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REVIEW

Erwinia spp. from pome fruit trees: similarities and differences among pathogenic and non-pathogenic species

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Abstract The number of described pathogenic and non-pathogenic *Erwinia* species associated with pome fruit trees, especially pear trees, has increased in recent years, but updated comparative information about their similarities and differences is scarce. The causal agent of the fire blight disease of rosaceous plants, *Erwinia amylovora*, is the most studied species of this genus. Recently described species that are pathogenic to pear trees include *Erwinia pyrifoliae* in Korea and Japan, *Erwinia* spp. in Japan, and *Erwinia piriflorinigrans* in Spain. *E. pyrifoliae* causes symptoms that are indistinguishable from those of fire blight in Asian pear trees, *Erwinia* spp. from Japan cause black lesions on several cultivars of pear trees, and *E. piriflorinigrans* causes necrosis of only pear blossoms. All these novel species share some phenotypic and genetic characteristics with *E. amylovora*. Non-pathogenic *Erwinia* species are *Erwinia billingiae* and *Erwinia tasmaniensis* that have also been described on pome fruits; however, less information is available on these species. We present an updated review on the phenotypic and molecular characteristics, habitat, pathogenicity, and epidemiology of *E. amylovora*, *E. pyrifoliae*, *Erwinia* spp. from Japan, *E. piriflorinigrans*, *E. billingiae,* and *E. tasmaniensis.* In addition, the interaction of these species with pome fruit trees is discussed.

Keywords *Erwinia amylovora* · *Erwinia pyrifoliae* · BSBP and BBSDP Japanese *Erwinia* spp. · *Erwinia piriflorinigrans* · *Erwinia billingiae* · *Erwinia tasmaniensis*

Introduction

Several species within the genus *Erwinia* are pathogenic to pome fruit trees. The fire blight disease of rosaceous plants was described in the late eighteenth century, and its causal agent (*Erwinia amylovora)* was identified in the late nineteenth century. In the last 15 years, some other *Ewinia* species that are pathogenic to pome fruit trees have been described, such as *E. pyrifoliae*, Japanese *Erwinia* spp. that cause bacterial diseases of pear (BSBP and BBSDP), and *E. piriflorinigrans*. Other *Erwinia* species found in pome fruit trees are non-pathogenic, such as *Erwinia billingiae* and *Erwinia tasmaniensis*. Our aim is not to exhaustively review the literature available on these species but rather to present a selection of the update information that focuses on their phenotypic and molecular characteristics, habitat, pathogenicity, and epidemiology, and to summarize the main characteristics of the *Erwinia* species from pome fruit trees.

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Pathogenic *Erwinia* species: general background of the diseases they cause and their hosts

Erwinia amylovora

In 1884, *E. amylovora* was recognized as the first bacterial plant pathogen by fulfilling Koch's postulates; however, the fire blight disease, which is caused by *E. amylovora*, had been observed in rosaceous plants since 1780 (van der Zwet and Keil 1979). This bacterium has been thoroughly studied due to its early description and significant economic impact. The name of the disease is descriptive of its major characteristic, which is a blackening of twigs, flowers, and leaves as if burned by fire. Infection of growing shoots often results in a typical "shepherd's crook" symptom. Depending on the part of the plant that is affected, the disease causes blossom, twig, leaf, fruit, trunk, collar, or rootstock blight with frequent ooze production (van der Zwet and Keil 1979; van der Zwet and Beer 1995). *E. amylovora* causes disease in plants of the *Rosaceae* family and has a wide host range inside this family. Although the taxonomy of *Rosaceae* is currently under revision (Hummer and Janick 2009), in the present review, we use the traditional division of four subfamilies grouped by fruit type. Fire blight has been described in approximately 200 species, which are included in 40 genera (van der Zwet and Keil 1979) and all four subfamilies of the *Rosaceae* family: *Maloideae* (syn. *Pomoideae*), *Rosoideae*, *Amygdaloideae* (syn. *Prunoideae*), and *Spiraeoideae*. In the last subfamily, the disease has been reported because of inoculations and not under natural infection conditions (van der Zwet and Keil 1979). The *Maloideae* subfamily's species are frequently affected and are also of high economic importance because *Pyrus*, *Malus*, *Cydonia*, *Eryobotria*, *Cotoneaster*, *Crataegus*, *Pyracantha,* and *Sorbus* species are the most common hosts. Fire blight has also been described in raspberry (*Rubus idaeus*) (Starr et al. 1951) and, more recently, in *Rosa rugosa* in Germany (Vanneste et al. 2002); both belong to the *Rosoideae* subfamily. In some cases, the bacterium has been reported in the United States (USA) and in South Germany as causing similar symptoms in members of *Amygdaloideae*, such as in Japanese plum (*Prunus salicina*) (Mohan and Thomson 1996), European plum (*Prunus domestica*) (Vanneste et al. 2002), and Pluot® (plum × apricot hybrid) trees naturally infected under high inoculum pressure (Mohan 2007). A plum isolate of *E. amylovora* has also been reported to cause symptoms after inoculation in other species in this subfamily, specifically, in cultivars of peach (*P. persica*), nectarine (*P. persica* var. *nucipersica*), sweet cherry (*P. avium*), and almond (*P. amygdalus*, syn. *P. dulcis*) (Mohan and Bijman 1999). These authors suggest that *Prunus* species could be susceptible to fire blight and that they may act as a reservoir for the pathogen. Recently, *E. amylovora* has again been reported in *Prunus* spp. in the Czech Republic (Korba and Šillerová 2010).

Although, in general, the strains of *E. amylovora* are pathogenic to apple and pear trees and the most common fire blight hosts, some strains of *E. amylovora* isolated from *Rubus* are not pathogenic to pear or apple trees (Heimann and Worf 1985; Powney et al. 2011; Ries and Otterbacher 1977; Starr et al. 1951).

Since its discovery, fire blight has been considered the most destructive disease of pome fruit trees and a limiting factor for apple and pear cultivation (Waite 1896). Since then, its significant economic impact has been confirmed every year, even one century later (Sobiczewski et al. 1997; Vanneste 2000). The importance of the economic losses it produces is related to weather conditions favourable to the development of the disease. For example, in the 1990s, more than a half million trees were destroyed in Italy alone (Vanneste 2000). In 1998, losses were estimated to be in excess of 68 million dollars (US) in the northwest United States and 10 million dollars (NZ) in the Hawke's Bay region of New Zealand (Vanneste 2000). Moreover, the use of streptomycin for control and the progressive accumulation of resistant strains have been estimated to bring additional losses of more than 100 million dollars (US) per year in the USA (Norelli et al. 2003).

Fire blight infection occurs when the host is in a susceptible condition, the pathogen inoculum is adequate, and the environmental conditions are suitable. The disease cycle has been described by various authors (Thomson 2000; van der Zwet and Beer 1995; van der Zwet and Keil 1979), and all agree that the primary route of infection of *E. amylovora* is through the blossoms. The pathogen enters the host via natural openings (nectarthodes) in the floral nectary (Wilson et al. 1990). Young shoots are also frequently infected through lenticels and stomata, but, more commonly, they are infected through wounds created by wind, insects, hail, or cultural practices. Later, *E. amylovora* can move into the branch, and systemic invasion may follow. The host attempts to limit the spread of infection, sealing off the diseased tissue by the deposition of cork layers in the cortex, resulting in the formation of cankers, in which *E. amylovora* can overwinter (Eden-Green and Billing 1974). The following spring, reactivation of plant growth results in an increased nutrient supply to the dormant bacterial population, which permits the pathogen multiplication. Bacterial ooze may be exuded from the cankers, providing an inoculum that can be transmitted to blossoms of the same or other plants. Different views persist on the endophytic growth phase of the pathogen and the optimal route of systemic migration. Some experimental evidence strongly suggests that the main route of migration of *E. amylovora* takes place in the

intercellular spaces of the parenchymal bark tissue (Gowda and Goodman 1970). In addition, the bacteria can invade and multiply in mature vessels for long distance transport down the tree (Suhayda and Goodman 1981), although the role of negative pressure in that movement is unknown. The pathogen needs some means of movement from vessels into the bark tissue, but this mechanism also remains unknown. See Billing (2011) for a re-examination of the available evidence on which these divergent views are based.

