Applied and Environmental Microbiology, July 2005, p. 3778–3785 Vol. 71, No. 7
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.7.3778–3785.2005
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Changes in Race-Specific Virulence in *Pseudomonas syringae* pv. phaseolicola Are Associated with a Chimeric Transposable Element and Rare Deletion Events in a Plasmid-Borne Pathogenicity Island

Luis A. Rivas,[1]† John Mansfield,[2] George Tsiamis,[2,3]‡ Robert W. Jackson,[4] and Jesús Murillo[1]*

Laboratorio de Patología Vegetal, Departamento de Producción Agraria, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Pública de Navarra, 31006 Pamplona, Spain[1]*; Department of Agricultural Sciences, Imperial College, Wye Campus, Ashford, Kent TN25 5AH, United Kingdom*[2]*; Department of Environmental and Natural Resources Management, University of Ioannina, 2 Seferi St., 30100 Agrinio, Greece*[3]*; and Centre for Research in Plant Science, University of the West of England, Coldharbour Lane, Frenchay, Bristol BS16 1QY, United Kingdom*[4]

Received 10 September 2004/Accepted 19 January 2005

Virulence for bean and soybean is determined by effector genes in a plasmid-borne pathogenicity island (PAI) in race 7 strain 1449B of *Pseudomonas syringae* pv. phaseolicola. One of the effector genes, *avrPphF*, confers either pathogenicity, virulence, or avirulence depending on the plant host and is absent from races 2, 3, 4, 6, and 8 of this pathogen. Analysis of cosmid clones and comparison of DNA sequences showed that the absence of *avrPphF* from strain 1448A is due to deletion of a continuous 9.5-kb fragment. The remainder of the PAI is well conserved in strains 1448A and 1449B. The left junction of the deleted region consists of a chimeric transposable element generated from the fusion of homologs of IS*1492* from *Pseudomonas putida* and IS*1090* from *Ralstonia eutropha*. The borders of the deletion were conserved in 66 *P. syringae* pv. phaseolicola strains isolated in different countries and representing the five races lacking *avrPphF*. However, six strains isolated in Spain had a 10.5-kb deletion that extended 1 kb further from the right junction. The perfect conservation of the 28-nucleotide right repeat of the IS*1090* homolog in the two deletion types and in the other 47 insertions of the IS*1090* homolog in the 1448A genome strongly suggests that the *avrPphF* deletions were mediated by the activity of the chimeric mobile element. Our data strongly support a clonal origin for the races of *P. syringae* pv. phaseolicola lacking *avrPphF*.

Many bacterial pathogens of plants and animals use the same strategies to infect and colonize hosts, producing a series of virulence proteins or effectors that are injected into host cell cytoplasm through a specialized type III secretion system (18, 43). Effector genes are highly diverse in sequence, length, and genome location, and their products have distinct biochemical activities that facilitate colonization of the host, suppression of host defense responses, and dispersion of the pathogen (5, 15). In bacterial plant pathogens, effectors were first described as avirulence genes because of their ability to elicit the hypersensitive response, a programmed cell death that occurs in resistant plants (17). Later, certain avirulence genes were shown to increase bacterial aggressiveness and, in some cases, to be essential for pathogenicity (6, 15); it appears that the primary roles of effectors are to suppress plant immune responses and to promote host cell death (7, 23). The functions of the effectors are usually additive and redundant (3, 4, 20, 55), and although the precise roles of many effectors have not been established, it is becoming increasingly clear than in many cases they are the main determinants of pathogenicity and host range (13, 19, 20, 45). Additionally, some effector genes are highly conserved among very different genera of plant and animal pathogens (5, 43), possibly suggesting a common origin. Genes coding for effectors are often located on pathogenicity islands (PAIs) (2, 20), suggesting that they were acquired by horizontal gene transfer. PAIs often carry genes coding for genetic mobility, such as genes coding for integrases, mobile elements, and transposases, and phage genes, which can mediate the integration, mobility, and rearrangement of the PAI (2). Such events, which can occur over long periods of time, can lead to the development of new pathogenic capabilities or to the emergence of new microbial species (36).

