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REVIEW

Functions and origin of plasmids in *Erwinia* species that are pathogenic to or epiphytically associated with pome fruit trees

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Abstract The genus *Erwinia* includes plant-associated pathogenic and non-pathogenic species. Among them, all species pathogenic to pome fruit trees (*E. amylovora, E. pyrifoliae, E. piriflorinigrans, Erwinia* sp. from Japan) cause similar symptoms, but differ in their degrees of aggressiveness, i.e. in symptoms, host range or both. The presence of plasmids of similar size, in the range of 30 kb, is a common characteristic that they possess. Besides, they share some genetic content with high homology in several genes associated with exopolysaccharide production and hence, with virulence, as well as in some other genes. Knowledge of the content of these plasmids and comparative genetic analyses may provide interesting new clues to understanding the origin and evolution of these pathogens and the level of symptoms they produce. Furthermore, genetic similarities observed among some of the plasmids (and genomes) from the above indicated pathogenic species and *E. tasmaniensis* or *E. billingiae*, which are epiphytic on the same hosts, may reveal associations that could expose the mechanisms of origin of pathogens. A summary of the current information on their plasmids and the relationships among them is presented here.

Keywords Plasmids · Genetic relatedness · Origin · Evolution · Functions

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Introduction

Genetic material in bacteria: chromosomes and plasmids

Bacterial genomes are formed by chromosomal and plasmid genetic material. Their size is directly proportional to the number of genes they contain and correlates with the bacterial lifestyle. Larger genomes are common in bacteria that face changing environmental conditions and occupy more complex and variable niches, whereas bacteria that are very specialised tend to have smaller genomes.

Bacterial chromosomes are circular and/or linear, covalently closed loops of double-stranded DNA, supercoiled and with a single origin of replication, and their numbers vary from one to several per cell. In prokaryotes, the frequency of repetitive sequences is low compared to other organisms, but on the contrary the content in mobile elements such as plasmids, phages, transposable elements or genomic islands is high (Russell 1998).

Plasmids are molecules of extrachromosomal DNA, capable of being replicated and transcribed autonomously (independently of the chromosomal DNA) that are very common in prokaryotic cells. They can also be circular or, less commonly, linear. Their size and copy number is very variable, although generally it decreases with increasing size. The number can vary from one copy to hundreds in high-copy number plasmids. Plasmid sizes range from a few kb to a few Mb and when they are close to the size of chromosomes are called megaplasmids (Rosenberg et al. 1982). Although there is still no general consensus as to the minimum size at which a plasmid is considered a megaplasmid, some authors suggest that it should be around 100 kb (Antón et al. 1995; Barton et al. 1995). Megaplasmids may be related to the survival strategies of the

bacteria in their special surroundings, as reported for halophilic bacteria (Argandoña et al. 2003). In plant-associated bacteria, plasmids with sizes ranging from 150 kb to 1.5 Mb are found in many species, such as *Rhizobium meliloti, Agrobacterium tumefaciens* and *Ralstonia solanacearum* (Burkardt and Burkardt 1984; Unger et al. 1985; Negishi et al. 1993).

Some authors hypothesise that recent events, thought to have occurred since the development of agriculture, led to the conversion of a non-pathogenic or a weak pathogen into a virulent pathogen after acquiring the genes needed for pathogenicity from a different bacterial species (Stukenbrock and McDonald 2008). Plant pathogens could emerge in agricultural ecosystems through several mechanisms, including host-tracking, host jumps, hybridisation and horizontal gene transfer. Some pathogens may have been domesticated along with their hosts during the development of agriculture, but some others appear to have emerged very recently and developed almost instantaneously following horizontal gene transfer or hybridisation (Stukenbrock and McDonald 2008). Currently, pathogen populations in modern agroecosystems are regularly challenged by plant resistance genes, pesticides, crop rotations, and a variety of other cultural practices aimed at reducing plant infections. Consequently, selected genotypes that can adapt to these management practices, including the ability to overcome host resistance genes and chemical treatments, may increase rapidly in frequency and be dispersed to neighbouring fields. Plasmids can play a role in these adaptations by providing bacterial cells with specific genes enabling them to survive or multiply under certain unfavourable conditions (Stukenbrock and McDonald 2008).

Plasmids are very common in phytopathogenic bacteria, conferring the host bacteria with different traits that enhance their fitness, adaptability to a specific niche, virulence or metabolic features, symbiotic abilities, resistance to certain antibiotics or other characteristics, which can improve their environmental adaptability and contribute significantly to their genetic evolution. An important characteristic is that they can be transferable genetic elements, moving from one organism to another (Sundin 2007). Usually they are not essential for the host bacterium, but in most cases they confer on the bacteria decisive features for their survival in the environment that provide them with selective advantages.

Horizontal gene transfer, also known as lateral gene transfer, is the exchange of specific genes or genomic regions between species that are normally reproductively isolated (de la Cruz and Davies 2000). Horizontal gene transfer can also result in unexpected gene homologies among organisms and, in the case of pathogens, in a patchy distribution of virulence genes among different phylogenetic lineages. Plasmids and conjugative transposons are very frequently transferred horizontally among species of bacterial strains (Panopoulos and Peet 1985).

The ability of bacteria to adapt to new environments most frequently results from the acquisition of new genes through horizontal gene transfer rather than by the alteration of gene functions through mutations. Most plasmids characterised in phytopathogenic bacteria are self-transmissible and can include insertion sequences and other transposable elements that contribute to the movement of sequences within and between genomes. These features convert plasmids into dynamic instruments of change for bacterial genomes and confer them with genetic plasticity and different advantages in host–pathogen interactions and environmental traits (Sundin 2007).

The roles of plasmids described in plant pathogenic bacteria are considered in terms of the phenotypes they confer to their host cells, although most of them are cryptic. Many of these genes are acquired from other bacteria via horizontal transfer, in which the plasmids play an important role as tools of genetic exchange that enable rapid evolution of the genome of the microorganisms (Sundin 2007). The genes they carry can be integrated into the host chromosome. Through integration events, the genetic content of plasmids can be stabilised within the genome and further disseminated.

Functions encoded by plasmids in plant pathogenic bacteria

Plasmids are not restricted to the bacterial species from which they originated due to the horizontal transfer of genes. Their genetic content is variable and they represent the flexible part of the bacterial genome and contribute to the ecological and pathogenic differences among strains of the same species, since they usually code for genomic islands (Sundin 2007). Plasmids, with their potential for inter-strain mobility, play an important role in bacterial interaction with plants and a substantial number of genes involved in pathogenicity and host specificity have been described in plasmids in some species or particular strains. Other characteristics frequently encoded in plasmids are toxin and hormone production, resistance to bactericides such as copper or antibiotics, and to UV irradiation (Vivian et al. 2001). Plasmids can also acquire a number of different antibiotic-resistance genes by means of integrons. Integrons are transposons that can carry multiple gene clusters, called gene cassettes that move as a unit from one piece of DNA to another. In this way, a number of different antibiotic-resistance genes can be transferred from one bacterium to another.

