REVIEW ARTICLE

The roles of plasmids in phytopathogenic bacteria: mobile arsenals?

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Overview

Plasmids are extrachromosomal elements of finite size, usually stably inherited within a bacterial cell line and potentially capable of transfer between strains, species or genera. The most widely used systems of grouping for plasmids rely on incompatibility between closely related replicons. This review surveys our current knowledge of plasmids among members of six Gram-negative genera of phytopathogenic bacteria (*Burkholderia*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Ralstonia*, *Xanthomonas*), but excludes *Agrobacterium*. A major aspect concerns the growing interest in the role of plasmids in pathogenicity and host specificity and the possible advantages of plasmid-borne locations for the genes involved. A range of plasmid-borne phenotypes, including toxin and hormone production, and resistance to bactericides, are reviewed. The role of mobile elements and their association with pathogenicity islands on plasmids and in the bacterial chromosome provide indicators of possible evolutionary routes to the acquisition of disease-inducing capabilities. The paucity of knowledge concerning almost all aspects of plasmid biology among phytopathogenic bacteria is implicit: we argue the need for more work in this somewhat neglected area, to provide a clearer understanding of the molecular strategies adopted by bacteria that enable them to cause disease and evade host defences among a wide range of important crop plants.

Background

Bacterial groups and scope of this review

It is over 10 years since Coplin (1989) published his comprehensive survey of plasmids in phytopathogenic bacteria. While this review remains an important source

for earlier work, it appears timely to revisit this relatively neglected area of microbiology. The development of PCR and the advent of relatively inexpensive DNA sequencing have contributed to a revival in interest about the precise contribution of plasmid-borne genes to the host–pathogen relationship. This review will focus on plasmids from bacteria among six genera (*Burkholderia*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Ralstonia* and *Xanthomonas*) that cause diseases in plants, but excludes *Agrobacterium* (see review by Kado, 1998). These pathogens cause a variety of symptoms in their plant hosts, ranging from soft rots through wilts, necroses and blights to knots and cankers, and some examples are shown in Table 1. Some genera contain species with complex subdivisions based largely on their host specificity, such as *Pseudomonas syringae*, which comprises some 51 pathovars (Young *et al.*, 1996) and the genus *Xanthomonas*, which comprises 20 groups identified by DNA–DNA hybridization (Vauterin *et al.*, 1995). Many recent nomenclatural changes have created a taxonomic minefield for those unfamiliar with this field of bacteriology; for example, the wilt pathogen *Ralstonia solanacearum* was previously *Pseudomonas* and *Burkholderia*, before assuming its current name (Yabuuchi *et al.*, 1995).

Plant-pathogenic bacteria were for many years regarded as essentially opportunists, entering plants via wounds and natural openings such as stomata, and multiplying in the intercellular spaces within plant tissues. Resistance in plants was associated with a form of localized, programmed host cell death at the site of infection termed the hypersensitive reaction (HR), which restricted the ability of the pathogen to survive and multiply (Richberg *et al.*, 1998). However, recent experimental results have indicated that the relationships between host and pathogen are much more intimate and subtle (Alfano & Collmer, 1997; Anderson *et al.*, 1999).

This review explains briefly how bacterial plasmids are

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Table 1. Symptoms associated with phytopathogenic bacterial groups

Symptom	Bacterium	Host plant	Plasmid traits
Blights, cankers, knots	<i>Ps. syringae</i> pathovars	Pathovar-specific ranges	<i>avr</i> , <i>vir</i> , coronatine, Sm ^r , Tc ^r , Cu ^r , UV ^r , hormones
Blights	<i>X. campestris</i> pathovars	Pepper, cotton and other hosts	<i>avr</i> genes, Cu ^r , Sm ^r , Tc ^r
Galls	<i>Er. herbicola</i> pv. <i>gypsophila</i>	<i>Gypsophila</i>	<i>hrp</i> and pathogenicity genes, hormones
Necrosis	<i>Er. amylovora</i>	Apple, pear and related ornamentals	Thiamin and EPS biosynthesis
Tissue soft rot	<i>B. cepacia</i>	Onion	Endopolygalacturonase production
Tissue soft rot of storage organs	<i>Er. carotovora</i>	Wide range, including potato	Cryptic
Wilt	<i>R. solanacearum</i>	Potato, tomato, banana, aubergine	Virulence
Wilt	<i>Pa. stewartii</i>	Corn	Cryptic

classified into groups and considers the relevance of these systems for plasmids from plant-pathogenic bacteria. The roles of plasmids found in plant-pathogenic bacteria are considered in terms of the phenotypes they confer on their host cells. These include pathogenicity and host specificity, toxin and hormone production, and resistance to bactericides such as copper and antibiotics, and to UV irradiation. We briefly review mobile elements associated with plasmids and the implications of these for evolving host-pathogen relationships. Attention is devoted to the pPT23A family of plasmids, which constitute the largest and best-studied type of plasmid found among these phytopathogenic bacteria. The review concludes by listing a number of areas where our knowledge concerning plasmids remains strictly limited and in need of renewed scientific support and effort.

Typing systems for plasmids

Plasmids were first discovered in enteric bacteria and from the late 1950s onwards were increasingly recorded in relation to antibiotic resistance (Datta & Hughes, 1983; Hughes & Datta, 1983). Typing systems were developed for plasmids, based on the observation that replicons sharing common replication functions were unable to stably co-exist, as independent plasmids, in the same cell: in general, when selection for the acquisition of a second plasmid was applied to the culture, the related replicon was either displaced or recombined with the incoming plasmid to form a cointegrate. In this way, systems which assigned plasmids to different incompatibility groups, were developed for *Escherichia coli* (groups assigned alphabetical letters; Hedges, 1974; Bukhari *et al.*, 1977) and *Pseudomonas aeruginosa* (P1–P23; Jacoby, 1977). These systems have proved to be functionally robust for medically related groups of bacteria, but there are indications that they may be less appropriate for plant-related bacteria. For example, among *Ps. syringae*, for which the most evidence is available, the commonest

replicon type appears to be the recently described pPT23A family (Gibbon *et al.*, 1999), which show similarities to the *Es. coli* ColE2-type plasmids. Related members of this famiFCly stably co-exist in the same strain and thus require direct identification of the replicon type by sequence analysis or hybridization. Currently, very little is known about the range and types of replicon in phytopathogenic bacteria, including the groups covered by this review.

In the case of *Erwinia*, since members of this genus are closely related to other enteric bacteria there is the potential to base replicon typing on the current systems for *Es. coli* (Couturier *et al.*, 1988). However, the cryptic plasmid (pCPP60) identified in *Erwinia amylovora* strain Ea322, while showing strong hybridization to IncFII and IncFIV replicons, and producing pili for F-plasmid-specific phages, did not show incompatibility with any IncF replicons tested (Steinberger *et al.*, 1990).

A small cryptic replicon pPS10 from *Pseudomonas savastanoi* pv. *savastanoi* (previously known as *Ps. syringae* pv. *savastanoi*; Young *et al.*, 1996) has been intensively used to study replication using a minimal replicon of 1267 bp, derived from the parent plasmid (Nieto *et al.*, 1990), but little use seems to have been made of this in plant-related studies of the original host bacterium.

Plasmids that have been identified from the six plant-related genera are shown in Table 2.