Short distance spread of fire blight has been reported by wind, rain, aerosols, insects (especially honey bees, but also other insects), and cultural practices. Long distance spread occurs mainly by the transport of latently infected or contaminated propagation material (Donat et al. 2007; Palacio-Bielsa et al. 2009; Sobiczewski et al. 1997; Thomson 2000). The role of birds in bacterial dissemination has been suggested (van der Zwet and Keil 1979) but never demonstrated.

It is necessary to take into account that most of, if not all, the information on the life cycle of *E. amylovora* has been provided by the isolation (or lack thereof) of *E. amylovora* colonies from several organs in different seasons, or by the visualization of bacteria by microscopy techniques after inoculation with green fluorescent protein-marked bacteria. The description of the induction of the viable but non-culturable (VBNC) state in *E. amylovora* by lack of nutrients and copper (and probably also by other factors) reported by Ordax et al. (2006), first in vitro and later in apple fruits (Ordax et al. 2009), adds complementary information about the hidden life of this pathogen. This information would likely modify the described cycle because resuscitation from such a state, which implies a recovery of pathogenicity, has been demonstrated even after nine-month VBNC state (Ordax et al. 2006).

Erwinia pyrifoliae

Bacterial isolates that cause necrosis on the Asian pear (*Pyrus pyrifolia*) cultivars (cvs.) Shingo and Mansamgil in Korea have been characterized and classified as the novel species *Erwinia pyrifoliae* (Kim et al. 1999). *E. pyrifoliae* is closely related to *E. amylovora* and induces very similar symptoms to those of fire blight, but, currently, it is reported to affect only Asian pear trees by natural infection. The host range of *E. pyrifoliae* may be broader, as disease symptoms were observed after inoculation of several commercial European pear cultivars (*Pyrus communis*) and apple (*Malus domestica* cv. Idared) (Kim et al. 2001b). *E. pyrifoliae* symptoms include black to brown stripes in the leaf midribs, dark brown leaf spots, and necrotic petioles, all of which may occur on large parts of the trees and affect entire branches, blossoms, and fruitlets (Rhim et al. 1999).

Although this pathogen currently is considered to have a restricted geographic distribution in Eastern Asia (Korea) (Kim et al. 1999; McGhee et al. 2002), its real distribution could be somewhat uncertain because specific surveys for *E. pyrifoliae* are rarely conducted (Smits et al. 2010a). It has been suggested that severe losses of fruit production may result from the disease (Rhim et al. 1999) and could be attributable to a reduction in foliage and loss of fruitlets.

The bacteria likely move into some parts of the plant in the same way as *E. amylovora*, but only scarce information is available on its biology; therefore, further studies of its epidemiology and survival are needed. It is likely that, as described for *E. amylovora*, long distance spread of *E. pyrifoliae* can also occur through latently contaminated propagation material (Jock et al. 2005).

Bacterial shoot blight of pear (BSBP), described as a fire blight-like disorder, was observed in the Asian pear (*Pyrus ussuriensis*) cv. Mishirazu and in European pear trees on the Japanese island of Hokkaido at the end of the 1970s (Tanii et al. 1981). Necrotic symptoms began at the base of the fruit and the leaf stalks, and the blossoms wilted and died. Young shoots were also affected, and ooze production was observed. The losses caused by this pathogen were not evaluated. The Japanese BSBP strains of *Erwinia* sp. were limited to restricted areas (Mizuno et al. 2000, 2010; Tanii 1983) and the disease was considered eradicated in 1999 (Mizuno et al. 2010).

The isolated bacteria were originally described as a pathotype of *E. amylovora.* The name *E. amylovora* pv. *pyri* was proposed (Tanii 1983), but it was not considered a valid name (Young et al. 1996). The taxonomic position of the Japanese strains was examined further, and 36 Japanese BSBP isolates were classified as *E. amylovora* biovar 4 based on differences in nine physiological and biochemical tests, including growth factors requirements, crater formation on high sucrose medium, hydrolysis of esculin, β-galactosidase activity, and acid production from cellobiose (Mizuno et al. 2000). Apparently, only two strains out of the 36 Japanese BSBP strains investigated by Mizuno et al. (2000) were included among the six Japanese *Erwinia* strains further investigated by Kim et al. (2001a), who concluded that these two strains were more closely related to *E. pyrifoliae* than to *E. amylovora*. Geider et al. (2009) complemented these results with new biochemical, molecular, pathological, and taxonomic studies using four strains, supporting the conclusion that the four Japanese BSBP strains were isolates of, or closely related to, *E. pyrifoliae* (Geider et al. 2009).

However, Mizuno et al. (2000), in their study of a larger collection of BSBP isolates, found homogeneous pathological characteristics among the 36 studied strains but some biochemical and physiological variability and large DNA–DNA hybridization differences in comparison with

E. amylovora. Nevertheless, they considered that these differences were not enough to separate the two groups into separate species and that the BSBP pathogen should be included in *E. amylovora* at the species level (Mizuno et al. 2010). Although they did not provide enough information to accurately analyze their data, it is difficult to definitively classify all the BSBP strains as *E. pyrifoliae,* and, in our opinion, new comparisons between a large collection of Japanese BSBP strains and the other described *Erwinia* species are still required. The complete genome sequence of one Japanese BSBP strain is now available (Park et al. 2011), but these authors did not compare it with the sequences of *E. pyrifoliae* or other *Erwinia* species. The analysis of the genomes of more BSBP *Erwinia* strains may provide insight into their genotypic diversity and help to further understand the evolutionary relationships among the BSBP strains, *E. pyrifoliae,* and *E. amylovora* (Smits et al. 2010a).

Japanese bacterial black shoot disease pathogen (Japanese BBSDP *Erwinia* sp.)

The differences among Japanese *Erwinia* strains have been confirmed by the recent isolation in Japan of another type of *Erwinia* sp. that produces bacterial black shoot disease (BBSDP) in European pear trees. The symptoms were caused by strains that were clearly different from *E. amylovora*, *E. pyrifoliae*, and other previously described *Erwinia* strains that cause the BSBP disease (Mizuno et al. 2010).

BBSDP was first observed in 2007 in young shoots of the European pear tree cv. La France in an orchard in Yamagata (Honshu island, Japan) (Mizuno et al. 2010). According to these authors, necrotic symptoms were observed in young shoots only, and the development of lesions stopped within 20 cm from the base of the shoots and did not affect the branches. In some cases, the lesions extended from the shoot through the petioles to the main vein of the leaves, but affected plants did not show blossom blight, fruitlet blight, or "shepherd's crook" of shoots, which are the typical symptoms of fire blight or BSBP. As the disease symptoms in the field were limited to young shoots and the disease did not spread to other areas of Japan, the causal agent of BBSDP may be a minor pathogen with low virulence (Mizuno et al. 2010). A comparison of these bacterial strains with *E. amylovora* and *E. pyrifoliae* has revealed that biochemical, serological, and pathogenic properties of the BBSDP causal agent differed from both *Erwinia* species (Mizuno et al. 2010). The BBSDP bacteria belong to the genus *Erwinia* and seem to be closely related to *E. amylovora* and *E. pyrifoliae* by 16S rRNA gene analysis. However, DNA–DNA hybridization reveals that the BBSDP pathogen does not exceed 70% similarity with either of the two aforementioned species, indicating that it belongs to another species. Now, the taxonomic classification of this pathogen is still uncertain. Due to its restricted distribution, little is known about the ecological traits of this pathogen or its epidemiology.