Some of the most relevant genes and phenotypes described in plant pathogenic bacteria that can be encoded in plasmids are:

- Pathogenicity and host-specificity genes. The most notable are avirulence (*avr*) and virulence (*vir*) genes and the *hrp* genes involved in a type III protein secretion system. The *hrp* genes are mainly coded by the chromosome, and only in *Pantoea agglomerans* (formerly *Erwinia herbicola*) pv. *gypsophilae* and *Ralstonia solanacearum* are the *hrp* genes coded in plasmids (Lichter et al. 1995; Nizan et al. 1997; Boucher et al. 1986). In contrast, the *avr* genes that have been described are evenly divided between plasmid and chromosomal locations (Vivian et al. 1997).
  A totally different system encoding for pathogenicity is the Ti plasmid of *Agrobacterium tumefaciens* which contains the T-DNA and *vir* regions that play a direct role in tumour induction and oncogenicity (Watson et al. 1975).
- *Toxins* One example is coronatine, whose genetic determinants in the species *Pseudomonas syringae* are generally located on plasmids (Cuppels and Ainsworth 1995).
- *Plant hormones* Genes for indoleacetic acid (IAA) production are located on the Ti plasmid in *A. tumefaciens* and also harboured by a plasmid in *P. agglomerans* pv. *gypsophilae* (Manulis et al. 1991; Clark et al. 1993). In *Pseudomonas savastanoi* pv. *savastanoi*, the IAA genes are located in plasmids in oleander strains, but they are chromosomically located in ash and most olive strains (Caponero et al. 1995).
- *Resistance to copper* Appears to be plasmid-borne in several phytopathogenic bacteria (*Xanthomonas* spp. or *X. campestris* pv. *vesicatoria*), *Pseudomonas syringae* pv. *syringae*; and *P. syringae* pv. *tomato*) (Stall et al. 1986; Bender and Cooksey 1986, 1987; Mellano and Cooksey 1988; Kidambi et al. 1995). It is generally located on large plasmids, with the exception of *X. arboricola* pv. *juglandis*, in which the genes are chromosomally located (Lee et al. 1994).
- *Resistance to antibiotics and other compounds* Plasmid-borne resistance to streptomycin has been detected in *P. syringae* pv. *papulans* and other Gram-negative bacteria (Norelli et al. 1991). Copper resistance is often linked to streptomycin resistance and dual resistance to both bactericides has been detected in conjugative plasmids of *P. syringae* pv. *syringae* (Sundin and Bender 1993).
- *Resistance to UV irradiation* Resistance determinants to UV light, *rulAB*, have been identified and characterised in plasmids in *P. syringae* (Sundin and Murillo 1999). They are homologous to the DNA repair operon, *umuDC,* in *E. coli* and they are harboured on pPT23A-like plasmids. These replicons are indigenous residents of the species *P. syringae* and also tend to encode determinants of importance in host–pathogen interactions. The precise role of the *rulAB* genes in protection against UV needs cautious assessment since it is clear from the work of Arnold et al. (2000) that these genes may no longer be functional. Consequently, their role among *Ps. syringae* strains, whether in protection against UV irradiation or as mobile regions of potential homology for integration of virulence genes, is still to be determined.
- *Insertion sequences (IS) and transposons* They have a role in the transfer and mobility of genes among bacteria. In some cases they can inactivate genes, such as the effect of IS*51* and IS*52* on the *iaaM* gene in *P. savastanoi* pv. *savastanoi*, leading an inability to induce gall formation (Comai and Kosuge 1983; Yamada et al. 1986) and IS*476*, which inactivates the avirulence gene *avrBs1* in *Xanthomonas spp.* (*X. campestris* pv. *vesicatoria*) (Kearney and Staskawicz 1990). Others may serve as sites of potential mobile homology, such as IS*1327* in *P. agglomerans* pv. *gypsophilae,* which is probably also involved in the horizontal acquisition of the IAA gene cluster in *P. savastanoi* pv. *phaseolicola* (Szabo and Mills 1984; Lichter et al. 1996; Vivian et al. 2001).

Few other phenotypes have been shown to be plasmid-derived among phytopathogenic bacteria, but given the size of several plasmids already described, the genes they harbour may play a role in many other characteristics that remain still unknown.

Brief description of phytopathogenic *Erwinia* sp. and their relationship with non-pathogenic *Erwinia* sp.

The genus *Erwinia* essentially comprises plant-associated bacteria and includes several species that are pathogenic to pome fruit trees. A more detailed description of the characteristics of each species can be found in this issue (see Palacio-Bielsa et al. 2011). The most important pathogenic species is *Erwinia amylovora*, causal agent of fire blight on a wide range of rosaceous hosts (pear, apple, quince, loquat, ornamental plants), and which is capable of producing symptoms in different organs of these plants. Once established in a tree, *E. amylovora* invades old tissues through the current season's growth. Death of infected branches can be so rapid that the leaves do not fall off the tree. Young trees of susceptible cultivars can be easily killed by the infection while mature trees can survive, but the economic losses are very high (van der Zwet and Keil 1979; Bonn and van der Zwet 2000). *Erwinia amylovora* is present worldwide, including in North America, Europe, the Eastern Mediterranean, and some countries in Pacific Asia and North Africa (Bonn and van der Zwet 2000; Shrestha et al. 2003).

Related species producing symptoms similar to those of fire blight in pome fruit trees described in the last decades include:

- *Erwinia pyrifoliae* A pathogen described in Asian pear and, thus far, only isolated in South Korea. Affected plants show symptoms very similar to fire blight in Nashi cultivars (Kim et al. 1999; Rhim et al. 1999). Some strains of the previously described bacterial shoot blight of pear (BSBP) in Japan were found to be closely related to the Korean pathogen *E. pyrifoliae* (Kim et al. 2001). Recently a polyphasic characterisation has assigned some strains of these *Erwinia* from Japan to *E. pyrifoliae* (Geider et al. 2009).
- *Erwinia piriflorinigrans* In 1999, necrotic pear blossoms were observed in pear trees of cvs. *Ercolini* (Coscia) and Tendral in Valencia, Spain, and the disease was also observed in subsequent years (López et al. 2011). The affected trees only presented infected blossoms similar to fire blight in spring, but these were the only symptomatic parts of the plant, in contrast to the disease caused by *E. amylovora* (Roselló et al. 2002, 2006). A new *Erwinia* species was described based on the characteristics of the strains isolated from these symptoms, and called *E. piriflorinigrans*. So far, the disease has been reported only from some orchards in Valencia, Spain.
- A new pathogenic *Erwinia* sp. that produces bacterial black shoot disease (BBSDP) on European pear has also been recently reported (Mizuno et al. 2010). So far, the disease is restricted to one island in Japan. Isolates of this new pathogen are closely related, but different to, *E. amylovora* and *E. pyrifoliae*. Their taxonomic classification is not yet defined. No information about the possible spread of this new pathogenic *Erwinia* is available.

The symptoms that all these pathogenic erwinias produce on pear are shown in Fig. 1. Other species of this genus isolated from pome fruit are *Erwinia billingiae* and *Erwinia tasmaniensis*. Due to the epiphytic occurrence of these latter species, they can grow competitively with *E. amylovora* on flowers and may be applied as antagonists for control of fire blight (Kube et al. 2010). All these *Erwinia* species are genetically and phenotypically closely related, although they can be distinguished by taxonomic criteria (Palacio-Bielsa et al. 2011).