Roles of plasmids

Pathogenicity and host specificity

The genes involved in pathogenicity and host specificity comprise two main groups, those termed avirulence (*avr*) and virulence (*vir*) genes (Vivian & Gibbon, 1997; Vivian *et al.*, 1997; Vivian & Arnold, 2000), and those involved with a type III protein secretion system, the 'harp' (*hrc/hrp*) genes (Bogdanove *et al.*, 1996; Lee, 1997; Galan & Collmer, 1999). The latter group of

Table 2. Plasmids found in Gram-negative phytopathogenic bacteria

Pathogen*	Designation	Size (kb)	Replicon type	Phenotype/genotype/comments	Reference
<i>B. cepacia</i> Strain ATCC 25416	pPEC320	200	NK	<i>pehA</i> endoglucanase	González <i>et al.</i> (1997)
<i>Er. amylovora</i> Strain EA322	pCPP60	56	IncF	Cryptic, low copy, conjugative	Steinberger <i>et al.</i> (1990)
Strain CA11	pEa34	34	NK	pEa29 carrying Sm ^r on Tn5393; conjugative	Chiou & Jones (1993)
Strain CA11	pEa29	28	NK	Loss results in thiamin prototrophy, reduced EPS and pathogenicity	Bereswill <i>et al.</i> (1992); McGhee & Jones (2000)
Strain CA3R	pEa8.7	8.7	IncQ	Su ^r , Sm ^r ; related to RSF1010	Palmer <i>et al.</i> (1997)
<i>Er. carotovora</i> pv. <i>carotovora</i>	pEC3	5.8	NK	Cryptic, multicopy, non-conjugative, mobilizable by incP plasmids	Nomura <i>et al.</i> (1996)
<i>Er. chrysanthemi</i> Strain NCPPB 377	pLS1	50	NK	Cryptic	Sparks & Lacy (1980)
Strain NCPPB 377	pLS2	4.8	NK	Cryptic	Sparks & Lacy (1980)
<i>Er. citreus</i> Strain ATCC 31623	pPZG500	3.8	NK	Cryptic, multicopy	Bilic & Delic (1997)
<i>Er. herbicola</i> pv. <i>gypsophilae</i>	pPATH	150	NK	Gall-formation; <i>etz</i> , <i>hrp</i> , <i>iaa</i> and <i>pthG</i> genes, IS1327	Lichter <i>et al.</i> (1995a); Ezra <i>et al.</i> (2000)
<i>Pa. agglomerans</i>	ND	50–126	NK	Sm ^r ; associated with <i>Er. amylovora</i> , but transfer not detected	Huang & Burr (1999)
<i>Pa. citrea</i>	pUCD5000	5.3	NK	Related to p15a and ColE1; contributes to pink coloration in pineapple	Pujol & Kado (1998)
<i>Pa. stewartii</i> Strain SS104	pDC140	34	pDC250	Cryptic	Coplin <i>et al.</i> (1985)
Strain SS104	pDC190	68	NK	Not involved in pathogenicity	Frederick & Coplin (1986)
Strain SW2	pDC250	52	pDC250	Cryptic, conjugative; designated pSW800	Coplin <i>et al.</i> (1985)
Strain SW2	pSW100	4.3	ColE1	Cryptic, mobilizable by pDC250	Fu <i>et al.</i> (1995)
Strain SW2	pSW200	4.4	ColE1	Cryptic, <i>mobCBAD</i> , 41 × 15 bp repeats	Fu <i>et al.</i> (1998)
Strain SW2	pSW500	35	NK	Cryptic, iterons, 7 × 16 bp repeats	Fu <i>et al.</i> (1996)
Strain SW2	pSW1200	106	IncY	Cryptic, RepA related to phage P1	Fu <i>et al.</i> (1997)
<i>Pseudomonas</i> spp. strain PyR19	pCPP519	83	pPT23A	<i>strA</i> , <i>strB</i> ; conjugative	Huang & Burr (1999)
<i>Ps. savastanoi</i> pv. <i>glycinea</i> Strain PG4180	p4180A	90	pPT23A	Coronatine	Bender <i>et al.</i> (1991)
Strain Pg83	pPg2	40	NK	Tp ^r	Leary & Trollinger (1985)
Strain MAFF301683	pETH2	105	NK	Ethylene	Watanabe <i>et al.</i> (1998)
<i>Ps. savastanoi</i> pv. <i>phaseolicola</i> Strain 1302A	pAV505	140	pPT23A	Curing results in loss of pathogenicity	Jackson (1997)
Strain 1449B	pAV511	154	pPT23A	Curing results in loss of pathogenicity; contains PAI with <i>vir</i> and <i>avr</i> genes	Jackson <i>et al.</i> (1999)
Strain 1449B	pAV512	32	pPT23A	Cryptic	R. W. Jackson, unpublished
Strain LR700	pMMC7105	150	NK	Integration/excision into host chromosome via repeat sequences; can form smaller plasmids	Quant & Mills (1984); Szabo & Mills (1984a, b)

Table 2 (cont.)

Pathogen*	Designation	Size (kb)	Replicon type	Phenotype/genotype/comments	Reference
<i>Ps. savastanoi</i> pv. <i>phaseolicola</i> kudzu					
Strain PK2	pPSP1	68	NK	Ethylene	Fukuda <i>et al.</i> (1992)
<i>Ps. savastanoi</i> pv. <i>savastanoi</i>					
Strain PS93	pPS10	10	NK	Cryptic, minimum replicon (1267 bp), iterons present, used to study <i>rep</i> function	Nieto <i>et al.</i> (1990)
Strain EW2009	pIAA1	52	NK	Indoleacetic acid; oleander pathogen	Comai & Kosuge (1980)
Strain PB213	pIAA2	72	NK	Indoleacetic acid; oleander pathogen	Soby <i>et al.</i> (1994)
Strain PB213	pCK1	42	NK	Cytokinin; oleander pathogen	MacDonald <i>et al.</i> (1986)
Strain EW1006	pCK2	105	NK	Cytokinin; olive pathogen	MacDonald <i>et al.</i> (1986); Powell & Morris (1986)
Strain NA1	ND	44	NK	Cytokinin; oleander pathogen	Caponero <i>et al.</i> (1995)
Strain OC17	ND	73	NK	Cytokinin; olive pathogen	Caponero <i>et al.</i> (1995)
Strain OD21	ND	97	NK	Cytokinin; indoleacetic acid; olive pathogen	Caponero <i>et al.</i> (1995)
<i>Ps. syringae</i> pv. <i>atropurpurea</i>					
Strain NIAES 1309	pCOR1	88	NK	Coronatine; conjugative	Sato <i>et al.</i> (1989)
<i>Ps. syringae</i> pv. <i>cannabina</i>					
Strain MAFF 302256	pETH1	110	NK	Ethylene	Sato <i>et al.</i> (1997)
<i>Ps. syringae</i> pv. <i>eriobotryae</i>					
Strain NAE6	ND	82	NK	Curing results in loss of pathogenicity; contains <i>vir</i> gene <i>psvA</i>	Kamiuntun (1995, 1999)
Strain NAE6	ND	94.5	NK	Cryptic, curing does not affect pathogenicity	Kamiuntun (1995)
<i>Ps. syringae</i> pv. <i>maculicola</i>					
Strain H3-6	pMAC1	83	NK	Coronatine	Zhu <i>et al.</i> (1995)
<i>Ps. syringae</i> pv. <i>morsprunorum</i>	ND	105	NK	Coronatine	Bender <i>et al.</i> (1991); Liang <i>et al.</i> (1994)
<i>Ps. syringae</i> pv. <i>papulans</i>					
Strain Psp36	pCPP501	108	NK	Sm ^r ; conjugative	Burr <i>et al.</i> (1988)
Strain Psp 47	PCPP511	89	NK	Sm ^r ; conjugative	Huang & Burr (1999)
<i>Ps. syringae</i> pv. <i>syringae</i>					
Strain A2	pPSR1	68	pPT23A	Cu ^r , Sm ^r , <i>rulAB</i> (UV ^r)	Sundin & Bender (1993)
Strain B3010	pPSR12	200	pPT23A	Cu ^r , Co ^r , As ^r ; confers stable mucoid colony form	Kidambi <i>et al.</i> (1995)
Strain J900	pOSU900	80	pPT23A	Cryptic, loss does not affect pathogenicity toward bean	Mukhopadhyay <i>et al.</i> (1990)
	pOSU221	8.2	Shuttle/pUC	Ap ^r based on pOSU900	Mukhopadhyay <i>et al.</i> (1990)
	pOSU222	9.7	Shuttle/pUC	Km ^r based on pOSU900	Mukhopadhyay <i>et al.</i> (1990)
Strain HS191	pCG131	55	pPT23A	Conjugative; may contribute to virulence	González <i>et al.</i> (1984)
<i>Ps. syringae</i> pv. <i>tabaci</i>					
Strain BR2	pBPW1	48	NK	Cryptic, loss does not affect pathogenicity toward bean	Obukowicz & Shaw (1983, 1985)
<i>Ps. syringae</i> pv. <i>tomato</i>					
Strain DC3000	pDC3000A	64	pPT23A	<i>vir</i> genes	G. Tsiamis, personal communication; Sesma <i>et al.</i> (2000)