Erwinia piriflorinigrans

Recently, this novel pathogen has been reported and described as the causative agent of necrotic pear blossoms (López et al. 2011), and strains have been isolated from the cultivars Ercolini (Coscia) and Tendral in Valencia, Spain. Although there is not much information about this species, it is considered to have a narrow spectrum of host species and organs. Based on data obtained by inoculations performed in the laboratory, only pear blossoms, but not pear shoots or fruitlets, apple trees, or other inoculated *Rosaceae* species showed necrotic symptoms (Roselló et al. 2006). However, complementary studies are needed to determine more accurately the affected species and cultivar susceptibility. The economic impact and distribution of this new pome fruit pathogen should also be investigated because, until now, it has only been reported in pear orchards in Spain, where the damages are only related to the necrosis of blossoms (Roselló et al. 2008; López et al. 2011).

Non-pathogenic species: general background

Both *E. billingiae* and *E. tasmaniensis* are considered part of the apple and pear microbiota, and their relatively recent discovery highlights the lack of information about their diversity, hosts, biology, and ecology. However, it is known that there are several mechanisms that confer to bacteria the ability to survive and colonize the plant environment, including synthesis of anchoring structures like exopolysaccharides, formation of biofilms, motility, and chemotaxis toward plant extracts. There are also other specific adaptations to counteract environmental stresses, such as nutrient limitation, which can be minimized, for example, by the ability to metabolize a broad range of nutrients or to produce iron chelators to overcome iron scarcity. Genetic studies suggest that at least some of the adaptative strategies described above may be present in epiphytic *E. billingiae* and *E. tasmaniensis* (Kube et al. 2008a, 2010). Rain, wind, and insects are thought to be main factors for their dissemination on plant surfaces and between plants; however, ecological studies on these two species have not yet been performed.

Erwinia billingiae

The current *E. billingiae* strains have had previously different names. First, *E. herbicola*-like non-pigmented

colonies were isolated from different types of lesions on pear, apple, cherry, hawthorn (*Crataegus* spp.), and elm (*Ulmus* spp.) trees. Such colonies were found in stem cankers, diseased blossoms, and immature fruits in the UK and were initially designated as "white *E. herbicola*" (Billing and Baker 1963). Based on a numerical analysis of phenotypic features, the strains of this species were assigned to *Pantoea agglomerans* (syn. *E. herbicola*) (Verdonck et al. 1987). However, according to DNA–DNA hybridization assays together with 16S rDNA sequence analysis, the non-pigmented strains have been reclassified as the novel non-pathogenic species, *E. billingiae* (Mergaert et al. 1999), within the genus *Erwinia* (Hauben et al. 1998). *E. billingiae* has a tendency to invade necrotic tissue of plants. English isolates were considered secondary invaders rather than primary pathogens, possibly helping to extend lesions but not to initiate them (Billing and Baker 1963).

Recently, strains belonging to this species have been isolated from apple trees in Poland, Germany, and Spain (Geider et al. 2008; López et al. unpublished data).

Erwinia tasmaniensis

E. tasmaniensis is another *Erwinia* species that has been reported as epiphytic and non-pathogenic. It has been isolated from the flowers and bark of apple and pear trees on three continents: Australia (Victoria, Tasmania, and Queensland) (Geider et al. 2006), Africa (Cape region of South Africa), and Europe (Germany) (Geider et al. 2008; Jakovljevic et al. 2008; Kube et al. 2008a). However, it is possible that *E. tasmaniensis* is present worldwide (Geider et al. 2008).

E. billingiae and *E. tasmaniensis* may occupy the same ecological niche as other pathogenic *Erwinia* species, and its accumulation in the environment (as in apple flowers) could interfere with the growth of other bacteria, such as *E. amylovora* (Jakovljevic et al. 2008). Assays for testing the antagonistic effect of *E. billingiae* and *E. tasmaniensis* against *E. amylovora* in immature pears and apple flowers have revealed that the application of high inoculum concentration of some strains can result in growth reduction of the pathogen (Geider et al. 2006, 2008; Jakovljevic et al. 2008; Kube et al. 2008a, b, 2010; Mohammadi and Geider 2007).

Phenotypical traits of *Erwinia* spp.

All of the species reviewed here belong to the family *Enterobacteriaceae* and the genus *Erwinia*, and thus, have common phenotypic characteristics: Gram-negative, rod shaped, motile by peritrichous flagella, facultative anaerobic growth, oxidase negative, catalase positive, and acid production from glucose, fructose, and galactose (Hauben and Swings 2005; Hauben et al. 1998). However, they also differ in some physiological and biochemical characteristics, as reported by several authors (Bereswill et al. 1997; Geider et al. 2006, 2009; Kim et al. 1999; López et al. 2011; Mergaert et al. 1999; Mizuno et al. 2010; Rhim et al. 1999; Roselló et al. 2006; Shrestha et al. 2003). It is necessary to consider that due to the different number of strains employed in these studies, the differences in methodologies utilized, and the limited knowledge available on the diversity within each species (especially for those recently described), there can be exceptions to the described specific biochemical and physiological characteristics. Table 1 shows some key phenotypic differences based on the most homogeneous data available.

Molecular traits of *Erwinia* spp.

In recent years, the complete genome sequences of strains belonging to *E. amylovora*, *E. pyrifoliae*, the BSBP *Erwinia* sp., *E. billingiae,* and *E. tasmaniensis* have increased our understanding of the genetic characteristics of each species and have allowed comparative genomic studies.

Erwinia amylovora

The genome sequences of the strains ATCC 49946, CFBP 1430, and ATCC BAA-2158 were recently published (Sebaihia et al. 2010; Smits et al. 2010b; Powney et al. 2011). Strain ATCC 49946 was isolated from apple in New York, USA (Sebaihia et al. 2010); strain CFBP 1430 was isolated in France from *Crataegus* sp. (Paulin and Samson 1973), and strain ATCC BAA-2158, which presents a restricted pathogenicity to *Rubus* sp., was isolated from thornless blackberry cultivars (*Rubus* sp. hybrids) in IL, USA (Ries and Otterbacher 1977).

All three strains have a circular chromosome of approximately 3.81 Mb with a 53.6% G + C content. However, the strains differ in the plasmid content, only sharing the pEA29 plasmid. Strain ATCC 49946 also harbours a larger plasmid, while the *Rubus* strain harbours two smaller plasmids.

Although the intraspecific genetic diversity of *E. amylovora* was considered low in previous decades, more recent molecular analyses have shown differences among strains (see another article in this issue by Pulawska and Sobiczewski).