Plasmids in *E. amylovora*

Genomic studies performed on strains of *E. amylovora* have revealed that although the species is very homogeneous at the biochemical and serological levels (Paulin 2000; Smits et al. 2010), there are differences among strains found in different analyses, including host range and virulence (Norelli et al. 1984; Cabrefiga and Montesinos 2005; Wang et al. 2009). The diversity that could explain such differences may primarily be attributed to the flexible genome that comprised plasmids. Several works have reported finding different plasmids in strains of this pathogen (Laurent et al. 1989; Chiou and Jones 1991; McGhee et al. 2002; Maxson-Stein et al. 2003; Foster et al. 2004), but such studies were performed using a low number of isolates from few geographic areas or countries. Consequently, there is a lack of information on the plasmid content of large collections of isolates of *E. amylovora* (Foster et al. 2004; Llop et al. 2008). This topic has acquired more attention in recent years since the discovery of strains lacking plasmid pEA29, which was thought to be universal (Llop et al. 2006, 2008, 2011; Mohammadi et al. 2009; Mohammadi 2010). The search for isolates lacking the pEA29 plasmid led to the discovery of new isolates with different plasmid contents (Llop et al. 2006, 2011). Thus, the genetic information encompassed by this extrachromosomal material could be more important in the life cycle, aggressiveness or fitness of *E. amylovora* than expected. Here, we describe current knowledge about the different plasmids found in *E. amylovora* strains, their possible functions and their comparison to other plasmids found in related pathogenic and non-pathogenic *Erwinia* species. A summary of the plasmids found in *E. amylovora* and the other pome fruit *Erwinia* species is shown in Table 1.

The plasmids found and reported in *E. amylovora* to date, in decreasing size order, are the following.

pEA72

This plasmid has been described in strain Ea273 (ATCC 49946), which has been sequenced recently (Sebaihia et al. 2010). The strain was isolated from an infected apple tree growing in an orchard in New York State (USA) in the 1970s. In a previous work on strains Ea273 and Ea322, a plasmid thought to have a size of 56 kb was reported and named pCPP60 (Steinberger et al. 1990). However, recent sequencing of strain Ea273 has revealed a plasmid with a size of 71,487 kb (GenBank accession number FN666577) and the designation of pEA72 was given instead. No specific genes relating to important characteristics that could be provided to the host bacterium are present and as its function is unknown it is considered cryptic.

pEI70

A plasmid of nearly 66 kb in size was found during screening of the plasmid content of Spanish strains of *E. amylovora* (Llop et al. 2006). This plasmid has important characteristics that make it unusual among the other plasmids found in this pathogen: (1) it is conjugative (Llop

Fig. 1 Symptoms produced on pear trees by the different pathogenic *Erwinia* species. **a** Symptoms caused by *E. amylovora* on a branch (Photo: IVIA, Spain). **b** Symptoms of *E. amylovora* on a shoot, producing the typical "shepherd's crook" (Photo: IVIA, Spain). **c** Necrotic symptoms caused by *E. pyrifoliae* (Photo: Bacterial Genetics and Biotechnology Laboratory). **d** Symptoms caused by *E. piriflorinigrans*. Necrosis on flowers of cv. *Ercolini* (Photo: IVIA, Spain). **e** Symptoms on young shoots of cv. La France inoculated with isolates of the new pathogenic *Erwinia* sp. from Japan (Photo from Mizuno et al. (2010))

et al. 2011); (2) it is widespread in 11 European countries, being present in 5–92% of strains analysed, depending on the country; and (3) more importantly, it has been observed that when introduced into low virulence strains of *E. amylovora*, it was able to induce faster development of symptoms than without it (Llop et al. 2008, 2011). It has been sequenced, but the annotation has not revealed known virulence genes. The sequencing yielded a consensus sequence of 65,840 base pairs, with an overall G+C content of 52.2% (GenBank accession number CP002951). A total of 70 open reading frames (ORFs) have been identified and annotated. It presents a high sequence identity to sequences of plasmid pEB102 from *E. billingiae* strain Eb661, and the protein RepA has 99% sequence identity to the RepA of the same plasmid, indicating a potential common origin. It is thought to provide a fitness advantage to the host bacterium, but its specific role has not yet been determined (Llop et al. 2011).

pCPP60

A plasmid from *E. amylovora* strain Ea322 (CFBP1368), isolated from *Crataegus* in France was described with a size of approximately 56 kb, and called pCPP60 (Steinberger et al. 1990). Other strains including Ea208, Ea220 and Ea273 from the USA and strain Ea321 from France (CFBP1367), were reported to also contain pCPP60. However, as explained above, the plasmid in strain Ea273 was renamed pEA72, while sequences of strain Ea321 now demonstrate that it harbours pEI70 (T. Smits, personal communication). As strains Ea321 and Ea322 were isolated from the same place and at the same time, they could be clonal, and the plasmid in strain Ea322 may also prove to be pEI70. The presence of pCPP60 in other strains, claimed to be in 25% of 40 isolates analysed from the USA and France (Steinberger et al. 1990), is misleading because pEI70 has not yet been found in the USA (Llop et al. 2011).

pEL60

This plasmid, described by Foster et al. (2004) has been reported in strains from Lebanon and was present in 47% of the 49 *E. amylovora* strains analysed from three plant hosts (apple, pear and quince). This plasmid has 60,145 bp with a G+C content of 51.5% (GenBank accession number NC_005246). Annotation of its sequence revealed 68 ORFs, but putative functions could only be assigned to 46 (68.7%) of them, encoding a variety of genes thought to

Table 1 Species of *Erwinia* from pome fruits, the plasmids they harbour and other characteristics

Species	Plasmid	Strain of origin	Size (bp)	Bacterial host plant	Country	Accession numbers	References
Erwinia amylovora	pEA72	Ea273	71,487	Apple	USA	FN666577	Sebaihia et al. (2010)
	pEI70	IVIA1614-2a	65,840	*Crataegus*	11 European countries	CP002951	Llop et al. (2008, 2011)
	pEL60	LebB66	60,145	Apple, pear	Lebanon	NC_005246	Foster et al. (2004)
	pCPP60[a]	Ea322	Near 56 kb	*Crataegus*	France	Na	Steinberger et al. (1990)
	pEA34	CA11	Near 34 kb	Pear, apple	Michigan, USA	Na	Chiou and Jones (1991, 1993)
	pEU30	UTRJ2	30,314	Pear, apple	USA	NC_005247	Foster et al. (2004)
	pEA29	Ea88	28,185	Pear	Washington, USA	AF264948	McGhee and Jones (2000)
		Ea273	28,243	Apple	New York, USA	FN666576	Sebaihia et al. (2010)
		CFBP1430	28,259	*Crataegus*	France	FN43411	Smits et al. (2010)
	pEA8.7	CAR3	Near 9 kb	Apple	California, USA	Na	Palmer et al. (1997)
	pEAR5.2	ATCCBAA2158	5,251	*Rubus* sp	Illinois, USA	FR719211	Powney et al. (2011)
	pEAR4.3	ATCCBAA2158	4,369	*Rubus* sp	Illinois, USA	FR719210	Powney et al. (2011)
	pEA2.8	IL-5	2,825	*Rubus sp*	Illinois, USA	AY123047	McGhee et al. (2002)
	pEA1.7	IH3-1	1,711	*Crataegus*	Louisiana, USA	AY123046	McGhee et al. (2002)
E. pyrifoliae	pEP36	Ep1/96	35,904/	Asian pear	Korea	AY123045	McGhee et al. (2002)
		Ep1/96	35,909			FP236829	Kube et al. (2010)
		DSM12163^T	35,901			FN392238	Smits et al. (2010)
	pEJ01	Ejp617	30,866	Asian pear	Japan	CP002125	Park et al. (2011)
	pEJ30	Ejp556	29,593	Asian pear	Japan	AY255829	Maxson-Stein et al. (2003)
	pJE03	Ejp617	6,417	Asian pear	Japan	CP002127	Park et al. (2011)
	pJE02	Ejp617	5,296	Asian pear	Japan	CP002126	Park et al. (2011)
	pEP05	Ep1/96	4,955	Asian pear	Korea	FP236828	Kube et al. (2010)
	pJE04	Ejp617	3,237	Asian pear	Japan	CP002128	Park et al. (2011)
	pEP03	Ep1/96	3,070	Asian pear	Korea	FP236827	Kube et al. (2010)
	pJE05	Ejp617	2,691	Asian pear	Japan	CP002129	Park et al. (2011)
	pEP2.6	Ep1/96	2,590	Asian pear	Korea	FN392236	Kube et al. (2010)
E. tasmaniensis	pET49	Et1/99	48,751	Apple	Tasmania	CU468131	Kube et al. (2008)
	pET46	Et1/99	46,159	Apple	Tasmania	CU468133	Kube et al. (2008)
	pET45	Et1/99	44,694	Apple	Tasmania	CU468132	Kube et al. (2008)
	pET35	Et1/99	35,494	Apple	Tasmania	CU468130	Kube et al. (2008)
	pET09	Et1/99	9,299	Apple	Tasmania	CU468128	Kube et al. (2008)
E. billingiae	pEB170	Eb661	169,778	Pear	UK	FP236830	Kube et al. (2010)
	pEB102	Eb661	102,323	Pear	UK	FP236826	Kube et al. (2010)
E. piriflorinigrans	pEPIR37	CFBP5888	Near 37 kb	Pear	Spain	Pending	Barbé et al. (in preparation)
	pEPIR5	CFBP5887	Near 5 kb	Pear	Spain	Pending	Barbé et al. (in preparation)