Table 2 (cont.)

Pathogen*	Designation	Size (kb)	Replicon type	Phenotype/genotype/comments	Reference
Strain PT23	pPT23A	101	pPT23A	Coronatine; conjugative; affects lesion size and <i>in planta</i> growth 4 d post-infection	Bender <i>et al.</i> (1989)
Strain PT23	pPT23B	83	pPT23A	Syringolide elicitors; affects lesion size and <i>in planta</i> growth 4 d post-infection	Sesma <i>et al.</i> (2001)
Strain PT23	pPT23C	65	NK	Cryptic	Bender & Cooksey (1986)
Strain PT23	pPT23D	35	pPT23A	Cu ^r ; non-conjugative	Cooksey (1987); Mills <i>et al.</i> (1993)
<i>R. solanacearum</i>					
Strain M4S	pJTSP1	6.6	NK	Low copy confers loss of pathogenicity and reduced EPS and endoglucanase production	Negishi <i>et al.</i> (1993)
Strain GMI1000	pVir	~ 2030	Mega-plasmid	<i>hrp</i> and <i>dsp</i> genes	Boucher <i>et al.</i> (1986); C. A. Boucher, personal communication
Strain mps5	pWI5	7.5	NK	Cryptic	Morales & Sequeira (1985)
<i>X. campestris</i> pv. <i>vesicatoria</i>					
Race 2 strain 81-23	pXvCu1	200	NK	Sm ^r , Cu ^r , <i>avrBs1</i> , conjugative	Stall <i>et al.</i> (1986); Swanson <i>et al.</i> (1988)
Strain Xv2	pXV2	14.6	IncW	Cryptic, non-conjugative	Wu & Tseng (2000)
Race 1 strain 71-21	pXV11	45	NK	<i>avrBs3</i> , conjugative	Bonas <i>et al.</i> (1989)
Race 1 strain 82-8	pXV12	ND	NK	<i>avrBs3-2</i>	Bonas <i>et al.</i> (1993)
	ND	68	NK	Sm ^r	Minsavage <i>et al.</i> (1990a)

ND, No designation given.

NK, Not known.

* Strains given where known.

genes, with two notable exceptions, which will be described later, are chromosomally encoded (Lindgren, 1997; Nizan *et al.*, 1997). It appears that the type III secretion system, also present in many animal pathogens, determines the production of a pilus-like structure, which is thought to deliver certain protein products, including Avr proteins, inside plant cells (Roine *et al.*, 1997; Taira *et al.*, 1999). In contrast, those *avr* genes that have been described are evenly divided between plasmid and chromosomal locations (Vivian & Gibbon, 1997). Avirulence genes were identified as conferring the ability to induce an HR in plant hosts that carry a matching gene for resistance (*R*), the so-called gene-for-gene theory, based on the original work of Flor (1971).

Distinct races, which differ in their abilities to cause disease in cultivated varieties (termed cultivars) of crop plants, have been identified for certain pathovars of *Ps. syringae* and *Xanthomonas campestris*. For example, nine races of *Ps. savastanoi* pv. *phaseolicola*, the cause of bacterial halo blight of bean (*Phaseolus* spp.), can be distinguished on the basis of their differential interactions with eight bean cultivars. From the pattern of

susceptible and resistant reactions, it has been deduced that six pairs of matching *avr* and *R* genes are responsible (Taylor *et al.*, 1996). As long as one pair of *avr/R* genes matches in pathogen and host, an HR results, while in the absence of this matching, disease is the outcome.

A substantial number of genes involved in pathogenicity and host specificity that have been assigned to plasmids in particular strains are shown in Table 3; some of these are also found as homologues in the chromosome of other strains. These include *avr* genes involved in the determination of gene-for-gene host specificity. Some of them may also be involved, together with *hrp* and *vir* genes and genes for extracellular enzyme production, in pathogenicity. Here we will briefly consider some examples, which strengthen the view that plasmids, with their potential for inter-strain mobility, play an important role in relation to the bacterial interaction with plants.

As already mentioned in the Background, phytopathogenic bacteria have a specialized secretion system, involved in delivery of proteins to plant cells, termed the

Table 3. Plasmid-borne effector genes

Pathogen	Effector gene	Plant interactor*	Plasmid	Plasmid size (kb)	Reference
<i>Ps. savastanoi</i> pv. <i>glycinea</i>	<i>avrC.RPG3</i>	Soybean	ND	150–200	Tamaki <i>et al.</i> (1988)
<i>Ps. savastanoi</i> pv. <i>phaseolicola</i>	<i>avrPphD</i>	Pea	pAV505	140	Wood <i>et al.</i> (1994); Arnold <i>et al.</i> (2001a)
	<i>avrPphF.R1</i>	Bean, soybean	pAV511	154	Jackson <i>et al.</i> (1999); Tsiamis <i>et al.</i> (2000)
	<i>avrPphC</i>	Soybean, bean	pAV511	154	Yucel <i>et al.</i> (1994b); Tsiamis <i>et al.</i> (2000)
	<i>avrD5</i>	Soybean	pAV511	154	Yucel <i>et al.</i> (1994a); Jackson <i>et al.</i> (1999)
	<i>virPphA</i>	Bean, soybean	pAV511	154	Jackson <i>et al.</i> (1999)
<i>Ps. syringae</i> pv. <i>eriobotryae</i>	<i>psvA</i>	Loquat	ND	82	Kamiunten (1999)
<i>Ps. syringae</i> pv. <i>lachrymans</i>	<i>avrD3</i>	Soybean	ND	90	Yucel <i>et al.</i> (1994a)
	<i>avrD4</i>	Soybean	ND	75	Yucel <i>et al.</i> (1994a)
<i>Ps. syringae</i> pv. <i>maculicola</i>	<i>avrRpm1.RPM1</i>	Arabidopsis	ND	NA	Dangl <i>et al.</i> (1992)
<i>Ps. syringae</i> pv. <i>pisi</i>					
Race 7	<i>avrPpiA2.R2</i>	Pea	pAV382	47	Gibbon <i>et al.</i> (1997)
Race 5	<i>avrPpiA3.R2</i>	Pea	pAV251	45	Gibbon <i>et al.</i> (1997)
Race 3	<i>avrPpiB1.R3</i>	Pea	pAV232	40	Cournoyer <i>et al.</i> (1995)
Race 1	<i>avrPpiB2.R3</i>	Pea	pAV212	66	Cournoyer <i>et al.</i> (1995)
Race 7	<i>avrPpiB3.R3</i>	Pea	pAV381	66	Cournoyer <i>et al.</i> (1995)
Race 1	<i>avrPpiE.RPS4</i>	Arabidopsis	ND	NA	Hinsch & Staskawicz (1996)
Race 4A	<i>avrPpiG</i>	Bean	pAV241	45	Arnold <i>et al.</i> (2001b)
<i>Ps. syringae</i> pv. <i>tomato</i>	<i>avrD1.RPG4</i>	Soybean	pPT23B	83	Kobayashi <i>et al.</i> (1990)
<i>X. campestris</i> pv. <i>vesicatoria</i>	<i>avrBsT</i>	Pepper	ND	41	Minsavage <i>et al.</i> (1990b)
Race 2 strain 81-23	<i>avrBs1</i>	Pepper	pXvCu1	200	Swanson <i>et al.</i> (1988)
Race 1 strain 71-21	<i>avrBs3</i>	Pepper	pXV11	45	Bonas <i>et al.</i> (1989)
Race 1 strain 82-8	<i>avrBs3-2</i>	Tomato	pXV12	NA	Bonas <i>et al.</i> (1993)
<i>X. campestris</i> pv. <i>malvacearum</i>					
Strain XcmH	<i>avrb6</i> plus 5 other <i>avr</i> genes	Cotton	pXcmH	90	De Feyter & Gabriel (1991); Yang <i>et al.</i> (1994, 1996)