Erwinia pyrifoliae

The genome sequence of the type strain DSM 12163^T, isolated from *P. pyrifolia* in South Korea (Kim et al. 1999),

Table 1 Selected phenotypic characteristics showing the differences among *Erwinia amylovora*, *E. pyrifoliae*, BSBP Japanese *Erwinia* sp., BBSDP Japanese *Erwinia* sp., *E. piriflorinigrans*, *E. billingiae* and *E. tasmaniensis* adapted from several authors

Characteristics	*E. amylovora*[a]	*E. pyrifoliae*[b]	BSBP *Erwinia* sp.[c]	BBSDP *Erwinia* sp.[d]	*E. piriflorinigrans*[e]	*E. billingiae*[f]	*E. tasmaniensis*[g]
Levan production	+[h]	−	+	ND	+	−	+
Tween 20 hydrolysis	−	−	ND	ND	+	+	−
Nitrate reduction	−	−	−	−	−	+	−
β-Galactosidase	−	−	−	+	+	+	+
Acid production from							
Sorbitol	+	+	+	+	−	+	−
Saccharose	+	+	+	+	+	−	+
Glycerol	−	−	+	+	+	−	−
D-Xylose	+	−	ND	ND	+	+	−
Adonitol	−	−	−	−	+	−	−
D-Mannose	−	−	ND	ND	−	+	−
L-Rhamnose	−	−	ND	ND	−	+	−
Methyl-α D-glucopyranoside	−	−	−	V[i]	+	−	−
Esculin hydrolysis	(+)	−	−	(+)	+	+	−
D-Maltose	−	−	ND	ND	−	+	−
D-Raffinose	−	−	ND	ND	+	−	−
D-Fucose	−	−	ND	ND	+	−	−
D-Arabitol	−	−	ND	ND	−	+	−

+ Positive, − Negative, *ND* not determined or uncertain results, *V* Variable, (+) Weak reaction. Data adapted from:

[a] Geider et al. (2006, 2009), López et al. (2011), Mizuno et al. (2010), Roselló et al. (2006)

[b] Geider et al. (2006), Kim et al. (1999), Rhim et al. (1999), Roselló et al. (2006), Shrestha et al. (2003)

[c] Mizuno et al. (2000)

[d] Mizuno et al. (2010)

[e] Roselló et al. (2006), López et al. (2011)

[f] Geider et al. (2006), López et al. (2011), Mergaert et al. (1999)

[g] Geider et al. (2006), López et al. (2011)

[h] Strains deficient in levan synthesis have been reported (Bereswill et al. 1997)

[i] Mainly positive (Mizuno et al. 2010)

has been recently obtained (Smits et al. 2010a). The *E. pyrifoliae* genome contains a circular chromosome of about 4.03 Mb with a G + C content of 53.41%. Four plasmids have also been identified (Maxson-Stein et al. 2003; McGhee et al. 2002; Rhim et al. 1999).

The genome sequence of the Japanese BSBP strain Ejp617 isolated from Asian pear has also been recently decoded (Park et al. 2011). The genome of this strain contains a circular chromosome of approximately 3.9 Mb with a G + C content of 56.43% and 5 plasmids. The coding region accounts for 84.68% of the total sequence and has 3,600 annotated coding sequences (CDSs) of an average length of 902 bp. (Park et al. 2011).

E. pyrifoliae intraspecific genetic diversity seems to be low, although few strains have been studied, and differences are mainly observed in plasmid content (Shrestha et al. 2007). Phylogenetic analysis from 16S rRNA sequences and alignments of parts of the housekeeping genes *gpd* and *recA* show that the species *E. pyrifoliae* is more related to *E. amylovora* than to *E. tasmaniensis* and is distantly related to *E. billingiae* (Geider et al. 2009). Moreover, a comparison between the genomes of the type strains of *E. pyrifoliae* (DSM 12163^T) and *E. tasmaniensis* (Et1/99^T) (Kube et al. 2008a) has been recently published (Smits et al. 2010a) and supports the previously reported differences between the two species.

Japanese BBSDP *Erwinia* sp.

Although the *Erwinia* strains causing the BBSDP disease in European pear trees are considered to be genetically closely related to *E. amylovora* and *E. pyrifoliae*, the BBSDP strains differ in DNA-DNA hybridization analysis from these two *Erwinia* species (Mizuno et al. 2010), as

indicated above. Therefore, information about their genome and more detailed taxonomic studies are still necessary to identify the pathogens at the species level.

Erwinia piriflorinigrans

The genome sequence of the type strain CFBP 5888^T is underway but not yet available. The DNA G + C content (50.5–51.1 mol%), differences in the 16S rRNA sequence, the *gdp* and *recA* gene sequences from two strains, biochemical differences, and DNA–DNA hybridization analyses confirm that the studied strains belong to a new species. In addition, their rep-PCR profiles are different from the profiles obtained for *E. amylovora* and *E. pyrifoliae* (López et al. 2011). The plasmid pattern of selected isolates provides only one band, except in one *E. piriflorinigrans* strain, CFBP 5887, that harbours two plasmids, one of about 29 kb and another of about 5 kb. The eight strains studied have one plasmid of a similar size but different than pEA29 of *E. amylovora*, as shown by restriction analysis profile and hybridization with some plasmid fragments using pEA29 as a probe (López et al. 2011).

Erwinia billingiae

The genome of Eb661^T, the type strain isolated from *P. communis* in the UK (Mergaert et al. 1999), has been recently reported (Kube et al. 2010). It contains one circular chromosome with a size of 5.1 Mb with a G + C content of 55.2 mol%, which is larger than the chromosome of the pathogenic species *E. pyrifoliae* and the non-pathogenic *E. tasmaniensis*. The sequenced *E. billingiae* strain contains two plasmids (Kube et al. 2010).

Erwinia tasmaniensis

The complete genome sequence of the type strain Et1/99^T, isolated from apple flowers in Tasmania (Geider et al. 2006), is available (Kube et al. 2008a). The G + C composition of the DNA of three Australian isolates is 50.5–54 mol% (Geider et al. 2006; Kube et al. 2008a). The *E. tasmaniensis* type strain genome harbours a 3.9 Mb circular chromosome and five plasmids.

Sequence analysis of the 16S rRNA genes indicates that the strains of this species are clearly separated from *E. amylovora*, *E. pyrifoliae,* and *E. billingiae* (Geider et al. 2006). Analysis of the housekeeping genes *gpd* and *recA* of Australian strains also confirms that they form a separate cluster from other *Erwinia* species (Geider et al. 2006).

A comparative study of ten *E. tasmaniensis* strains from Australia, South Africa, and Germany showed that their 16S rRNA sequences are identical except for a one-nucleotide change in two strains from South Africa and one strain from Germany. Based on the sequences of the *wbdN* gene, which encodes a glycosil transferase, the isolates can be differentiated into two groups: (1) the Australian and the African strains and (2) the German strains (Geider et al. 2008).

Main molecular traits related to pathogenicity, virulence, and fitness in *Erwinia* species

Numerous studies have been conducted to elucidate the mechanisms, factors, and other aspects that lie behind the pathogenicity and virulence of *E. amylovora* as well as the genes involved in symptom development, colonization, protection against plant defences, and other bacteria–plant interaction events. *E. amylovora* modifies the epiphytic habitat in the stigma through a pathogenesis-related process, which increases the host resources available to itself and, coincidentally, to non-pathogenic competitors (Johnson et al. 2008). Until now, the different factors found to be involved in the pathogenicity and virulence of *E. amylovora* have been structural elements, such as exopolysaccharides, secretion systems, pathogenicity genes, and other genes, that provide advantages in metabolism, colonization, and competition against other bacteria (Bugert and Geider 1995; Eastgate 2000; Oh and Beer 2005; Oh et al. 2005; Zhao et al. 2009). Recently, some comparisons of the complete genomes of different *Erwinia* species have been performed (Kube et al. 2010, Smits et al. 2010a, b, 2011) with the goal not only to resolve the *Erwinia* genes responsible for basic aspects of the genus biology and its major phenotypic traits, but also to identify the genetic bases that could explain the differences observed in host ranges and in pathogenicity. It is also necessary to consider that other factors could influence the level of expression of virulence genes (Wang et al. 2010). A hypothesis on the evolution of virulence factors in *E. amylovora* has been generated using comparative genomics (Smits et al. 2011): the ancestral origins of several virulence factors have been found, including levan biosynthesis, sorbitol metabolism, three type III secretion systems (T3SS), and two type VI secretion systems (T6SS). Other genes seem to have been acquired after a divergence of pathogenic species, including a second flagellar gene and two glycosyltransferases involved in amylovoran biosynthesis. Genetic analysis has revealed signatures of foreign DNA, suggesting that horizontal gene transfer is responsible for some of the differential features between the species *E. amylovora*, *E. pyrifoliae,* and *E. tasmaniensis* (Smits et al. 2011).