[a] Recent data obtained in the sequencing of several *E. amylovora* strains indicate that this plasmid, thought to be present in several strains, corresponds in reality to two different ones: it has been renamed pEA72 in strain Ea273 from USA (Sebaihia et al. 2010) and it has been reported as pEI70 in some strains from Europe (Llop et al. 2011). See text for details

be involved in maintenance and conjugation functions. The most notable aspects of pEL60 are its relationships with other enterobacterial IncL/M plasmids and its high similarity to the *Citrobacter freundii* plasmid pCTX-M3. It is not related to the plasmid found in some *E. amylovora* strains (Steinberger et al. 1990), as a labelled pEL60 probe did not hybridise with *Eco*RI digested total plasmid DNA from *E. amylovora* Ea273. pEL60 encodes genes for a *mucAB*-like mutagenic DNA repair system that encodes tolerance to UV radiation (Sundin and Murillo 1999). It

may be of environmental significance to *E. amylovora*, because mutagenic DNA repair determinants confer UV radiation tolerance that may enhance bacterial fitness on plant surfaces.

pEA34

This plasmid was reported from some strains of *E. amylovora* with resistance to streptomycin (SmR), isolated during a study on resistance to this antibiotic in Michigan (USA) (Chiou and Jones 1991, 1993) and seems to be present only in strains from there. It has a size of ca. 34 kb and seems that SmR is due to the presence of a transposon (Tn*5393*) that contains the genes encoding this resistance. pEA34 likely originated from the insertion of the transposon into a 28 kb plasmid present in some strains of *E. amylovora*. Hybridisation studies indicated that pEA34 and this plasmid were not related to the pEA29 plasmid (Chiou and Jones 1993). Although this transposon appears to be restricted to plant-associated bacteria, the SmR genes of Tn*5393* are identical to the genes found in at least 14 genera of Gram-negative animal and human pathogens worldwide (Sundin and Bender 1996). The plasmid pEA34 has not been fully sequenced.

pEU30

This plasmid was reported from several strains from Washington, Utah and Oregon, in the USA (Foster et al. 2004) where it was present in 41.0% of the analysed strains. Plasmid pEU30 contains 30,314 bp with a G+C content of 48.2%; its annotation revealed 25 putative ORFs, and 23 of these (92.0%) had homology with existing gene sequences (GenBank accession number NC_005247). The genetic content of pEU30 is similar to that of other plasmids inhabiting plant pathogenic bacteria, but there are no specific genes related to virulence or fitness reported (Foster et al. 2004). In Fig. 2 the maps of this plasmid and pEL60 are shown in the way normally employed to observe the gene composition they harbour.

pEA29

This plasmid was first described by Merckaert et al. (1982) and since then the information about its presence and functions is abundant. For many years it was assumed that all pathogenic *E. amylovora* wild strains should carry plasmid pEA29, due to its high stability, role in virulence and non-transmissibility (Laurent et al. 1989; Falkenstein et al. 1989; McGhee and Jones 2000). Nevertheless, in Spain, wild strains lacking this plasmid were found in symptomatic hosts (Llop et al. 2006) and these strains showed similar virulence levels to strains harbouring this

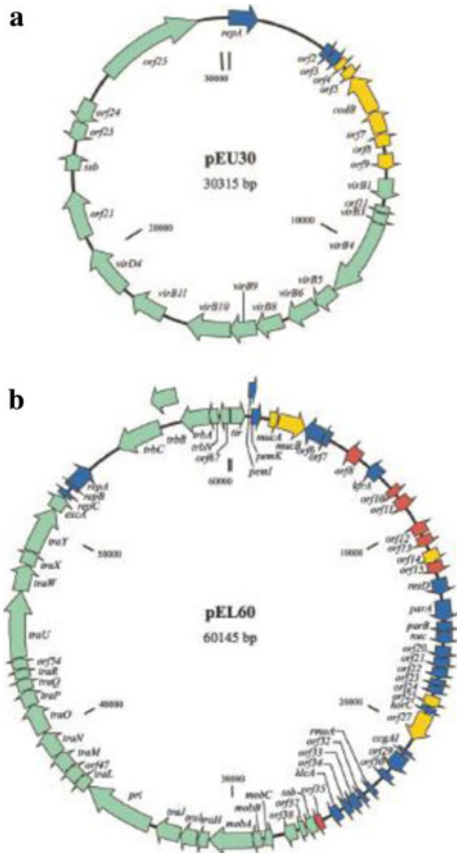

Fig. 2 Circular genetic maps of the 30,314-bp plasmid pEU30 (**a**) and 60,145-bp plasmid pEL60 (**b**) and their 25 and 68 identified ORFs, respectively. Genes are colour coded on the basis of function as follows: *blue* replication and stability, *yellow* ecological fitness, *red* transcriptional regulators, *green* conjugation- or plasmid-specific functions. The number or identity of each ORF is located inside the *circle*. From Foster et al. (2004)

plasmid. The complete sequence of plasmid pEA29 from *E. amylovora* strain Ea88 showed it has 28,185 bp with a 50.2% G+C content [the GenBank accession number from strain Ea88 is AF264948 (McGhee and Jones 2000), whereas the EMBL accession number for pEA29 from ATCC49946 is FN666576 (Sebaihia et al. 2010), and from strain CFBP1430 it is FN43411 (Smits et al. 2010)]. There are differences in the sizes of plasmids in strains from different origins due to a variable number of the short sequence repeats (SSRs) harboured. Thus, its size can actually vary from 27.6 to 34.9 kb (Schnabel and Jones 1998; Kim and Geider 1999; McGhee and Jones 2000). Thirteen ORFs that encoded predicted proteins with similarities to known proteins of other bacteria were identified, along with two ORFs related to hypothetical proteins found in GenBank and six ORFs with no similarities to the existing GenBank entries. Annotation revealed the following: predicted products of ORF with similarity to the thiamine biosynthetic genes *thiO*, *thiG*, and *thiF*; a *betT* gene encoding for choline transport; an *msrA* gene