ND, Not designated.

NA, Not available.

* Plant in which the gene has an effect.

type III system. The cluster of genes involved in this pathway is chromosomally located in *Ps. syringae*, *X. campestris* and *Er. amylovora*; however, in *Erwinia herbicola* pv. *gypsophilae* the *hrp* genes are on a 150 kb plasmid, pPATH (Lichter *et al.*, 1995a; Nizan *et al.*, 1997) and in *R. solanacearum* these genes are on a megaplasmid, pVir (previously designated pWi; Boucher *et al.*, 1986). It is worth noting that there are two phylogenetically distinct sets of *hrp* genes, one found in *R. solanacearum* and *X. campestris*, and the other found in *Ps. syringae* and *Er. amylovora*; the plasmid-borne counterparts in *R. solanacearum* and *Er. herbicola* pv. *gypsophilae* are similar to their respective chromosomal homologues.

The type III systems serve to deliver effector proteins into plant cells and these include both *avr* and *vir* gene products. Until recently no clearly defined virulence role

had been assigned to *avr* gene products in plant pathogens and the only 'virulence' genes identified were for those specifying extracellular enzymes, plant hormones and toxins (see below). Attempts to identify *vir* genes by mutagenesis of the pathogen, followed by screening for loss of pathogenicity in a compatible host plant, almost invariably resulted in the isolation of *hrp* mutants (Boucher *et al.*, 1985; Niepold *et al.*, 1985; Lindgren *et al.*, 1986; Malik *et al.*, 1987). A number of *avr* genes were shown to confer varying degrees of fitness in their interaction with the plant, but none showed clear loss of pathogenicity when mutated (Lorang *et al.*, 1994; Ritter & Dangl, 1995). Some early indications that the failure to obtain single gene mutations affecting pathogenicity alone, as opposed to *hrp* genes, were provided by Yang *et al.* (1996), who showed that, in the cotton (*Gossypium* spp.) pathogen *X. campestris* pv. *malvacearum*, a number of plasmid-

borne *avr* genes together redundantly encoded the ability to cause watersoaking in the host plant.

An early report of plasmid involvement in host-specific pathogenicity genes in *Ps. syringae* pv. *pisi* by Mazarei & Kerr (1991) was based on superficial evidence and has not yet been confirmed among the type races. In contrast, there is convincing evidence available from the halo-blight pathogen of bean, *Ps. savastanoi* pv. *phaseolicola*, which comprises nine races, eight of which harbour large plasmids of about 150 kb. Curing of one of these plasmids, pAV511, from a race 7 strain resulted in the loss of virulence toward bean cultivars. The cured strains, however, continued to elicit an HR in bean, implying that the chromosomal type III system remained functional and was delivering some signal to the plant that resulted in the elicitation of a defence reaction. Characterization of the region on pAV511 responsible for virulence led to the identification of a low G+C, 30 kb pathogenicity island (PAI), comprising several previously identified *avr* genes, together with a number of potential *vir* genes and sequences resembling mobile elements (Jackson *et al.*, 1999).

One of the potential *vir* genes, designated *virPphA*, when introduced into the cured race 7 strain RW60 was shown to restore virulence to bean, while conferring avirulence to certain soybean (*Glycine max*) cultivars. This, together with the common regulatory features shown by *virPphA* and *avr* genes in *Ps. syringae*, implies that this gene can be regarded as a virulence gene for bean and that the soybean is capable of recognizing its product. Recently, a gene showing dual *avr/vir* gene behaviour was isolated from the pPATH plasmid of *Er. herbicola* pv. *gypsophilae* (Ezra *et al.*, 2000).

A similar duality of function has been shown for a second gene (*avrPphF*) from the PAI on pAV511: *AvrPphF* acts as a virulence factor for bean cv. Tendergreen, an avirulence determinant for bean cv. Red Mexican (which carries the matching *R1* resistance gene), but enhances the HR seen in the cured race 7 strain on the bean cv. Canadian Wonder. A third gene from pAV511, *avrPphC*, which controls avirulence toward soybean, blocks the activity of *avrPphF* in cv. Canadian Wonder, but not that in cv. Red Mexican (Tsiamis *et al.*, 2000). Thus, the fortuitous curing of a single large native plasmid in the bean pathogen has provided significant insights into the complexity of interactions between pathogen genes and a range of host plants.

Curing of an approximately 82 kb native plasmid by heat shock (32 °C) from a strain of *Ps. syringae* pv. *erobotryae*, the causal agent of stem canker of loquat (*Eriobotrya japonica*) led to loss of pathogenicity on the host plant (Kamiunten, 1995). A single gene, *psvA*, was capable of restoring pathogenicity. The gene, with a low % G+C and potential *hrpL* promoter sequence, showed no similarity to other genes in the databases, except for a small portion of the N terminus of the putative protein product, with similarity to *AvrA* (Kamiunten, 1999). Like *virPphA*, *psvA* is flanked by a gene with similarity

to transposases and comparison of the DNA flanking *psvA* shows there to be further similarity in the non-coding regions (J. Murillo, R. W. Jackson & A. Vivian, unpublished). An important question regarding the evolution of host specificity is whether there are mechanisms that facilitate the acquisition and/or exchange of *vir* genes at common target sites in the bacterial genome; this topic is addressed later in the section on mobility of DNA sequences.

Race change is observed when a plant pathogen extends its host range as a result of the loss or inactivation of an avirulence determinant. Race change due to loss of a plasmid carrying *avrPpiB* was detected in *Ps. syringae* pv. *pisi* (Bavage *et al.*, 1991), while a similar plasmid loss from race 1 strains of *X. campestris* pv. *vesicatoria* involving *avrBs3* resulted in extension of host range (Kousik & Ritchie, 1996). A more recent report of race change in *Ps. savastanoi* pv. *phaseolicola* found a > 40 kb region of DNA, containing *avrPphB* (Jenner *et al.*, 1991), that appeared to have inserted and then excised from a tRNA-lysine gene (Jackson *et al.*, 2000). Analysis of the left junction revealed homology with plasmid *rep* and *par* genes and an unknown plasmid gene from the human periodontal pathogen *Actinobacillus actinomycetemcomitans*. Kiewitz *et al.* (2000) simultaneously reported an almost identical phenomenon in clinical isolates of *Ps. aeruginosa*, involving a chromosomal insertion/excision of a 106 kb plasmid. Further analysis of the *Ps. savastanoi* pv. *phaseolicola* deletion should help to elucidate whether the region containing *avrPphB* also has the potential to exist as an independent plasmid.