An important aspect of the genetics of virulence in *E. amylovora* is the study of genes involved in the development of plant symptoms. Some works using gene

expression techniques (IVET, subtractive hybridization) and other data have increased the knowledge of the genes acting in the pathogenicity progression. In a study on infection with immature pear tissue, a process that requires the major pathogenicity factors of *E. amylovora*, about 400 pear fruit-induced (*pfi*) genes were specifically induced. They were identified and separated into nine putative function groups: host–microbe interactions (3.8%), stress response (5.3%), regulation (11.9%), cell surface (8.9%), transport (13.5%), mobile elements (1.0%), metabolism (20.3%), nutrient acquisition and synthesis (15.5%), and unknown or hypothetical proteins (19.8%). Virulence genes already described in *E. amylovora* were found to be up-regulated during this process, including components of the type III secretion system (*hrp/hrc*), the effector gene *dspE*, type II secretion, levansucrase (*lsc*), and regulators of levansucrase and amylovoran biosynthesis (Zhao et al. 2005).

In this section, which discusses molecular traits related to virulence, we compare only the species for which complete genomes have been reported. We describe the data available on *E. amylovora* and compare them with the data on *E. pyrifoliae*, *E. tasmaniensis*, and *E. billingiae*. Properties playing a role in interaction of the different *Erwinia* species with plants are shown in Table 2.

Secretion systems and effectors

Type III Secretion Systems (T3SSs) and related genes

Multiple-component Type III secretion systems (T3SSs) widely distributed among proteobacterial pathogens of plants, animals, and humans, are present in *E. amylovora* and constitute a fundamental virulence determinant. They deliver proteins that act as pathogenicity factors into the extracellular space or the cytoplasm. In other bacteria, T3SSs have been found to be critical for the establishment of non-pathogenic host relationships with plants and insects (Kube et al. 2010, Smits et al. 2010a).

Primary, or "classical," Hrp-T3SS

The hypersensitive response and pathogenicity (Hrp) T3SS, encoded by the so-called pathogenicity island PAI-1, is an established pathogenicity factor in *E. amylovora* (Oh et al. 2005, Smits et al. 2010b). The ability of *E. amylovora* to cause disease in susceptible host plants and to elicit the hypersensitive response (HR) in resistant varieties in hosts and in non-host plants depends on the presence of a functional T3SS encoded by the *hrp* gene cluster included in PAI-1 (Klement 1982; Goodman and Novacky 1994; Steinberger and Beer 1988; Barny et al. 1990). *E. amylovora* has a secretion apparatus that delivers effector proteins into host plants (He et al. 2004; Kim and Beer 2000; Oh et al. 2005). At least three proteins are known to be secreted through this apparatus: (a) harpin, a major HR elicitor also involved in pathogenicity (Barny 1995; Dong et al. 1999; Wei et al. 1992); (b) DspA/E, an essential pathogenicity determinant (Gaudriault et al. 1997); and (c) HrpW, which shares similarities with harpin but acts as a negative effector of the HR mechanisms (Gaudriault et al. 1997).

Genes involved in PAI-1 and pathogenicity

To infect plants successfully, *E. amylovora* requires products of juxtaposed *hrp* and *dsp* (disease-specific) genes (Bogdanove et al. 2000; Kim and Beer 2000). An approximately 22-kb region, essential for the Hrp phenotype, contains the three regulatory operons *hrpXY*, *hrpS*, and *hrpL* and the three type III secretion operons *hrpA*, *hrpC*, and *hrpJ* (Oh et al. 2005). Expression of these genes is controlled by environmental signals, including pH, temperature, and nutrient concentration. Conditions that favour expression are similar to those found in the plant apoplast (Wei et al. 1992). Mutations in the *hrpN* (harpin)

Table 2 Comparison of some properties of *E. amylovora*, *E. pyrifoliae*, *E. piriflorinigrans*, *E. billingiae* and *E. tasmaniensis* playing a role in their interactions with plants (modified from Geider 2006)

Species	Capsular EPS	Levan formation from sucrose	AHL synthesis	AI-2 Production	Virulence on apple/pear	HR on tobacco
E. amylovora	+	+	−	+	+/+	+
E. pyrifoliae	+	−	−	+	−/+	+
E. piriflorinigrans	ND	+	ND	ND	−/+	+
E. billingiae	+	−	+	+	−/−	−
E. tasmaniensis	−	+	−	+	−/−	+

AHL acyl-homoserine lactones, *AI-2* autoinducer 2, *HR* hypersensitivity response, *ND* Not determined

gene result in greatly reduced virulence and HR-eliciting activity.

hrp genes The entire Hrp PAI-1 is divided into four distinct DNA regions: the *hrp/hrc* region, the Hrp effectors and elicitors (HEE) region, the Hrp-associated enzymes (HAE) region, and the island transfer (IT) region (Oh and Beer 2005). The *hrp* genes can be classified into three categories. The first category encodes regulatory proteins that control the gene expression of other *hrp* genes. The second category encodes components of a protein secretion system called the Hrp (type III) secretion pathway. In addition, the third category encodes other proteins that are secreted, such as the HR-elicitor protein, harpin (Kim and Beer 2000; Oh et al. 2005; Wei and Beer 1993).

dsp genes Along with the *hrp* genes, other genes required for pathogenicity are dispensable for the HR elicitation (Barny et al. 1990; Bellemann and Geider 1992; Vanneste et al. 1990). These genes are called disease-specific (*dsp*) genes, and they are located next to the *hrp* cluster in the same pathogenicity island. In *E. amylovora*, the DspA/E protein has been implicated in the generation of oxidative stress during disease and the suppression of callose deposition (Boureau et al. 2006). DspA/E is known as a pathogenicity factor in *E. amylovora* because *dspA/E* mutants are not pathogenic to apple shoots, immature pear fruit slices (Bogdanove et al. 1998), or pear seedlings (Gaudriault et al. 1997). Initially, DspA/E was not considered an HR elicitor, but recently it has been shown to elicit HR following its transient expression in *Nicotiana benthamiana* (Boureau et al. 2006; Oh 2005).

The Hrp-T3SS of *E. amylovora* strain CFBP 1430 is composed of 27 genes arranged in three transcriptional units with additional regulatory genes. This strain carries two notable singletons (ORFU1 and ORFU2) located between the *hrpA* and *hrpS* genes. Its *hrp* region is located between the *hrp* effectors and elicitors (HEE) region and the Hrp-associated enzymes (HAE) region. The HEE region includes 2 harpin genes (*hrpN* and *hrpW*), a gene coding for a secreted effector essential for *E. amylovora* pathogenicity (*dspA/E*), and a set of chaperone genes (ORFA, ORFB, ORFC, and *dsp B/F*). The HAE region of strains CFBP 1430 and ATCC 49946 is comprised of the *hrp*-associated systemic virulence genes (*hsvABC*), the *hrpK* gene (encoding a putative T3SS translocator), and two genes encoding proteins of unknown function (Smits et al. 2010b).