encoding for the enzyme methionine sulfoxide reductase; a putative methyl-accepting chemotaxis gene; an aldehyde dehydrogenase gene; a *hns* DNA-binding gene; a LysR-type transcriptional regulator; and *parA* and *parB* partitioning genes. The plasmid encodes a thiamine biosynthesis operon and several candidate genes that could affect virulence and survival in plants (McGhee and Jones 2000). Thiamine prototrophy has also been associated with pEA29 (Chiou and Jones 1993), which could play a role in the physiology or metabolism of extracellular polysaccharide production, and these traits are associated with full virulence of the pathogen (McGhee and Sundin 2008). Strains cured of this plasmid exhibited reduced virulence, thiamine auxotrophy, and altered exopolysaccharide production on minimal medium without thiamine (Falkenstein et al. 1989; Laurent et al. 1989; McGhee and Jones 2000). *Pst*I and *Kpn*I restriction patterns for pEA29 isolated from fruit tree strains of *E. amylovora* were homogenous, but different from those for pEA29 isolated from *Rubus* (raspberry) strains (McGhee and Jones 2000), although the number of strains analysed was too small to generalise. Sequencing data have shown that pEA29 plasmids from genotypically diverse strains of *E. amylovora* share 99% sequence identity (100% coverage) (Ea88, McGhee and Jones 2000; Ea273, Sebaihia et al. 2010; CFBP1430, Smits et al. 2010).

pEA8.7

This plasmid was first reported and analysed after a survey performed in California (USA) looking for strains showing SmR (Palmer et al. 1997). It was initially thought that resistance was due to the plasmid pEA34, as was the case with strains from Michigan described above, but in fact it was observed that the same genes of resistance (*strA-strB*) present in pEA34 were also harboured in a new plasmid, which was approximately 9 kb in size and was named pEA8.7. Analyses by PCR, sequencing, hybridisation, and restriction data indicate that pEA8.7 from strain CA3R is closely related, if not identical, to the broad host-range plasmid RSF1010. RSF1010-like plasmids have been reported in a wide variety of clinical bacteria, and have also been detected in *P. agglomerans* isolates from apple orchards in New Zealand (Palmer et al. 1997; Vanneste, personal communication). The plasmid has not yet been fully sequenced.

Other small plasmids

Two small plasmids were observed in *E. amylovora* strain IH3-1, isolated from *Crataegus* (McGhee et al. 2002); one of which has been sequenced and named pEA1.7, because it has a size of 1,711 bp (GenBank accession number AY123046). It has an ORF with 74% nucleotide similarity to the *rom* gene of the pEP2.6 plasmid from *E. pyrioliae*, but no origin of replication similar to that of pEP2.6 was detected. Three additional ORFs do not exhibit homology to known genes. No information is available for the other plasmid (McGhee et al. 2002).

Plasmids in *E. amylovora* strains from *Rubus*

Three small plasmids (pEA2.8, and two others of unknown size and function) have been found in *E. amylovora* strain IL-5 isolated from *Rubus* (McGhee et al. 2002). Plasmid pEA2.8 is 2,825 bp in size (GenBank accession number AY123047). Its *oriV* sequence presented 84% nucleotide similarity to the origin of replication found in the pEP2.6 plasmid from *E. pyrifoliae*. It also contained an ORF coding for the ampicillin resistance protein beta lactamase. Two additional ORFs do not exhibit sequence homology to existing entries in GenBank. The other two plasmids from this strain hybridise with the *ori* region of plasmid pEP2.6, but no further information is available.

Recent sequencing of the genome of strain ATCC BAA-2158, restricted to *Rubus* spp., has shown the presence of three circular plasmids: pEA29 (28,138 bp with 50% G+C), pEAR5.2 (5,251 bp with 52.2% G+C), and pEAR4.3 (4,369 bp with 51.5% G+C) (Powney et al. 2011). Plasmid pEAR5.2 (EMBL accession number FR719211) encodes for six ORFs and pEAR4.3 (EMBL accession number FR719210) encodes for four ORFs. Both seem to be unique to strain ATCC BAA-2158 at the moment, and share 88 and 89% sequence identity (57 and 53% coverage) with pEP5 of *E. pyrifoliae* DSM 12163T (Smits et al. 2010), respectively.

Latest screenings of plasmid content in *E. amylovora* isolates from different collections are bringing to light strains harbouring novel plasmids, such as that of ca. 60 kb found in isolates from Poland (Pulawska, personal communication; this issue). These screenings will increase our knowledge about the extrachromosomal material present in *E. amylovora* and the genetic information they provide to the host bacteria.

Plasmids in *Erwinia pyrifoliae*

The plasmid content of this pathogen of Asian pear (Rhim et al. 1999) has been studied quite extensively, despite its recent discovery and the availability of only a few strains (Smits et al. 2010). Since the first reports of this new pathogen, plasmids have been found in different isolates, and have been compared to the plasmids described in *E. amylovora* (Rhim et al. 1999; McGhee et al. 2002; Shrestha et al. 2007). The plasmid profiles of 25 strains of *E. pyrifoliae* were investigated by Shrestha et al. (2007) and

four groups were observed, suggesting intraspecific plasmid diversity. The *E. pyrifoliae* strains showed diverse plasmid profiles and restriction patterns typical of larger plasmids.

Variability in plasmid content was also observed in strains of *Erwinia* from Japan causing BSBP, some of which are now classified as *E. pyrifoliae* (Maxson-Stein et al. 2003).

To date, the following six plasmids and their possible functions have been reported from *E. pyrifoliae*.

pEP36

Plasmid pEP36 (EMBL accession number FN392238) was the most commonly encountered among the 25 isolates analysed from this species (Shrestha et al. 2007). A larger plasmid has been found in only two strains, but so far no information is available regarding its relatedness to pEP36. Only a part of Tn5394 (*tnpA* gene), which is specific to the pEP36 plasmid, was detected by hybridisation, indicating that this transposon was also present in the larger plasmid (Shrestha et al. 2007). The number of nucleotides of the 36 kb plasmid from strain Ep1/96 varies from 35,904 to 35,909 bp (GenBank accession numbers AY123045 and FP236829, respectively), depending on the sequencing project (McGhee et al. 2002; Kube et al. 2010) with a G+C content of 49.6%, and 32 ORF. Strain DSM 12163^T possesses a plasmid of 35,901 bp (GenBank accession number FN392238; Smits et al. 2010). Plasmid digestion with *Bam*HI enzyme generates several DNA fragments, which clearly differentiate it from the *E. amylovora* pEA29 plasmid (Rhim et al. 1999). One of the nine *Bam*HI restriction sites detected in pEP36 corresponds to the unique *Bam*HI site in pEA29. However, pEP36 was found to be incompatible with clones containing the reciprocal origin regions of pEA29. The sequencing of pEP36 has shown that the same RepA protein was present in both, which explains the incompatibility observed. Sequence analysis also showed that pEP36 carried the transposon Tn5394 of approximately 6.4 kb in size. Genes similar to thiamine biosynthesis operon genes of pEA29 were found in this plasmid, but the possibility that pEP36 contributes to the virulence of *E. pyrifoliae* remains to be determined.

pEJ30

This plasmid was reported from strain Ejp556 (Maxson-Stein et al. 2003; Geider et al. 2009) and its complete nucleotide sequence was determined to be 29,593 bp with a G+C content of 49.7% (GenBank accession number AY255829). The sequencing revealed that this plasmid was nearly identical to plasmid pEP36 and was closely related to the non-transferable plasmid pEA29 from *E. amylovora*. The annotated sequence of pEJ30 was very similar to that of pEP36 (in terms of conservation of ORFs and gene order). Major differences of pEJ30 compared to pEP36 include the presence of an additional ORF in pEJ30 (ORF EJ8), the absence of transposon Tn*5394*, which accounts for the difference in size of pEP36 (6 kb), and a slightly different insertion site and reversed orientation of the insertion sequence IS*285*.