Many plant pathogens, particularly the soft-rot pathogen *Erwinia carotovora* and some pathovars of *X. campestris*, secrete extracellular enzymes, which are specified by chromosomal genes. However, in the pathogen *Burkholderia cepacia*, particular strains appear to be adapted to different environments: some (BCP) causing bulb-rot of onion (*Allium cepa*), others (BCC) opportunistic human infections and others (BCS) colonizing soil. Production of an endopolygalacturonase in a BCP strain was shown to be due to a gene, *pehA*, located on a 200 kb plasmid. Mobilization of this plasmid to other BCP strains conferred enhanced maceration of onion, while transfer to a BCC strain conferred some slight ability to macerate onion (González *et al.*, 1997).

Toxins

Phytopathogenic bacteria produce a variety of toxins that affect the host plant, often causing chlorosis and stunting. The genetic determinants for one of these, coronatine, are generally located on plasmids, as shown in Table 4. However, among a majority of strains of *Ps. syringae* pv. *maculicola* examined, the genes were chromosomal (Cuppels & Ainsworth, 1995). The toxin is a polyketide, coronafacic acid, coupled by an amide bond to a cyclopropyl amino acid, coronamic acid. Production is thermoregulated, in a manner consistent with its expression during plant infection, which is

Table 4. Plasmid-borne coronatine genes in *Ps. syringae* and *Ps. savastanoi*

Pathovar*	Strain	Plasmid	Reference
<i>glycinea</i>	PG4180	p4180A	Bender <i>et al.</i> (1991, 1993)
<i>atropurpurea</i>	NIAES 1304 (= PA1304)	pCOR1	Sato <i>et al.</i> (1983); Bender <i>et al.</i> (1991)
<i>maculicola</i>	H3-6	pMAC1	Zhu <i>et al.</i> (1995)
<i>maculicola</i>	ICMP4981	ND	Cuppels & Ainsworth (1995)
<i>morsprunorum</i>	(Various)	ND	Bender <i>et al.</i> (1991); Liang <i>et al.</i> (1994)
<i>tomato</i>	PT23.2	pPT23A	Bender <i>et al.</i> (1987)

ND, Not designated.

* *glycinea*, pathovar of *Ps. savastanoi*; others, pathovars of *Ps. syringae*.

Table 5. Plasmid-borne genes for plant hormone production

Gene	Pathogen	Plasmid	Hormone	Reference
<i>efe</i>	<i>Ps. savastanoi</i> pv. <i>glycinea</i>	pETH2	Ethylene	Watanabe <i>et al.</i> (1998)
	<i>Ps. savastanoi</i> pv. <i>phaseolicola</i>	pPSP1	Ethylene	Fukuda <i>et al.</i> (1992)
	<i>Ps. syringae</i> pv. <i>cannabina</i>	pETH1	Ethylene	Sato <i>et al.</i> (1997)
<i>etz</i>	<i>Er. herbicola</i> pv. <i>gypsophilae</i>	pPATH	Cytokinin	Lichter <i>et al.</i> (1995a)
<i>iaaH</i>	<i>Er. herbicola</i> pv. <i>gypsophilae</i>	pPATH	IAA	Clark <i>et al.</i> (1993)
	<i>Ps. savastanoi</i> pv. <i>savastanoi</i>	pIAA2	IAA	Comai <i>et al.</i> (1982); Yamada <i>et al.</i> (1986)
<i>iaaL</i>	<i>Ps. savastanoi</i> pv. <i>savastanoi</i>	pIAA1	IAA	Glass & Kosuge (1986)
<i>iaaM</i>	<i>Er. herbicola</i> pv. <i>gypsophilae</i>	pPATH	IAA	Clark <i>et al.</i> (1993)
	<i>Ps. savastanoi</i> pv. <i>savastanoi</i>	pIAA2	IAA	Comai <i>et al.</i> (1982); Yamada <i>et al.</i> (1986)
<i>ipt</i>	<i>Ps. savastanoi</i> pv. <i>savastanoi</i>	ND	Cytokinin	Caponero <i>et al.</i> (1995)
<i>ptz (ipt)</i>	<i>Ps. savastanoi</i> pv. <i>savastanoi</i>	pCK2*	Cytokinin	MacDonald <i>et al.</i> (1986)

ND, Not designated (detected on a plasmid of 44 kb in oleander, and on plasmids ranging from 35 to 97 kb in olive strains).

* Described as pCK1 by Powell & Morris (1986), but as pCK2 by MacDonald *et al.* (1986).

optimal at 18 °C (Palmer & Bender, 1993; Ullrich & Bender, 1994).

In most strains of *Ps. savastanoi* pv. *glycinea* and *Ps. syringae* pv. *tomato*, coronatine production is specified by a 30 kb *cor* gene cluster located on large 90–100 kb plasmids (Bender *et al.*, 1996). Interestingly, plasmid-borne *cor* genes in *Ps. syringae* were always associated with strains capable of utilizing sorbitol (Cuppels & Ainsworth, 1995). Coronatine is a virulence factor, enabling producer strains to form larger lesions and develop higher *in planta* populations; in this way it appears to confer a selective advantage in the natural habitat of these bacteria (Bender *et al.*, 1987; Mittal & Davis, 1995). It was recently shown that the coronafacic acid gene cluster (located on plasmid pPT23A) in *Ps. syringae* pv. *tomato* PT23 was required for the expression of unknown virulence determinants on the closely related plasmid pPT23B, offering a possible explanation for the maintenance of this gene cluster prior to its incorporation into the coronatine gene cluster (Sesma *et al.*, 2001).

Hormones

It is perhaps not surprising that phytopathogenic bacteria have acquired the ability to produce certain plant hormones and examples are listed in Table 5. Indoleacetic acid (IAA) is a plant growth regulator that affects cell proliferation. In bacteria it is synthesized via indoleacetamide and the genes involved are found on the T-DNA of *Agrobacterium tumefaciens*, the cause of crown gall disease in many dicotyledonous plants. In this case, the bacteria make use of the plant host to manufacture this compound as a nutritional source for the invading bacteria: transfer of the T-DNA to the plant cells permits bacterial gene expression *in planta*, a mechanism that does not appear to occur with other plant pathogens (Patten & Glick, 1996).

However, a number of phytopathogenic bacteria cause outgrowths on their plant hosts, termed knots or galls, and here we will consider two examples. The first is that of *Ps. savastanoi* pv. *savastanoi*, strains of which infect olive (*Olea europaea*), oleander (*Nerium oleander*) and ash (*Fraxinus excelsior*), causing knots and resulting in

Table 6. Plasmid-borne bactericide resistance genes

Gene	Pathogen	Plasmid	Compound*	Reference
<i>copA–D</i>	<i>Ps. syringae</i> pv. <i>tomato</i>	pPT23D	Cu	Mellano & Cooksey (1988)
ND	<i>Ps. syringae</i> pv. <i>syringae</i>	pPSR12	Cu, As, Co	Kidambi <i>et al.</i> (1995)
<i>strA strB</i>	<i>Ps. syringae</i> pv. <i>syringae</i>	pPSR14	Cu, STR	Sundin <i>et al.</i> (1994)
<i>strA strB</i>	<i>X. campestris</i> pv. <i>vesicatoria</i>	ND (68 kb)	STR	Minsavage <i>et al.</i> (1990a)
<i>tetB</i>	Orchard epiphytes†	ND	TET	Schnabel & Jones (1999)
<i>tmp</i>	<i>Ps. savastanoi</i> pv. <i>glycinea</i>	pPg2	TMP	Leary & Trollinger (1985)

ND, Not designated.