Additional T3SS effectors

The *E. amylovora* strain CFBP 1430 genome reveals three singleton genes identified as putative T3SS effectors that may contribute to the broader host range of *E. amylovora*: the *eop2* gene, which encodes the T3SS helper protein Eop2; the effector HopPtoC; and the effector homologous to AvrRpt2, which, in *E. amylovora*, contributes to its ability to infect immature pear fruit (Smits et al. 2010b; Zhao et al. 2006).

Comparison with other *Erwinia* species

If the data on *E. amylovora* are compared with the data on the other *Erwinia* species, it can be observed that the genome of the pathogenic *E. pyrifoliae* DSM 12163^T contains one T3SS closely related to the *hrp/dsp* cluster of *E. amylovora*. The *hrp*-related region in DSM 12163^T is composed of 25 genes organized in four operons. The number and arrangement of these genes are similar to those of the known *hrp* pathogenicity island found in *E. amylovora* except for the absence of ORFU1 and ORFU2. The HEE and HAE regions are organized identically to *E. amylovora*. The HEE region includes the harpin genes *hrpN* and *hrpW*, the gene *dspA/E* (encoding a secreted effector essential for *E. amylovora* virulence), and the chaperone genes *orfA*, *orfB*, *orfC,* and *dspB/F*. The HAE region comprises the *hrp*-associated systemic virulence genes (*hsvABC*), the *hrpK* gene (encoding for a putative T3SS translocator), and two genes encoding proteins of unknown function as in *E. amylovora* (Smits et al. 2010a). However, in another strain of the same species, *hsv*A and *hsv*C are present but not *hsv*B (although a sequence coding for a putative capsular exopolysaccharide synthesis protein has been identified) (Kube et al. 2010).

The genome of the non-pathogenic *E. tasmaniensis* type strain Et1/99^T harbours a T3SS related to the *hrp/dsp* cluster of *E. amylovora*. The *hrp* genes are organized as an Hrp pathogenicity island. Compared to the *hrp/hrc* region of *E. amylovora*, the genome of *E. tasmaniensis* contains the same genes as *E. amylovora* and in the same order except for two metal-dependent hydrolases. Thus, this non-pathogenic strain shows a nearly complete *hrp/hrc* system. The HEE region includes the harpin genes *hrpN* and *hrpW*, the gene *dspA/E* (encoding a secreted effector essential for *E. amylovora* virulence), the genes encoding for the secreted EpoB proteins, and the chaperone gene *dspB/F*. These genes are present in conserved order and content compared with *E. amylovora*. However, the HAE region is missing, and the *hrpK* gene encoding a putative T3SS translocator is not present (Kube et al. 2008a). The *E. tasmaniensis* Australian strains studied were able to induce HR in tobacco leaves after conditioning in an inducing medium that is assumed to increase the expression of *hrp* genes (Jakovljevic et al. 2008) and also after cultivation in King′s medium B (López et al. unpublished data). The presence of *hrp* genes in the genome of *E. tasmaniensis* may also play a role in its epiphytic fitness and the bacterium could produce symptoms in certain hosts under some conditions, although

the ability to induce HR on non-host plants is not the only factor required for virulence.

The genome of the *E. billingiae* type strain Eb661^T does not contain a region homologous to a T3SS (Kube et al. 2010). The lack of ability of *E. billingiae* to induce a hypersensitive response (HR) in tobacco leaves may explain its inability to cause disease symptoms in plants (Jakovljevic et al. 2008).

"*inv/spa*-type" T3SS-PAI-2 and PAI-3

In low G + C regions, the *E. amylovora* strain CFBP 1430 contains two *inv/spa*-type T3SS islands (PAI-2 and PAI-3). Each island consists of more than 20 genes, and their function is still unknown, but it has been reported that they may not be directly involved in virulence in plants (Zhao et al. 2009, Smits et al. 2010b). PAI-2 is not in the genome of *E. pyrifoliae*, but parts are present in *E. tasmaniensis*. *E. billingiae* carries the *srfABC* gene cluster, like the other three *Erwinia* spp., but lacks the instrumentation for a T3SS (Kube et al. 2010). PAI-3, found in the *E. amylovora* strain CFBP 1430, is related to the complete *inv/spa* systems recently identified in *E. pyrifoliae* and *E. tasmaniensis*, in which the genes are separated into two clusters that complement themselves to form a complete injection system. However, PAI-3 is not present in *E. billingiae* (Smits et al. 2010a, b).

Type I secretion system (T1SS)

The T1SS *prtADEF* encodes an excreted protease (PrtA) and its export system (PrtDEF), and it is only present in the genome of *E. amylovora* CFBP 1430, not in the other species. For *E. tasmaniensis*, its absence has been interpreted to be one factor related to its lack of virulence in pome fruit trees (Kube et al. 2008a), but its absence in the pathogenic *E. pyrifoliae* suggests that it could be a specific contributor to host-range virulence in *E. amylovora* rather than an absolute virulence determinant (Smits et al. 2010b).

Type II secretion system (T2SS)

A T2SS, induced during an immature pear assay (Zhao et al. 2005), has been found in the genomes of *E. amylovora* CFBP 1430, *E. pyrifoliae,* and *E. tasmaniensis* but has not been described in *E. billingiae*. Because a T2SS had been detected in one of the non-pathogenic species, Zhao et al. (2005) have suggested that it is not an absolute virulence factor. This conclusion is supported by a mutational analysis that was performed with the *E. amylovora* strain Ea1189, demonstrating that the T2SS system has no role in pathogenicity toward immature pear or apple seedlings (Zhao et al. 2005). The presence of a chitin-binding domain in the putative ChiV protein suggests that the T2SS system could contribute to a yet-to-be-identified interaction with insect vectors of *E. amylovora* CFBP 1430 (Smits et al. 2010b).

Type V secretion system (T5SS)

A simple protein export machinery is built by the T5SS, and, because of the self-assembly and self-export, it is termed an auto-transporter. Most of the effector proteins released are involved in adherence, invasion, and degradation. The non-pathogenic *E. billingiae* is the only species in which these genes have been identified (Kube et al. 2010).

Type VI secretion system (T6SS)

T6SS gene clusters have recently been found to be widespread among pathogenic and non-pathogenic Gram-negative bacteria (Smits et al. 2011). This secretion system is involved in several processes related to pathogenesis; nevertheless, other processes facilitated by the T6SS include adherence, cytotoxicity, host-cell invasion, intracellular growth within macrophages, and survival and persistence within the host (Cascales 2008).

In the *E. amylovora* strain CFBP 1430, three T6SS gene clusters have been identified (Smits et al. 2010b). Clusters 1 and 2 are highly similar to the T6SS clusters of *E. pyrifoliae*, *E. tasmaniensis,* and *E. billingiae* with the exception of some genes encoding hypothetical proteins that do not belong to the core genes of T6SSs and some differences in the genes encoding VgrG effector proteins in cluster 1. Cluster 2 also shows variations in gene content (Kube et al. 2010; Smits et al. 2010a), whereas cluster 3 is absent in these three *Erwinia* species.

Metabolism

Exopolysaccharide (EPS) biosynthesis

Several metabolic factors are considered to play an important role in the symptoms of *E. amylovora*-infected plants, including synthesis of EPS and related products (Kube et al. 2010).