Recently, the entire genome of another *E. pyrifoliae* strain was sequenced (Ejp617) along with the plasmids it contains (Park et al. 2011). Five plasmids were sequenced and the largest, pJE01 (30,866 bp; GenBank accession number CP002125), which contains 34 predicted ORFs was compared to pEJ30, and it was concluded that they were almost identical (Park et al. 2011).

Small plasmids

Several small plasmids have been found and sequenced by different authors (Rhim et al. 1999; McGhee et al. 2002; Park et al. 2011) from strain Ep1/96 (pEP2.6, pEP05 and pEP03) and strain Ejp617 (pJE02, pJE03, pJE04, and pJE05). The sequence of plasmid pEP2.6 indicated that it was a 2,590 bp ColE1-like plasmid, but in the strain DSM 12163^T this plasmid was slightly larger (2,610 bp). It also contained an ORF with 45% similarity to a *rom* gene found in *E. coli* plasmid pEC157 (GenBank accession number AF432497). Three additional hypothetical ORFs were located on pEP2.6, but none had similarity to the existing GenBank entries. The ColE1-related origin of replication probe of the pEP2.6 plasmid hybridised with plasmid DNA in some *E. pyrifoliae* strains, but failed to do so with the DNA from others, indicating that the presence of these plasmids is limited to certain isolates.

Plasmids pEP05 and pEP03 are 4,955 and 3,070 bp in size, respectively. They were also classified as ColE1-type plasmids based on the presence of an *oriV* origin of replication, as were plasmids pJE02 (5,296 bp) pJE03 (6,417 bp), pEJ04 (3,237 bp) and pJE05 (2,691 bp). Plasmid pJE02 contains six ORFs with one mobilisation protein A (*mobA*), whereas no GenBank BLAST hits were found in the cases of pJE03, pJE04, and pJE05. Plasmid pEP03 shares a region with plasmid pEP2.6. The rest of the plasmid contains a large region that is similar (78% identity) to a portion of plasmid pEP05 that encodes three ORF, two of which are hypothetical proteins and one encodes Hcp, a putative type VI secretion system (T6SS) effector protein (Smits et al. 2010).

The annotated sequences of *E. pyrifoliae* strain Ep1/96 plasmids are deposited in Genbank/EMBL/DDBJ under accession numbers FP928999 (pEp2.6), FP236827 (pEP03), FP236828 (pEP05), FP236829 and AY123045 (both pEP36) and the sequences from strain DSM 12163 plasmids in EMBL are: FN392239 (pEP5), FN392237 (pEP3) and FN392236 (pEP2.6).

The sequences of the plasmids pJE01, pJE02, pJE03, pJE04, and pJE05 from strain Ejp617 have been deposited in GenBank under accession numbers CP002125, CP002126, CP002127, CP002128, and CP002129, respectively.

Plasmids in *E. piriflorinigrans*

The information about the plasmid content in this species, first reported in 2002 (Roselló et al. 2002), is limited because only a plasmid screening of eight strains from this species has so far been performed. The plasmid pattern of these isolates generated only one band, larger than the 29 kb plasmid of *E. amylovora*, but similar in size to pEP36 of *E. pyrifoliae*. The plasmid was named pEPIR37 (Barbé et al. manuscript in preparation). One *E. piriflorinigrans* strain (CFBP5887) harboured two plasmids, one of ca. 36 kb and another of ca. 5 kb, named pEPIR5 (López et al. 2011). Restriction analyses were performed using the *Bam*HI restriction enzyme and the plasmid profiles obtained showed the same pattern for all the *E. piriflorinigrans* strains, but were different to those of pEA29 and pEP36. Sequencing and genetic analyses of these plasmids are currently being undertaken.

Plasmids in *E. tasmaniensis*

Only strain Et1/99 of *E. tasmaniensis* has been sequenced and annotated. Besides the 4 Mb chromosomal sequence, five plasmids were characterised, which encode gene functions for autonomous plasmid transfer, bacteriocin-like proteins and synthesis of antibiotic precursors (Kube et al. 2010). The plasmids were named pET09 (9,299 bp), pET35 (35,494 bp), pET45 (44,694 bp), pET46 (46,159 bp) and pET49 (48,751 bp) and they may play a role in fitness of the bacteria on plant surfaces. Plasmid pET45 harbours an *hns* gene in addition to the two chromosomal copies, while plasmid pET46 encodes klebicin-like proteins and a type IV pilus system. Several of these plasmids show the potential for conjugal transfer (pET35, pET45, pET46 and pET49). The GenBank accession numbers are: pET09 (CU468128), pET35 (CU468130), pET45 (CU468132), pET46 (CU468133), pET49 (CU468131). No data on screening of plasmid content in other strains of this species are available.

Plasmids in *E. billingiae*

Strain Eb661 of *E. billingiae* has been sequenced and annotated (Kube et al. 2010). The authors found two plasmids in this strain: pEB170 (169,778 bp, a G+C content of 52.3% and 220 ORF) and pEB102 (102,323 bp, 51.7% G+C and 114 ORF). Plasmid pEB170 shows the potential for conjugal transfer, whereas no mobilisable or transfer genes are harboured by pEB102. The GenBank accession numbers are FP236826 (pEB102) and FP236830 (pEB170). No additional information on plasmid content in other isolates is available.

Comparative analyses of plasmids of similar size

As observed in this brief description of the plasmid content in different *Erwinia* species, all pathogenic and non-pathogenic strains harbour plasmids of different sizes, present in variable numbers. Among the pathogenic species, one striking characteristic is the presence of a plasmid with a similar size of around 30 kb (plasmids pEA29 in *E. amylovora*, pEJ30 and pEP36 in *E. pyrifoliae*, and pEPIR37 in *E. piriflorinigrans*). All but pEPIR37 have been sequenced entirely (McGhee and Jones 2000; Maxson-Stein et al. 2003; Smits et al. 2010) and comparative sequence analyses found that similar numbers of common ORFs were present in approximately the same order and orientation (Fig. 3). Among the 21 putative ORFs conserved between pEJ30 and pEP36, 12 of these were also found in pEA29 from *E. amylovora* Ea88. The gene content of these plasmids is highly conserved, sharing *thiO* (glycine oxidase), *thiS* (sulphur carrier protein), *thiG* (thiazole synthase) and *thiF* (adenylyl transferase) in conserved order. These genes are also present in the pEPIR37 plasmid from *E. piriflorinigrans*, and were also found in the chromosome of *E. tasmaniensis*.