Pseudomonas syringae pv. phaseolicola infects a wide variety of legumes and causes economically important losses in common bean (*Phaseolus vulgaris* L.) worldwide (39, 47). Control of this pathogen is difficult, and management is based mostly on the use of pathogen-free seed and tolerant or resistant cultivars. The most common strains contain a large DNA region for the biosynthesis of a non-host-specific toxin, phaseolotoxin, which is generally used for specific detection of the pathogen (38, 41). Cultivar-specific resistance is based on the

* Corresponding author. Mailing address: Laboratorio de Patología Vegetal, Departamento de Producción Agraria, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Pública de Navarra, 31006 Pamplona, Spain. Phone: 34 948 169133. Fax: 34 948 169732. E-mail: jesus@unavarra.es.

† Present address: Centro de Astrobiología (CSIC-INTA), Crta. de Ajalvir, Km 4, 28850 Torrejón de Ardoz, Madrid, Spain.

‡ Present address: Department of Environmental and Natural Resources Management, University of Ioannina, 2 Seferi St., 30100 Agrinio, Greece.

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TABLE 1. Strains of *P. syringae* pv. phaseolicola used in this work

Strain(s)	Place of isolation, year, and host	Race[a]	Presence of *avrPphF*	Reference or source
1281A	United Kingdom, 1984, *Phaseolus coccineus*	1	+	47
882	United States, 1975, *P. vulgaris*	2	−	47
1301A	Tanzania, 1984, *P. vulgaris*	3	−	24
1302A	Rwanda, 1984, *P. vulgaris*	4	−	47
1448A	Ethiopia, 1985, *P. vulgaris*	6	−	49
1448AR	—[b]	6	−	10
1449B	Ethiopia, 1985, *Lablab purpureus*	7	+	47
1674A	New Zealand, 1970, *Vigna angularis*	7	+	HRI[c]
2656A	Lesotho, 1990, *P. vulgaris*	8	−	47
2709A	Malawi, 1990, *P. vulgaris*	9	+	47
2732E	Colombia, 1990, *P. vulgaris*	9	+	HRI
Hb-1b	Unknown, unknown, *P. vulgaris*	uk	−	53
M2/1	Germany, 1977, *P. vulgaris*	uk	+	53
18 strains	South Africa, 1991–2002, *P. vulgaris*	uk	+	T. Goszczynska
52 strains	South Africa, 1991–2002, *P. vulgaris*	uk	−	T. Goszczynska
12 strains	Spain, 1993–2000, *P. vulgaris*	uk	−	38
CYL246	Spain, 1996, *P. vulgaris*	uk	−	38
CYL376	Spain, 2003, *P. vulgaris*	uk	−	38

[a] uk, unknown.
[b] Rif^r strain derived from 1448A.
[c] HRI, Horticultural Research International, Wellesbourne, United Kingdom.

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specific interaction of the products of a resistance gene in the host and an avirulence (effector) gene in the pathogen. Mutations that render an avirulence gene ineffective often do not have a major impact on pathogenicity, but they may suppress elicitation of resistance in a previously resistant plant species or cultivar. The spontaneous appearance of new virulence in a pathogen population, usually referred to as race change, occurs under field conditions and is a major cause of failure of disease control against bacterial pathogens (34). So far, nine races of *P. syringae* pv. phaseolicola have been identified based on their interactions with a set of eight differential bean cultivars, and five pairs of resistance and avirulence genes have been postulated to explain the gene-for-gene interactions (24, 48). Different combinations of the five putative genes determine the range of cultivars that can be infected by the pathogen (i.e., determine race). Three of the avirulence genes, *avrPphB*, *avrPphE*, and *avrPphF*, have been cloned based on their abilities to cause the hypersensitive response in cultivars carrying the matching dominant genes for resistance (*R3*, *R2*, and *R1*, respectively) (24, 32, 51). Remarkably, unlike *avrPphB* and *avrPphF*, alleles of *avrPphE* are present in all strains of *P. syringae* pv. phaseolicola, but they display an avirulence phenotype only in races 2, 4, 5, and 7 (44). Abolition of avirulence is due to single base pair changes in races 1, 3, 6, and 9 and occurs via a 104-bp insertion in the allele of *avrPphE* in race 8. Conversely, *avrPphB* is harbored only by races 3 and 4 and is part of a large (>40-kb) chromosomal region that is bordered by $tRNA^{Lys}$ genes (22, 24). A race change phenomenon occurs upon deletion of the *avrPphB*-containing region, resulting in an extended host range in previously resistant cultivars of bean and soybean (*Glycine max* L.) (22).

