsirigotaki et al., 2017; Smets et al., 2019), once the 15 Sec-dependent *E. amylovora* proteases have crossed the inner plasma membrane, we inferred that they could either remain in the periplasm or be further translocated through the outer membrane as membrane bound or free proteins. Nevertheless, in view of the gene expression profiles during the infection of immature pears, 11 of the 15 proteases tested showed significantly increased levels of gene expression, implying their potential roles in the context of plant infection. In particular, there were six (EaPrt1, 3, 7, 9, 10, and 12) that could conceivably function during the early stages of *E. amylovora* infection, since they showed the highest fold increase in gene expression at 8 hpi. In contrast, EaPrt2, 6, 11, and 13, whose gene expression profiles showed either a lack of significant variation or a continuous decrease, might have no or only minor roles in plant infection.

While the involvement of proteases in the virulence of phytopathogenic bacteria is less well understood than their involvement in the virulence of animal and human bacterial pathogens, several studies indicate that phytopathogenic bacteria have evolved extracellular metalloproteases to invade host plants (Figaj et al., 2019). One well-studied example is found in species of *Pseudomonas*, which secretes the metalloprotease AprA into the plant apoplast to specifically recognize and degrade the bacterial flagellin monomer, a strong inducer of innate immune responses, thus enabling the bacterium to evade host immune defense mechanisms (Bardoel et al., 2011; Pel et al., 2014). Moreover, the bacterial extracellular metalloproteases are presumed to participate in the degradation of the plant cell wall, the first barrier against plant pathogens (Figaj et al., 2019). *Pectobacterium carotovorum* produces an extracellular

metalloprotease that catalyzes the degradation of plant cell wall structural proteins in vitro (Feng et al., 2014), as does *X. campestris* pv. *campestris* (Dow et al., 1998). In this study of 11 Sec-dependent *E. amylovora* proteases potentially involved in host infection, three (EaPrt4, 12, and 14) were designated metalloproteases based on the MEROPS protease database (Rawlings et al., 2018), and merit further investigation. Of particular interest was EaPrt7, a putative serine protease that showed the most significantly upregulated level of gene expression during the infection of immature pears, suggesting its significance during host plant infection. EaPrt13, an ortholog of HtrA/DegP, was not included in the 11 proteases with potential roles in host infection. In contrast to the well-known role of HtrA/DegP in the virulence of medically significant plant pathogenic bacteria (Yorgey et al., 2001; Frees et al., 2013; Figaj et al., 2019), EaPrt13 seemed not to be implicated in plant infection because its gene expression decreased continuously over the course of a 24-h infection.

5 Conclusions

In this study, a total of 15 putative Sec-dependent secretory proteases, including 2 cysteine proteases, 3 metalloproteases, and 10 serine proteases, were identified from *E. amylovora* on a genome-wide scale. Of these putative extracytoplasmic proteases, 11 showed significantly increased levels of gene expression when *E. amylovora* interacted with mature pears, and were purported to be engaged in plant infection. While the extracytoplasmic localization site, as well as the specific biological function of each putative protease, remains to be determined, the data from this study suggest that *E. amylovora* has evolved a suite of Sec-dependent extracytoplasmic proteases to disrupt host homeostasis via their proteolytic activities, thereby promoting plant infection.

Contributors

Wang-bin ZHANG, Wen-jun ZHAO, and Wei-min LI conceived the project. Wang-bin ZHANG, Hai-lin YAN, Zong-cai ZHU, Chao ZHANG, Pei-xiu DU, and Wen-jun ZHAO performed the experiments. Wang-bin ZHANG, Hai-lin YAN, Wen-jun ZHAO, and Wei-min LI analyzed the data. Wen-jun ZHAO and Wei-min LI contributed reagents and materials. Wang-bin ZHANG, Wen-jun ZHAO, and Wei-min LI wrote the manuscript. All authors have read and approved the final version of the manuscript, and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Wang-bin ZHANG, Hai-lin YAN, Zong-cai ZHU, Chao ZHANG, Pei-xiu DU, Wen-jun ZHAO, and Wei-min LI declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 Primers used to amplify the DNA sequences that encode the putative signal peptides of Sec-dependent secretory proteases

Table S2 Primers used for RT-qPCR assay

Table S3 Putative Sec-dependent secretory proteins identified from *Erwinia amylovora* CFBP1430

中文概要

题　目：梨火疫菌 Sec 依赖分泌蛋白酶的全基因组鉴定及其在侵染幼梨过程中的基因表达分析

目　的：鉴定参与梨火疫菌侵染的重要 Sec 依赖分泌蛋白酶。

创新点：构建了梨火疫菌 Sec 依赖分泌蛋白酶编码基因在侵染寄主植物过程中的时序表达图谱。

方　法：利用生物信息学与大肠杆菌 PhoA 检测体系两者结合，在全基因组水平筛选并鉴定梨火疫菌的 Sec 依赖分泌蛋白酶；利用逆转录实时定量聚合酶链反应（RT-qPCR），分析 Sec 依赖分泌蛋白酶编码基因在梨火疫菌侵染寄主植物过程中转录表达的时序变化。

结　论：梨火疫菌含有完整的 Sec 分泌系统，可由此分泌至少 15 种蛋白酶，其中 11 种蛋白酶可能在病原菌侵染寄主植物过程中发挥功能。

关键词：梨火疫菌；Sec 依赖分泌系统；蛋白酶；基因表达；侵染