The following specific features shared by these plasmids of ca. 30 kb in *Erwinia* spp. have been reported:

- Short-sequence DNA repeats (SSR) conserved between pEJ30 and pEP36. The sequences of these SSR were different to those found in pEA29 (Jock et al. 2003) but the repeat ATTACAGA present in all *E. amylovora* strains was found to be reiterated 3–15 times in pEA29. The *E. pyrifoliae* strains from Japan contained the major repeat sequence GGATTCTG, which was reiterated 16–24 times in the pEJ30 plasmid. The sequence ATTACAGG, which resembles the SSR of *E. amylovora*, was reiterated two or three times in *E. pyrifoliae* strains from Japan, differing only in the terminal nucleotide (G instead of A) from the *E. amylovora* repeat sequence.
- A common *Bam*HI restriction site in plasmids pEJ30, pEP36 and pEA29 located at the beginning of the *repA* gene was highly similar in all three plasmids.
- The order and organisation of 21 presumptive genes in pEP36 and pEJ30 was also very similar.

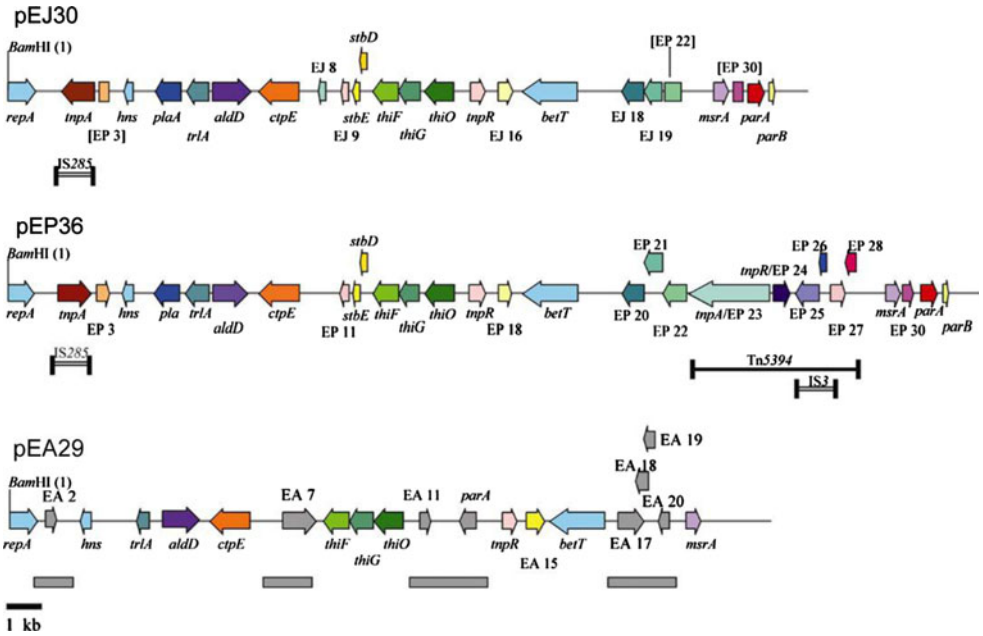

Fig. 3 Linear genetic maps for plasmid pEA29 from *Erwinia amylovora* Ea88 (accession no. AF264948), pEP36 from *E. pyrifoliae* Ep1/96 (AY123045), and pEJ30 from *E. pyrifoliae* strain Ejp556 (AY255829). A common *Bam*HI site was used as the origin for each map. Putative genes were inferred from sequence data in databases and *arrows* with similar colour indicate similar genes and their order. *Brackets* indicate pseudogenes. The *bottom row of boxes* indicate regions of pEA29 that were not detected in the other plasmids. Taken from: Genetic analysis of a pathogenic *Erwinia* sp. isolated from pear in Japan (Maxson-Stein et al. 2003)

- Plasmids pEJ30 and pEP36 showed several IS elements and transposons not found in pEA29. The transposon Tn*5394* was present in pEP36, but not in pEJ30.

The similarity between these ca. 30 kb plasmids from pathogenic pome fruit *Erwinia* raises the question as to whether these plasmids play the same function as pEA29 in *E. amylovora*. Thiamine genes are related to extracellular polysaccharide production and are involved in exacerbating the symptoms that pEA29 causes in *E. amylovora* (McGhee and Sundin 2008) and are also present in plasmids pEP36, pEJ30 and pEPIR37. Consequently, we could ask: do other plasmids elicit the same increase in symptoms development? To address this question, plasmids pEP36, pEJ30 and pEPIR37 should be introduced into *E. amylovora* pEA29 cured strains and inoculation assays performed to determine whether the *E. amylovora* strains with these plasmids show similar symptom development and incidence of disease as in the reference strains, and whether these are more extensive than the strains lacking pEA29. The results would demonstrate if these plasmids of ca. 30 kb from different species of *Erwinia* can have the same function in pathogenicity as pEA29, as suggested by their high genetic similarities.

Apart from the high similarity of plasmid genetic content and the possible similar functioning that these 30 kb plasmids could provide, the relationship observed among other medium and small plasmids in *Erwinia* species gives the impression of a common background shared among pear-associated *Erwinia* spp., both pathogenic and non-pathogenic. The high genetic identity between plasmids pEI70, from the pathogen *E. amylovora* and pEB102, from the epiphytic species *E. billingiae* indicates that lateral transfer of near entire extrachromosomic material could take place between species sharing host and niche (Llop et al. 2011). This genetic similarity among plasmids of other species may also be observed, supporting the mosaic nature and mobility of plasmid DNA. Analyses of *E. amylovora* plasmid pEA8.7 have shown that it was closely related or identical to a broad host-range plasmid RSF1010 found in many species of bacteria, but this was the first report of its presence in plant pathogenic bacteria (Palmer et al. 1997). Plasmid pEP2.6 from *E. pyrifoliae* strain Ep1/96 contained sequences found in small plasmids of *E. amylovora* strains IL-5 and IH3-1 (McGhee et al. 2002).

The SmR provided by pEA34 to some *E. amylovora* isolates is an example of how a particular trait can originate from and be extended by horizontal gene transfer to different plasmids. Thus, the first SmR strains reported contained both pEA34 and the almost ubiquitous non-conjugative plasmid, pEA29 (Chiou and Jones 1991). More recently, SmR strains in which Tn*5393* is present in pEA29 were observed, suggesting that the transposon could have moved via transposition from pEA34 to pEA29. However, almost all of the strains containing Tn*5393* in pEA29 had lost pEA34.

Plasmid pEA34 may have arisen from the insertion of a transposable element into an existing plasmid or by some *E. amylovora* strains acquiring a new plasmid (Chiou and Jones 1991). The possible origin and distribution of pEA34 has been studied (Chiou and Jones 1993) and a 34 kb plasmid resembling it was not reported among strains of *E. amylovora* examined for cryptic plasmids (Falkenstein et al. 1988; Steinberger et al. 1990). Finally, it was concluded that plasmid pEA34 could have originated from the

insertion of Tn5393 into a 28 kb plasmid (not described here), because such plasmid with DNA homologous to pEA34 was detected in a streptomycin-sensitive strain of *E. amylovora* (EL01). Hybridisation analyses indicated that the 28 kb plasmid and plasmid pEA34 were not related to pEA29 (Chiou and Jones 1993).