Genetic analysis of *P. syringae* pv. phaseolicola strain 1449B showed that its ability to produce disease in bean is dependent on the presence of a PAI localized to a 150-kb plasmid (20). Genetic analysis and partial sequencing of the PAI showed that it includes several effector genes, as well as a diverse assortment of mobile elements (20, 21, 51). One of the effector genes, *virPphA*, is essential for induction of typical water-soaking symptoms on bean, while it also confers avirulence for several soybean cultivars (20). *avrPphF* is also essential for induction of disease symptoms on soybean, which is not a common host for *P. syringae* pv. phaseolicola. *avrPphF* has also been found to confer virulence for the bean cultivar Tendergreen (51). The crystal structures of the two proteins from the *avrPphF* operon have been resolved recently (42). The structure of the ORF1-encoded protein is very similar to that of chaperones from diverse bacterial pathogens of animals. Also, the structure of the protein encoded by ORF2 is novel and mushroom-like, and the head exhibits structural homology to the catalytic domain of bacterial ADP ribosyltransferase toxins and contains two clusters of surface-exposed residues that are necessary both for virulence and to trigger disease resistance mediated by the bean *R1* gene. Despite the potential importance of *avrPphF* to the virulence of *P. syringae* pv. phaseolicola for certain bean cultivars, sequences hybridizing to *avrPphF* are absent from the type strains of races 2, 3, 4, 6, and 8 (51). The importance of the *avrPphF-R1* interaction for the control of bean halo blight prompted a comparative analysis of the *avrPphF*-containing PAI in strains other than the well-characterized strain 1449B (21). In addition to hybridization and PCR amplification experiments, we were able to utilize the newly available genomic sequence of 1448A (http://www.tigr.org). We found that the organization of the PAI is generally well conserved in strains of *P. syringae* pv. phaseolicola and that *avrPphF* is lost from the PAI due to large deletions mediated by a chimeric mobile element. Our results indicate that strains lacking *avrPphF* were probably derived from a unique deletion event.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The strains of *P. syringae* pv. phaseolicola used in this work are described in Table 1. *E. coli* DH5α, used for cloning, was cultured at 37°C using Luria-Bertani medium (40), and *P.*

syringae was cultured at 28°C using medium B (26). Cosmid pAV520 harbors the PAI of *P. syringae* pv. phaseolicola 1449B cloned in pLAFR3 (51).

General molecular techniques. Standard molecular biology techniques were used (40). Genomic DNA was extracted using a DNA isolation kit (Puregene; Gentra Systems) according to the manufacturer's instructions. For Southern blots, genomic DNA digested with appropriate restriction enzymes was separated by electrophoresis in 0.8% agarose gels and transferred to positively charged nylon membranes (Roche Diagnostics, Basel, Switzerland). Cloned PCR amplification products to be used as probes were amplified from the vector, separated by electrophoresis, and purified from the gel using a GFX PCR DNA purification kit (Amersham Pharmacia Biotech). Preparation of labeled probes with digoxigenin, Southern hybridization, and detection of hybridization signals were carried out with a DIG DNA labeling and detection kit (Roche Diagnostics). A gene library of *P. syringae* pv. phaseolicola 1448AR was prepared using cosmid pLAFR3, essentially as described previously (52).

Nucleotide sequences were obtained using an ABI 310 sequencer (Perkin-Elmer) or were determined at MWG Biotech AG (Ebersberg, Germany). Searches for sequence similarity in the National Center for Biotechnology Information databases were done using the BLAST algorithms (1), and sequences were aligned using the ClustalX program (50). Sequence data from the *P. syringae* pv. tomato DC3000 genome and from the unfinished *P. syringae* pv. syringae B728a genome were obtained from the websites of The Institute for Genomic Research (http://www.tigr.org) and the DOE Joint Genome Institute (http://www.jgi.doe.gov), respectively. Preliminary sequence data for the *P. syringae* pv. phaseolicola 1448A genome were obtained from The Institute for Genomic Research (http://www.tigr.org) as part of the NSF Tomato-*Pseudomonas* Functional Genomics project (http://pseudomonas-syringae.org). Sequencing of *P. syringae* pv. phaseolicola 1448A was accomplished with support from the National Science Foundation.

When possible, the designations of insertion sequences are the IS Finder Database designations (http://www-is.biotoul.fr/).