Amylovoran is a complex capsular EPS constituted by a repeating unit of three galactose residues, glucuronic acid, and another galactose residue that is substituted with other components (acetyl groups, pyruvate) (Geider 2000). Amylovoran affects plants primarily by plugging the vascular tissues, thus inducing wilt of shoots (Sjulin and Beer 1978). It is considered a pathogenicity factor (because amylovoran-deficient mutants of *E. amylovora* lack pathogenic ability) and also, it might protect the bacteria against host defence reactions (Bellemann and Geider 1992; Bugert and Geider 1995). The biosynthesis of

amylovoran requires a cluster of 12 *ams* genes situated in the genome of *E. amylovora* (Bugert and Geider 1995). A two-component signal transduction system, the regulator of capsular synthesis (Rcs) RcsCBD phosphorelay system, is present in the *E. amylovora* genome where it is supposed to regulate the biosynthesis of amylovoran and is essential for virulence (Zhao et al. 2009). Differences between the amylovoran structures and the genomes of *E. amylovora* strains from *Rubus* and other hosts have been reported (Maes et al. 2001; McManus and Jones 1995; Powney et al. 2011).

Levan is a homopolymer of fructose that can be synthesized with the enzyme levansucrase. It serves as a shield against recognition by plant defence reactions (Geier and Geider 1993). In its metabolic pathway, the secreted levansucrase cleaves sucrose into glucose and fructose, which is later polymerized to levan. Levan is not strictly necessary for the pathogenicity of *E. amylovora* (Kube et al. 2010), and strains deficient in levan synthesis have been found in nature (Bereswill et al. 1997).

Amylovoran and levan are necessary to build a complex structure that plays multiple roles during the colonization with *E. amylovora* of host plants. EPS-embedding bacteria enhance plant colonization by preserving the bacteria from dry conditions and water loss and by protecting against plant defences and copper stress (Barny et al. 1990; Bellemann and Geider 1992; Ordax et al. 2010a; Steinberger and Beer 1988; Vanneste et al. 1990). Exopolysaccharides themselves also play a role in the survival of *E. amylovora* during copper stress (Ordax et al. 2010a).

Pyrifolan is an EPS (Geider 2000) that has the same sugar composition in the repeating units as amylovoran and presents identical linkages (except the lack of a second side chain of glucose), but some authors think that the high degree of similarity of the chemical structures of both EPSs may not justify the name of pyrifolan by analogy to amylovoran (Kim et al. 2002).

E. pyrifoliae produces only pyrifolan. There is no chemical or genetic information on EPSs from *E. piriflorinigrans*, but it produces levan. *E. billingiae* possesses a gene cluster on its chromosome for the synthesis of capsular polysaccharides and also produces an EPS but does not produce levan (Geider 2006; Jakovljevic et al. 2008; Kim et al. 2001a); it possesses the encoding gene for levansucrase (*lsc*), but does not have the genes that encode the expression of this enzyme or the genes encoding for the levanase enzyme (Kube et al. 2010), which is another enzyme involved in levan metabolism. *E. tasmaniensis* does not produce a detectable amount of capsular exopolysaccharide, although a gene cluster related to the *ams* region of *E. amylovora* is present in the *E. tasmaniensis* type strain (Kube et al. 2008a). Nevertheless, the ability of *E. tasmaniensis* to form levan is an advantage for reducing high sucrose levels in the environment, such as in nectar (Jakovljevic et al. 2008). The polyfructan may act as a protection against the plant cell reaction, and the released glucose is a suitable carbon source (Jakovljevic et al. 2008).

Sugar metabolism

Sorbitol Another factor that influences virulence in *E. amylovora* is the metabolism of sugars. Rosaceous plants contain sorbitol and sucrose as storage and transport carbohydrates, and the distribution of these carbohydrates is dependent on the environmental conditions, species, and plant tissue (Blachinsky et al. 2006; Zhang and Geider 1999). Sorbitol is the dominant sugar alcohol in such plants and is used for carbohydrate transport rather than sucrose, which is utilized in many other plants. An operon, *srl*, necessary for sorbitol metabolism, has been identified in *E. amylovora*. This operon consists of six genes: three are required for sorbitol uptake (*srlA, srlB,* and *srlE*), one encodes a protein that converts sorbitol to fructose (*srlD*), and two other genes are regulatory (*srlM* and *srlR*).

Some authors believe that fire blight is restricted to members of the *Rosaceae* family because the presence of sorbitol could be a prerequisite for *E. amylovora* to colonize plants (Plouvier 1963). It has been described that rosaceous plants, especially apple and pear, use not only sucrose but also sorbitol for the transport and storage of carbohydrates (Wallaart 1980). Disruption of sorbitol uptake in mutants results in loss of pathogenicity in apple seedlings (Aldridge et al. 1997), but the mutants were virulent in immature pear fruits (Qazi et al. 2004). Moreover, sorbitol can be a good carbon source to synthesize and to increase amylovoran synthesis in *E. amylovora* (Belleman et al. 1994). However, other authors have indicated that sorbitol content in apple tree tissues has no major influence on the disease severity or progression of *E. amylovora* (Duffy and Dandekar 2008).

Interestingly, *E. pyrifoliae,* the BSBP and BBSDP *Erwinia* species from Japan, and *E. billingiae* also metabolize sorbitol (Mergaert et al. 1999; Mizuno et al. 2010; Rhim et al. 1999; Smits et al. 2010a), but neither the pathogenic species *E. piriflorinigrans* nor the epiphyte *E. tasmaniensis* metabolize it (Geider et al. 2006; Kube et al. 2008a; López et al. 2011). The *E. tasmaniensis* type strain Et1/99^{T} completely lacks the sorbitol operon (Kube et al. 2008a, b). Moreover, some strains differing in sorbitol utilization have been described for the non-pathogenic species *E. billingiae* (Mergaert et al. 1999).

Sucrose Sucrose metabolism is also important for the colonization of plants and the highest concentration of this sugar is found in the nectaries of host plants, which are assumed the main entry site for *E. amylovora* (Pusey et al.

2008; Wilson et al. 1990). This pathogen can metabolize sucrose via the secreted levansucrase, which polymerizes the homopolysaccharide levan and releases glucose from sucrose (Geier and Geider 1993; Gross et al. 1992). Five open reading frames (ORFs) are involved in the metabolism of sucrose. The corresponding genes are named *scrK*, *scrY*, *scrA*, *scrB*, and *scrR* in analogy to other *scr* regulons (Bogs and Geider 2000). Mutants of *E. amylovora* in the *scr* operon are avirulent (Kube et al. 2010). The external release of glucose does not substitute for a deficiency in sucrose metabolism, which is strictly required for pathogenicity. Reduced virulence of sorbitol and sucrose mutants could be due to a low level of both nutrients in xylem vessels.

The sucrose operon is also present in the genome of *E. pyrifoliae* and *E. tasmaniensis*, which carry a second copy of *scrAB*. All these genes are absent in the genome of *E. billingiae* (Kube et al. 2010).

Xylitol *E. billingiae* can metabolize xylitol, a sugar alcohol widely distributed in nature (Kube et al. 2010). However, *E. amylovora*, *E. tasmaniensis* (with exceptions) and *E. pyrifoliae* cannot metabolize it (López et al. 2011).

Iron acquisition

Iron uptake in bacteria is regulated globally by the ferric uptake receptor Fur, which specifically regulates the biosynthesis and uptake of iron-affinity siderophores (compounds with a high affinity for iron that bind Fe^{3+} under limiting conditions). Iron-bound siderophores are taken in through receptors in the outer membrane, and iron is delivered to the cells. Under iron-poor conditions, *E. amylovora* produces and secretes cyclic desferrioxamines (DFOs), hydroxamate-type siderophores. For uptake of these siderophores, *E. amylovora* produces siderophore receptors, such as FoxR for DFOE (Oh and Beer 2005). Iron acquisition systems are important for virulence in *E. amylovora*, and a second role for desferrioxiamine was determined as a major factor in the protection of *E. amylovora* against oxidative conditions (Oh and Beer, 2005). *E. pyrifoliae* and the non-pathogenic species *E. tasmaniensis* share a common set of genes for siderophore production: ferrioxamine receptor (encoded by the gene *foxR*); siderophore biosynthetis enzyme L-lysine 6-monooxygenase (NADPH) (encoded by the gene *dfoA*); and siderophore biosynthesis protein, probably alcaligin (encoded by gene *alcA*). However, only ferrioxamine receptor is present in *E. billingiae* (Kube et al. 2010).