This research on the possible origin of genes for SmR, an important characteristic for the survival of the bacterium, and their exchange by several transfer systems is a perfect example of the ways in which genes disseminate through horizontal transfer. The donor strain could be a non-pathogenic species that has the same ecologic niche as the pathogenic one, as may be the case between plasmids pEB102 and pEI70, as explained above, or there can be a transfer between non-pathogenic and pathogenic isolates of the same species. As an example, the Tn*5393* transposon, involved in conferring SmR on *E. amylovora*, has been shown to be widely distributed among other SmR plant pathogens and is also carried by other bacteria from the phyllosphere (Chiou and Jones 1993; Sundin and Bender 1995). In addition, a number of enterobacterial species harbouring this transposon were isolated from apple flowers and leaves, with *P. agglomerans* being the most prevalent species. It has been hypothesised that *P. agglomerans* could be the source of the SmR plasmid pEA34, which was later acquired by *E. amylovora* (Chiou and Jones 1993).

Several of the plasmids reported above show the potential for conjugal transfer, such as pEI70 from *E. amylovora*, pEB170 of *E. billingiae*, pET35, pET45, pET46, pET49 of *E. tasmaniensis* and pEL60 of *E. amylovora* from Lebanon, and others carry *mob* genes and may contain an oriT to be mobilised by Tra proteins of other plasmids (plasmids pEP05 and pEt46 from *E. pyrifoliae* and *E. tasmaniensis*) (Kube et al. 2010). This indicates the wide variability of genetic interchange available in this genus, but it could also include genetic material from other genera sharing the same niches.

On the other hand, the importance of stable plasmids as epidemiological markers has only been explored in the case of pEI70 (Llop et al. 2011), but knowledge of stable and non-conjugative plasmids present in strains of one area could provide information about possible sources of inoculum.

Plasmid and evolution: hypotheses

Horizontal transfer is an important mechanism for genetic exchange. In this sense, the existence of the SSR array in the genome of *E. amylovora* and *E. pyrifoliae* could intervene in DNA replication (Bramhill and Kornberg 1988), DNA repair (Strand et al. 1993), and recombination, favouring genomic evolution, although this remains to be proved. The presence of near identical plasmids (pEI70 and pEB102) between pathogenic and epiphytic erwinias, or the close relationship of pEL60 with other enterobacterial IncL/M plasmids suggests that the plant pathogen *E. amylovora* can access the gene pools of other enteric bacteria through horizontal transfer. IncL/M plasmids are widespread among enteric pathogens, they typically encode resistance to multiple antibiotics, and they harbour mobile elements including integrons and IS elements associated with antibiotic-resistance genes (Prodinger et al. 1996; Villa et al. 2000).

Other plasmids, such as pEU30 and pEL60, may represent DNA that can spread efficiently with limited effects on the reproductive fitness of their bacterial host. Coexistence of pEU30 or pEL60 with their *E. amylovora* hosts could be a system for acquisition of novel genetic determinants that would improve the ecological fitness of bacteria (Foster et al. 2004).

The presence of IS elements in some derivatives of pEA29 and of transposon Tn*5394* in pEP36, also suggests that horizontal transfer has played a role in the evolution of these plasmids. The presence of such similar plasmids in different species from distant geographic origins having common hosts, which cause relatively similar symptoms, raises the following question: how did pEP36, pEJ30, pEPIR37 and pEA29 become established in their respective hosts? Although there is evidence of horizontal transfer of plasmids with genes for SmR in *E. amylovora* (Chiou and Jones 1995), horizontal transfer of pEA29 has not been proven, and no genes involved in conjugative transfer were found in either plasmid. A helper plasmid may have taken part in transfer of non-transferable plasmids, but until spontaneous horizontal transfer of pEPIR37, pEP36, pEJ30 and pEA29 can be demonstrated, the origin of pEA29-related plasmids in their hosts through horizontal transfer remains a conjecture (McGhee et al. 2002). As non-transferable plasmids, inherited vertically within a bacterial cell line, the evolution of this family of pEA29-related plasmids is suggestive of the evolution of these pathogens (Maxson-Stein et al. 2003).

Do pEA29 and other similar plasmids confer a fitness advantage on their host bacteria? The answer to this question is not yet known. Although plasmids may carry genes beneficial to their bacterial hosts, they may also reduce fitness. The introduction of pEA29 to several *E. amylovora* strains naturally lacking it appears to enhance the fitness of some, but not all, strains. In general, strains without pEA29 are substantially less aggressive than strains containing it (Mohammadi et al. 2009). However, some strains lacking pEA29 and having instead other plasmids, such as pEI70, or even strains without any plasmids can show similar virulence levels to isolates harbouring pEA29 (Llop et al. 2011). Since it has been

reported in *A. tumefaciens* that strains with several plasmids were more virulent than strains with a single plasmid (Ogawa and Mii 2001), it is tempting to wonder whether plasmids found in different strains of pathogenic *Erwinia* might also have an additive role in virulence.

It would also be interesting to determine if ancestral strains of *E. amylovora* harbour the pEA29 plasmid. It is intriguing to speculate that *E. amylovora* evolved by acquiring plasmids from other bacteria through horizontal gene transfer with subsequent selection-based deletion of the gene(s) for conjugal transfer. It is possible that the ancestral lines were plasmid-free and less aggressive to their host plants, and became more pathogenic by acquiring plasmid DNA from other microbial sources. Thus, plasmid-free strains may represent ancestral strains rather than a more recent lineage as suggested by Ochman et al. (2000).

The examination of other pEA29-related plasmids in strains of *E. pyrifoliae*, *E. piriflorinigrans* and in other *Erwinia* species, such as those recently described from Japan (Mizuno et al. 2010), and in the non-pathogenic species, will provide further insight into the evolution of these plasmids and their bacterial hosts.

Conclusions

The genus *Erwinia* includes plant-associated pathogenic and non-pathogenic species of *Enterobacteriaceae*, some of which have been reported in pome fruit trees. Important pathogens such as *E. amylovora*, the causative agent of fire blight of *Rosaceae*, *E. pyrifoliae* that causes bacterial shoot blight of Asian pear, and *E. piriflorinigrans* that causes necrosis on pear blossoms, belong to this genus. Other species such as *E. tasmaniensis* and *E. billingiae* are epiphytic bacteria and may be used as antagonists for fire blight biocontrol (Kube et al. 2010). All these species harbour plasmids of similar sizes in variable numbers, but although the role of the genetic information they contain is known for some of them, for many others it remains to be revealed. Plasmids in *Erwinia* species from pome fruits are very common, and further screenings of worldwide isolates will probably augment those so far described. Such screenings and the sequencing of more plasmids may reveal other possible features that they provide to the host bacteria, facilitating a better understanding of the epidemiology and life cycle of these pathogens, and possibly the genetic relationship between pathogenic and non-pathogenic species. Although there are still many cryptic plasmids, the data available suggest that the common background in the genes harboured by different plasmids and their genetic exchanges could explain the evolution and the presence of several new characteristics in some *Erwinia* strains.

Plasmids inherited vertically, and maintained as residents in the long-term should co-evolve with their bacterial hosts (Sundin 2007). Although the different species of pome fruit pathogenic erwinias seem to have distant geographic origins, *E. amylovora* being widespread but other species showing a restricted habitat until now, they elicit more or less similar symptoms in these pome fruit hosts and have a pool of plasmids that share high genetic similarity. The origin of these plant pathogenic bacteria, and the way they evolve from a possible epiphytic life to an increasingly aggressive pathogen is a very interesting subject. The studies on pome fruit pathogenic and non-pathogenic *Erwinia* spp., including their plasmids, could provide an insight into how an important pathogen such as *E. amylovora* evolves through its interactions with other bacteria. Whole genome sequence analyses of more strains, together with the use of advanced molecular tools, will elucidate the means of exchange of genetic material among different but related species.

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