* As, arsenate; Co, cobalt; Cu, copper; STR, streptomycin; TET, tetracycline; TMP, trimethoprim.

† Identified only as *Pa. agglomerans* (= *Er. herbicola*) with TetB or *Pseudomonas* spp. with TetA, TetC or TetG.

economic losses for olive in particular (Wilson, 1935; Smidt & Kosuge, 1978). The strains appear to be essentially host-specific, and while the IAA genes occur on plasmids in oleander strains, they are chromosomally located in ash and most olive strains (Caponero *et al.*, 1995). There is no evidence that the genes for IAA production are transferred to the plant and it is probable that the ability to infect these hosts does not reside solely in their ability to produce IAA; however, the basis of their host specificity remains unknown. Olive and oleander strains, but not ash strains, produce a further hormone, cytokinin, specified by the *ipt* (*ptz*) gene in *Ps. savastanoi* pv. *savastanoi* (MacDonald *et al.*, 1986; Powell & Morris, 1986).

IAA production is also specified on a large 150 kb plasmid, pPATH, in *Er. herbicola* pv. *gypsophila*, which causes galls on gypsophila (*Gypsophila* sp.) (Manulis *et al.*, 1991; Clark *et al.*, 1993). On the same plasmid, the *etz* gene specifies the production of a second hormone, a cytokinin, which affects the size of galls (Lichter *et al.*, 1995a, b). Adjacent to the *etz* gene, an 'insertion-like' element, IS1327, could have been involved in the horizontal acquisition of the phytohormone gene cluster (Lichter *et al.*, 1996). The pPATH plasmid also harbours a cluster of *hrp* genes, as described above (Nizan *et al.*, 1997).

Recently, Glickmann *et al.* (1998) investigated the production of IAA among 57 strains representing *Ps. syringae* pathovars from eight of the nine genomospecies groups identified by Gardan *et al.* (1999). Most produced IAA, particularly in the presence of tryptophan. In general, high-level production of IAA, which was limited to a small number of strains, correlated with the presence of homologues of the genes *iaaM* and *iaaH* from *Ps. savastanoi* pv. *savastanoi*; in pathovars *myricae* and *photiniae* these genes were located on plasmids. In contrast, many strains hybridized with a probe for the *iaaL* gene for IAA-lysine synthase, which was often plasmid-borne. The authors concluded that the majority of strains appeared to produce IAA by an alternative pathway, possibly involving indolepyruvate as an intermediate (Glickmann *et al.*, 1998).

Among certain strains of the bean pathogen *Ps. savastanoi* pv. *phaseolicola* which infect a tropical weed called kudzu (*Pueraria lobata*) (Goto & Hyodo, 1987), a plasmid, pPSP1, has been shown to carry a gene, *efe*, for the production of ethylene, a hormone involved in the ripening of fruit (Fukuda *et al.*, 1992). The *efe* gene was also found on plasmids in *Ps. syringae* pv. *cannabina* and *Ps. savastanoi* pv. *glycinea* (Sato *et al.*, 1997; Watanabe *et al.*, 1998; Table 5).

The production of hormones is frequently host-specific and the location of such genes may simply reflect their precise mode of acquisition – whether by the stable maintenance of an incoming plasmid or by some transposition or recombination event involving the transient acquisition of a plasmid.

Copper and antibiotics

Bordeaux mixture, primarily a fungicide based on copper plus lime, whose accidental discovery is recounted by Schumann (1991), has been used to control plant disease in crops for over 100 years. Plasmid-borne resistance to copper has been found in several phytopathogenic bacteria, including *X. campestris* pv. *vesicatoria* pathogenic on pepper (*Capsicum annuum*), in *Ps. syringae* pv. *syringae* pathogenic to ornamental fruit trees [mainly pear (*Pyrus callieriana*)] and *Ps. syringae* pv. *tomato*, a pathogen of crucifers and tomato (*Lycopersicon esculentum*) (Stall *et al.*, 1986; Bender & Cooksey, 1986, 1987; Mellano & Cooksey, 1988; Kidambi *et al.*, 1995). The genes specifying resistance to copper appear to be widely conserved among the two genera (Voloudakis *et al.*, 1993) and are generally located on large plasmids, with the exception of a walnut (*Juglans regia*) pathogen, *X. campestris* pv. *juglandis*, in which they are chromosomal (Lee *et al.*, 1994). Table 6 lists examples of plasmid-borne resistance to bactericides used in agriculture.

Resistance to streptomycin, which has been used since the late 1950s to control disease in fruit orchards, was detected in the pathogen *Ps. syringae* pv. *papulans* and a number of other Gram-negative bacteria present in apple (*Malus* sp.) orchards in the USA (Norelli *et al.*,

1991). Common fluorescent epiphytic bacteria, which were not associated with disease, were found to transfer streptomycin resistance efficiently to the pathogen, *Ps. syringae* pv. *papulans*, in the laboratory, suggesting that these bacteria may provide a reservoir for streptomycin resistance (Huang & Burr, 1999).

Copper resistance is often linked to streptomycin resistance and dual resistance to these bactericides was detected on conjugative plasmids in *Ps. syringae* pv. *syringae*, ranging in size from 68 to 220 kb (Sundin & Bender, 1993). One such determinant is the Sm^r transposon, Tn5393, first identified in *Er. amylovora* (Chiou & Jones, 1993) and subsequently identified as the basis of streptomycin resistance seen in *Ps. syringae* and *X. campestris* (Sundin & Bender, 1995). Insertion sequences, IS1133 in *Er. amylovora* and IS6100 in *X. campestris*, increase the expression of the resistance genes, leading to higher resistance among the bacteria (Chiou & Jones, 1993; Sundin & Bender, 1995).

In a very revealing study, Sundin & Bender (1996) examined the relationships between bacteria and their plasmids among strains of *Ps. syringae* pv. *syringae* isolated from nurseries for the propagation of ornamental pear (*Pyrus calleryana* cv. Aristocrat). They isolated nine strains, each harbouring a pOSU900 replicon [Mukhopadhyay *et al.*, 1990; now known to belong to the pPT23A family (Gibbon *et al.*, 1999)] and determined that six of the plasmids carried only Cu^r, two carried Cu^r plus Sm^r and one carried only Sm^r. The bacterial genomes were compared by arbitrarily primed PCR (AP-PCR) amplification of total DNA and a similarity matrix based on the resulting banding patterns enabled the generation of a dendrogram reflecting the relatedness of the strains. Using restriction patterns to compare the plasmid replicons from the same strains, a further dendrogram was obtained that showed a remarkable similarity to the strain dendrogram, suggesting that there had been little or no recent plasmid transfer between these strains. This in turn implies that the spread of resistance to copper and streptomycin may owe more to the agency of transposable elements rather than to plasmid transfer *per se* (Sundin & Bender, 1996).

Resistance to UV irradiation

Many *Ps. syringae* pathogens exist as epiphytes on plant leaf surfaces, without causing visible disease symptoms. However, it is clear that this phase, in many cases, is critical for the spread of the pathogen and for the development of disease under favourable environmental conditions (Hirano & Upper, 2000). Recent studies have indicated that for growth under conditions of environmental stress, such as dry leaf surfaces, pathogenic epiphytes have a better growth fitness compared to non-pathogenic epiphytes, although this advantage disappeared under moist conditions (Wilson *et al.*, 1999). One aspect of life on surfaces exposed to sunlight is the effect of UV light, which is classified by photobiologists into UV-A (320–400 nm) and UV-B (290–320 nm). Exposure to the longer wavelength UV-A causes indirect damage

to DNA via the generation of chemical intermediates such as reactive oxygen species, whereas UV-B causes direct damage through the formation of DNA photo-products (Eisenstark, 1989; Friedberg *et al.*, 1995).