Flagellar systems

E. amylovora is motile by peritrichous flagella, and its motility is dependent on temperature, pH, and other environmental signals. Swarming is a form of social motility along surfaces, and swarmer cells are normally hyper-flagellated and require extracellular components, such as EPS and surfactants, to enable mass migration (Zhao et al. 2009). It has been confirmed that swarming motility is also required for, and enhances, the virulence of *E. amylovora* (Wang et al. 2010). Two sets of genes encoding flagellar biosynthesis and chemotaxis-related proteins were found in the genome of *E. amylovora* CFBP 1430. A complete gene set comprises four gene clusters, and the related operons resemble those found in other *Erwinia* spp., including *E. pyrifoliae* and *E. tasmaniensis*. The second gene set contains a single gene cluster and matches an analogous region found in the *E. pyrifoliae* type strain. This second set is absent in the *E. tasmaniensis* type strain; however, *E. tasmaniensis* does contain the quorum-sensing (QS) genes *expRI* in an equivalent position in its genome. Both *E. amylovora* and *E. pyrifoliae* lack a QS signal-generating enzyme (Smits et al. 2011).

Fimbriae

All the *Erwinia* species, except *E. billingiae*, possess a comparable incomplete gene cluster of K88 (F4) fimbriae genes. These fimbriae have been identified as virulence factors in enterotoxigenic *E. coli* strains. However, the incomplete cluster found in these *Erwinia* species has not yet been linked to pathogenicity (Kube et al. 2010).

The *E. tasmaniensis* genome shows a nearly complete Type I fimbrial gene cluster, which is not present in *E. pyrifoliae* or *E. billingiae* (Kube et al. 2010). Enterobacterial Type I fimbriae are involved in cell attachment and adhesion to surfaces and lead to bacterial aggregation and biofilm formation (Blumer et al. 2005); Enterobacterial Type I fimbriae can also help in plant colonization and environmental fitness. The *E. tasmaniensis* genome presents multiple clusters encoding fimbriae, which are largely absent in the genomes of *E. amylovora* CFBP 1430 and *E. pyrifoliae* (Smits et al. 2011). As *E. tasmaniensis* does not produce capsular EPS, the fimbriae may replace the capsules to allow bacterial aggregates (Kube et al. 2010). In addition, the colonization of host surfaces may be supported by a type IV pilus system, which is present in its plasmid pET49. Such a pilus system can provide effective flagella-independent movement in biofims (Kube et al. 2008a).

Quorum-sensing (QS) systems

Chemical communication, or QS, regulates many key bacterial traits, such as production of virulence factors, motility, and symbiosis. Two principal QS systems are known in Gram-negative bacteria and are defined by the chemical nature of the autoinducer (AI) involved. The AI-1

system utilizes N-acyl homoserine lactones (AHLs), produced by the LuxI family of proteins, as signal molecules. Synthesis of AHLs is involved in cell-to-cell communication strategies to face competitive environmental conditions in different bacteria. A second QS system, based on the production of an AI-2 signal molecule (a furanosyl borate) controlled by the LuxS protein, is also widespread among Gram-negative and Gram-positive bacteria and is putatively involved in cross-species bacterial communication (Smits et al. 2010a).

For both systems, the genetic endowment of *E. amylovora* CFBP 1430 is analogous to the ones found in *E. pyrifoliae* and *E. tasmaniensis* (Smits et al. 2010b). Synthesis of an AHL was detected in *E. billingiae* but not in *E. tasmaniensis*, *E. amylovora*, or *E. pyrifoliae* (Jakovljevic et al. 2008). Genes similar to those involved in the synthesis of AI-2 were found in *E. billingiae*, *E. amylovora*, and other *Erwinia* species (Mohammadi and Geider 2007). However, both *E. amylovora* and *E. pyrifoliae* lack a corresponding signal-generating enzyme for AI-1 signals, and they lack essential accessory genes involved in the signal response for AI-2 (Smits et al. 2010a, b).

Biofilms

Biofilms protect the associated bacterial cells from rapid fluctuations in environmental conditions and also enable an accelerated rate of horizontal genetic exchange, so bacteria in biofilms may be protected from external stresses, such as antibiotics and host defences (Koczan et al. 2009). It has already been demonstrated that *E. amylovora* can form biofilms on abiotic surfaces and in planta and that amylovoran but not levan is necessary for biofilm formation, although levan also plays a role in biofilm formation (Koczan et al. 2009). Microscopic observations suggest the presence of biofilms in very different biotic surfaces, such as mature apple fruit peduncles and the Mediterranean fruit fly *Ceratitis capitata* (Ordax et al. 2010b). It is not yet demonstrated that the other *Erwinia* species are able to form biofilms in vitro or in planta, but very likely they also have the ability to form them.

CRISPR-associated genes

Clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated sequence (Cas) proteins constitute a putative prokaryotic RNA-interference-based immune system protecting against bacteriophages and plasmids. The *E. amylovora* CFBP 1430 genome contains eight genes with homology to *cas* genes of the *E. coli* subtype, and orthologues thereof have been detected in the genome of *E. pyrifoliae* but not *E. tasmaniensis*. However, the latter strain's genome contains *cas* genes of the Ypest subtype, and orthologs of these were found in *E. pyrifoliae* as well (Smits et al. 2011).

Other extracellular factors

Necrosis factors

Virulence factors such as the cytotoxic necrotizing factors Cnf1 and Cnf2 are encoded by the *cnf1* and *cnf2* genes, which have been identified in *E. amylovora*, *E. pyrifoliae*, and *E. tasmaniensis* but not in *E. billingiae*. These factors are proteins, and they have been suggested to weaken defence responses and therefore help bacterial spread in plants (Kube et al. 2010).

Proteases

In contrast to many species of *Erwinia*, *E. amylovora* lacks the ability to degrade cell wall components by the action of carbohydrate-degrading enzymes. However, this bacterium produces and secretes a metalloprotease, PrtA, which helps the colonization of apple leaves (Oh and Beer, 2005). Interestingly, the protease operon for the secreted enzyme PrtA is not represented in the genomes of *E. pyrifoliae*, *E. tasmaniensis*, or *E. billingiae*, although these three species share a common set of genes for other proteases that can help plant colonization and nutrient acquisition (Kube et al. 2010).

Plasmid content

Differences in plasmid content have been reported among the pome fruit pathogenic and epiphytic *Erwinia*, but they are detailed in another review in this issue (see Llop et al. in this issue).

General conclusions

The goal of this review is to present a comparative analysis of the most relevant features of *E. amylovora* and the more recently described pathogenic species *E. pyrifoliae* (Kim et al. 1999), *E. piriflorinigrans* (López et al. 2011), and the *Erwinia* spp. described in Japan (Mizuno et al. 2000, 2010), with the non-pathogenic *E. billingiae* (Mergaert et al. 1999) and *E. tasmaniensis* (Geider et al. 2006).

The six species share several host plants and have common metabolic and physiological capabilities. Although the biology and life cycle of the more recently described species are not sufficiently understood to be able to draw definitive conclusions, the information from the genomes of the studied *Erwinia* species suggests that they share many common genes but show important differences related to the

production of pathogenicity factors, which correlate with their pathogenic or epiphytic biology.

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