Plasmid-borne resistance determinants, *ruAB*, homologous to DNA repair operons *umuDC* in *Es. coli*, have been identified and characterized in *Ps. syringae* (Sundin *et al.*, 1996). These genes confer resistance to UV-B and are widely distributed among pathovars and strains of *Ps. syringae*: in 49 strains with plasmid-borne *ruAB*-hybridizing sequences, 48 also hybridized to a pPT23A-specific probe, indicating a high correlation between this replicon type and carriage of these determinants (Sundin & Murillo, 1999). However, caution is needed in the interpretation of these data since it is clear from the work presented below (Arnold *et al.*, 2000, 2001b) that these genes may no longer be functional. In consequence, the question arises of the precise role of the *ruAB* genes among *Ps. syringae* strains, whether in protection against UV or as mobile regions of potential homology for integration of virulence genes.

Mobility of DNA sequences

A number of insertion sequences (IS) found on plasmids in plant pathogens are shown in Table 7. Some were detected because they appear to specifically inactivate certain genes, such as IS51 and IS52, which inactivate the *iaaM* gene in *Ps. savastanoi* pv. *savastanoi*, leading to loss of the ability to induce gall formation (Comai & Kosuge, 1983; Yamada *et al.*, 1986), and IS476, which inactivates the avirulence gene *avrBs1* in pXvCu1 in strains of the pepper pathogen *X. campestris* pv. *vesicatoria* (Kearney & Staskawicz, 1990). Others may serve as sites of potentially mobile homology, such as IS1327 in pPATH of *Er. herbicola* pv. *gypsophilae* and RSII in *Ps. savastanoi* pv. *phaseolicola* strain LR700, the latter being involved in integration and excision of the plasmid, pMMC7105 (Szabo & Mills, 1984a, b).

IS801 (previously RSI) from *Ps. savastanoi* pv. *phaseolicola* strain LR700 was reported to show a predilection for the target sequence TGAAC (Romantschuk *et al.*, 1991). This element belongs to the IS91 family (Mendiola *et al.*, 1992) and was recently found to be present in an ORF showing features of an *avr/vir* gene in race 2, but not in race 1 strains of *Ps. savastanoi* pv. *phaseolicola*. Attempts to show that the cultivar specificity of these two strains resulted from the insertion in the ORF were unsuccessful, but further experiments might reveal whether this gene functions as an *avr* gene towards non-host plants or as a *vir* gene towards bean. Interestingly, transpositions of this element are permanent and the target sequence was considered likely to be TGNAC, where N is any of the four nucleotides (González *et al.*, 1998).

An exciting and intriguing discovery of a 'Russian Doll' effect was found in the chromosome of the pea-blight pathogen *Ps. syringae* pv. *pisi* race 2, where an 8.5 kb fragment was detected, which was absent from a race 4B

Table 7. Plasmid-borne insertion sequence elements in Gram-negative phytopathogenic bacteria

IS	Size (bp)	IR (bp)	Family*	Pathogen	Comment	Reference
51	1311	26	IS3	<i>Ps. savastanoi</i> pv. <i>glycinea</i>	One copy downstream of the CMA cluster in p4180A	Alarcón-Chaidez <i>et al.</i> (1999)
				<i>Ps. savastanoi</i> pv. <i>savastanoi</i>	Multiple copies; inactivates <i>iaaM</i>	Comai & Kosuge (1983); Yamada <i>et al.</i> (1986)
52	1209	9/10	IS5	<i>Ps. savastanoi</i> pv. <i>savastanoi</i>	Few copies; inactivates <i>iaaM</i>	Yamada <i>et al.</i> (1986)
53	2568	27	IS21	<i>Ps. savastanoi</i> pv. <i>savastanoi</i>	In IS51	Soby <i>et al.</i> (1993)
100	1053	20/28	IS21	<i>Ps. savastanoi</i> pv. <i>phaseolicola</i>	Flanking avirulence/virulence genes in the pathogenicity island of plasmid pAV511	Jackson <i>et al.</i> (1999)
476	1225	26	IS3	<i>X. campestris</i> pv. <i>vesicatoria</i>	Present in Cu ^r strains	Kearney & Staskawicz (1990)
801	1517	0	IS91	<i>Ps. savastanoi</i> pv. <i>phaseolicola</i>	Race-specific	Romantschuk <i>et al.</i> (1991) González <i>et al.</i> (1998)
870	1074–1085	0	IS630	<i>Ps. savastanoi</i> pv. <i>glycinea</i>	Three copies of IS801-like sequences flank the coronatine cluster in p4180A	Alarcón-Chaidez <i>et al.</i> (1999)
911	1250	25/36	IS3	<i>Pa. agglomerans</i>	Associated with Tn10; Tc ^r	Schnabel & Jones (1999)
1133	1231	27	IS3	<i>Er. amylovora</i>	Associated with Tn5393; Sm ^r	Chiou & Jones (1993)
1327	812	17	IS6	<i>Er. herbicola</i> pv. <i>gypsophilae</i>	Related to IS26 in <i>Proteus vulgaris</i> , only on pPATH	Lichter <i>et al.</i> (1996)
1416	1322	29	IS3	<i>B. glumae</i>	Four copies in <i>B. glumae</i>	Hasebe <i>et al.</i> (1998)
1417	1335	17	IS3	<i>B. glumae</i>	Widespread but restricted to <i>B. glumae</i> and related species	Hasebe & Iida (2000)
1418	865	15	IS5	<i>B. glumae</i>	Widespread but restricted to <i>B. glumae</i> and related species	Hasebe & Iida (2000)
1419	1215	36	Novel	<i>B. glumae</i>	Widespread but restricted to <i>B. glumae</i> and related species	Hasebe & Iida (2000)
6100	880	14	IS6	<i>X. campestris</i> pv. <i>vesicatoria</i>	Associated with Tn5393; Sm ^r	Sundin & Bender (1995)

*Based on data of Mahillon & Chandler (1998).

strain. This 8.5 kb fragment contained genes normally associated with plasmids, such as *rulAB*. The *rulB* gene was disrupted by a 4.3 kb fragment, including *avrPpiA1* and three genes associated with transposons and phages. Flanking the 4.3 kb fragment were slightly imperfect, 12 bp inverted repeats and the 8.5 kb region was bounded by 7 bp slightly imperfect direct repeats (Arnold *et al.*, 1999, 2000). Homologues of *avrPpiA1* and the 4.3 kb region are present on plasmids in *Ps. syringae* pv. *psii* races 5 and 7 (Arnold *et al.*, 2000). The DNA sequence clearly provides evidence of repeated insertions and topologically the 4.3 kb region containing *avrPpiA1* appears similar to a transposable element. However, a functionally mobile element has yet to be demonstrated and these may represent relatively ancient events. The recent identification of a chromosomal PAI in *Ps. savastanoi* pv. *phaseolicola* race 4 strains provides further evidence of the mobility of genes involved in pathogenicity (Jackson *et al.*, 2000).

Other plasmid-borne traits

Apart from those described above, remarkably few other phenotypes have been shown to be plasmid-

determined among phytopathogens. There is an interesting report of thiamin prototrophy associated with pEa29 (previously described as a 28 kb plasmid; Chiou & Jones, 1993), suggesting that in this case the plasmid plays a role in the physiology or metabolism of *Er. amylovora* (McGhee & Jones, 2000).

The control of extracellular polysaccharide (EPS) production has been associated with plasmid pEa29 in *Er. amylovora* (Bereswill *et al.*, 1992) and with pJTPS1 in *R. solanacearum* (Negishi *et al.*, 1993). However, recent studies in *R. solanacearum* have shown that high-level transcription of *eps* requires the products of at least seven regulatory genes that are chromosomally located (Garg *et al.*, 2000). In both cases, these traits are associated with full virulence of the pathogen.

Pantoea citrea causes a post-harvest disease of pineapple (*Ananas comosus*) called pink disease, which produces a pink coloration in canned pineapple due to the production of chromogenic 2,5-diketo gluconate from glucose (Pujol & Kado, 2000). Although the genes *gdhA* and *gdhB* are chromosomal, plasmid pUCD5000 was required for full expression of the pink colour (Pujol & Kado, 1998).

The pPT23A family of replicons

The first example of a pPT23A-like replicon was the cryptic 80 kb plasmid, pOSU900, isolated from *Ps. syringae* pv. *syringae* strain J900. Loss of this plasmid from the strain did not affect its pathogenicity toward bean. Two shuttle vectors for use with *Ps. syringae* and *Es. coli* were developed, but appear to have been little used (Mukhopadhyay *et al.*, 1990). However, the pPT23A family is undoubtedly the best-characterized group of plasmids among phytopathogenic bacteria (Gibbon *et al.*, 1999). Replicons of this type have so far only been identified in *Ps. savastanoi*, *Ps. syringae* and in a plasmid, pCPP519, from the yellow-pigmented, non-fluorescent epiphytic *Pseudomonas* sp. strain PyR19 (Huang & Burr, 1999). In pathovars of *Ps. savastanoi* and *Ps. syringae*, pPT23A-like plasmids often share large regions of homology at the DNA level (Murillo & Keen, 1994; Sundin & Bender, 1996; Glickmann *et al.*, 1998; Sesma *et al.*, 1998). This is surprising on two counts. Firstly, replicons sharing extensive regions of homology might be expected to undergo frequent recombination, leading to rearrangements, deletions and integration events, which might well result in significant instability of plasmids. Secondly, it was not clear how apparently related replicons stably co-exist, given the utility of the incompatibility typing systems used elsewhere. However, in general, plasmid profiles among strains of *Ps. syringae* appear to be remarkably stable (A. Vivian, unpublished observations over some 20 years). Indeed, attempts to obtain cured derivative strains were only successful with the advent of electroporation (Murillo & Keen, 1994).

Recent work has, however, provided some insights into the reasons for these apparent contradictions. A number of *rep* genes from pPT23A-like plasmids has shown them to be related to ColE2-type replicons (Hiraga *et al.*, 1994; Gibbon *et al.*, 1999), but to differ in being substantially larger, with an additional 100 residues at their C termini (Gibbon *et al.*, 1999). Among members of the ColE2-type plasmids, two incompatibility determinants appear to function. The IncA specificity is due to the reliance on an antisense RNA, produced by the gene *incA*, that regulates the production of Rep protein, which is essential for replication. The RepA protein in ColE2-type plasmids functions *in trans* to activate replication by binding to a sequence at the replication origin in a plasmid-specific manner and synthesizing a primer RNA for the initiation of DNA synthesis by DNA polymerase. IncB incompatibility is due to competition for the Rep protein among origins of the same binding specificity. Five IncA and four IncB incompatibility specificities were identified and the specificity of interaction of the Rep proteins and origins appeared to be determined by insertion or deletion of single nucleotides and the substitution of several nucleotides at specific sites in the origins, with corresponding changes in the sequences found in the C-terminal regions of the Rep proteins (Hiraga *et al.*, 1994).

Thus, plasmids closely related in terms of their DNA

sequences have the ready potential to establish compatibility within a bacterial cell. Given this situation, homologous recombination between plasmids may occur with some frequency and might permit the maintenance of a level of heterogeneity in certain genes; however, no evidence for such mechanisms has appeared to date. Nevertheless, even relatively rare recombination events, particularly if these were (even indirectly) plant-inducible, could offer an explanation for the appearance of novel strains with changed host specificity or increased virulence.

To date, the precise mechanism of replication control is unknown for the pPT23A family and putative antisense RNA has not been detected, but it is not uncommon for strains of *Ps. syringae* to carry up to six plasmids sharing a pPT23A-like *rep* replicase gene (Sesma *et al.*, 1998). It has been suggested that compatibility between closely related replicons could be due to a combination of sequence divergence within *rep* and *ori*, which would enable the maintenance of binding specificity and loss or modification of other Inc determinants, such as IncA and IncC (Sesma *et al.*, 1998). The presence in one strain of closely related plasmids could indicate an ancestral duplication event and concomitant modification of a pre-existing plasmid.

Subsequently, the phylogenetic relationships, based on an analysis of the *repA* gene sequences, were examined among 14 pPT23A-like plasmids from 5 *Ps. syringae* pathovars. Two genes of this type have been completely sequenced and their predicted peptides show relatively high sequence conservation from the start to residue 373, but are poorly conserved from residue 374 to the end of the peptide at residue 437 (Gibbon *et al.*, 1999). Consequently, regions at the 5' end (including 100–120 bp of upstream sequence) and 3' ends of the gene were sequenced and a combined phylogenetic tree based on sequence identity revealed four groups, with plasmids from the pathovars *syringae* and *tomato* being found in more than one group. Some *repA* genes had deletions in their upstream regions associated with a 9 bp direct repeat sequence (Sesma *et al.*, 2000).

So do these plasmids represent mobile arsenals? Transfer of plasmid pCPP519 has been shown *in vitro* from a non-pathogenic *Pseudomonas* sp. (PyR19) to *Ps. syringae* pv. *papulans* and a number of similar plasmids were transmissible, both within *Ps. syringae* pv. *papulans* strains and to non-fluorescent, yellow *Pseudomonas* strains; however, failure to detect common R plasmids in the two types of strain suggests that plasmid transfer between these co-habiting epiphytes of apple orchards may rarely occur (Huang & Burr, 1999). This view is also supported by the phylogenetic studies mentioned above that provide evidence that chromosomes and plasmids of some *Ps. syringae* strains have co-evolved (Sundin & Bender, 1996). The use of bactericides and the subsequent spread of resistance to copper and streptomycin on a transposon clearly demonstrates the role of such elements in situations of strong genetic selection. Perhaps the plasmids act as 'sponges',

soaking up useful genetic determinants carried into the strains by the agency of transposons. In this context, it will be interesting to see if more examples can be identified of the integrative disruption of *rulB* on plasmids: this gene appears to be a potential 'hot-spot' for such events involving the exchange of *avr/vir* genes (Arnold *et al.*, 2001b).

Concluding remarks

What we know of plasmids in plant-pathogenic bacteria is outlined in this article; what we do not know about plasmids in these groups is much more extensive. We would pose the following questions to address these unknowns. What other types of replicon might be found? What are the functions of the cryptic plasmids, given that their carriage must impose a load on their bacterial hosts? What is the nature of replication control in the pPT23A family and does this have implications for the evolution of the pathogen in relation to its plant host? What are the main agencies of gene mobility and what evolutionary timescales are involved? What factors are driving plasmid evolution among these pathogen groups?

In summary, this whole area provides inquisitive scientists with a range of opportunities to study many aspects of basic biology, such as replicon maintenance, recombination or apparent lack of it between homologous DNA sequences, pathogenicity islands and the significance of their distinctive properties in terms of function, that could have important consequences for the more effective and environmentally friendly control of plant disease.

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