PCR analysis. PCR primers avrD3For (5′-CAAACGTAGGTCAACGCCAAAGC-3′), anchoring in the *avrD* gene promoter, and avrPphC2Rev (5′-CCATTCGAGCATGGTGCAGTTC-3′), which binds 80 nucleotides (nt) before the stop codon of *avrPphC*, were used for amplification of the region bordering the deletion encompassing *avrPphF*. Primers avrPphC2Rev and avrPphFRev (5′-TCAGACCGAACTCTCAGACA-3′), which starts in the stop codon of *avrPphF* ORF1, were used to examine DNA conservation in *avrPphF* and *avrPphC*. Other PCR primers were designed from previously published sequences. Amplification was carried out using either *Taq* DNA polymerase (Biotaq; Bioline Ltd., London, United Kingdom) or Ready To Go PCR beads (Amersham Pharmacia Biotech).

Nucleotide sequence accession numbers. The nucleotide sequences obtained in this work have been deposited in the EMBL/GenBank databases under the following accession numbers: AY603426 for the 31,068-nt insert of cosmid pAV520; AY699267 (strain 882, race 2), AY699268 (strain 1301A, race 3), AY512597 (strain 1302A, race 4), AY512596 (strain 1448A, race 6), and AY699266 (strain 2656A, race 8) for the borders of the 9.5-kb deletion; AY512599 (strain CYL246, race unknown) and AY512598 (strain CYL376, race unknown) for the borders of the 10.5-kb deletion; and AY699269 (strain 1281, race 1) for the PAI region between *avrPphF* and *avrPphC* lacking *tnpJR1*.

RESULTS

The absence of *avrPphF* from the PAI is due to a large deletion. Race 6 strain *P. syringae* pv. phaseolicola 1448A is virulent for the bean cultivar Red Mexican (*R1*) and lacks DNA homology with either open reading frame of *avrPphF* (51). In order to study the reasons for the absence of *avrPphF* from this and other strains of *P. syringae* pv. phaseolicola, we first examined the conservation of the PAI in strains 1448A and 1449B, which contains *avrPphF*.

A cosmid library of strain 1448AR was screened by colony hybridization using a probe corresponding to the complete coding sequence of *virPphA* (20). Cosmid clone p19U1 hybridized to the probe and also produced specific amplification products using primers for *avrD*, *avrPphC*, and ORF4, which are included in the PAI in strain 1449B, indicating that they are adjacent in the 1448A genome. This clone was analyzed further by restriction digestion with BamHI, BglII, EcoRI HindIII, PstI, SmaI, and SspI, by hybridization with probes specific for genes included in the PAI, and by PCR. The restriction patterns were identical for the 1448A genome and the cosmid insert except for the insert borders, indicating that no major reorganization had occurred during cloning. Additionally, there was general colinearity in the gene order in cosmids p19U1 and pAV520 (Fig. 1), which contains the PAI from the race 7 strain 1449B, indicating that the organization of the PAI is conserved at least in races 6 and 7 of *P. syringae* pv. phaseolicola. However, the analysis of pAV520 and p19U1 with SspI indicated that the absence of *avrPphF* from p19U1 was associated with the lack of an approximately 10-kb DNA fragment between the *avrD* and *avrPphC* genes (Fig. 1), which could have been the result of either a deletion in strain 1448A or an insertion in strain 1449B.

Comparison of the nucleotide sequence of pAV520 (31,068 nt) with the closed genome sequence of strain 1448A confirmed the results described above, showing that the PAI from the race 6 strain 1448A lacks a continuous 9,471-bp fragment (including positions 7601 to 17071 in pAV520) between the *avrD* and *avrPphC* genes (Fig. 1). To confirm the difference between the PAIs, the DNA between the *avrD* and *avrPphC* genes was amplified by PCR with primers avrD3For and avrPphC2Rev, which flank a 14-kb fragment in pAV520. As expected, no amplification product was obtained using pAV520 as the template, although a strong 4.6-kb fragment was consistently amplified from p19U1 and from genomic DNA of strain 1448A. Restriction analysis and partial sequencing of the 1448A amplicon confirmed the absence of a 9.5-kb fragment encompassing *avrPphF* (Fig. 1).

The sequence surrounding the borders of the 9.5-kb discontinuity was examined in detail. The left border was flanked in strain 1448A and pAV520 by a 1,010-nt putative chimeric mobile element (Fig. 1 and 2). The first 295 nt of this element corresponded to a truncated copy of IS*Psy16* (http://www-is.biotoul.fr/) and was 86.2% identical to the 5′ end of IS*1492* from *P. putida* (accession no. AJ288910). The remaining 715 nt corresponded to a truncated copy of an insertion sequence that was designated IS*Psy17* (http://www-is.biotoul.fr/) and exhibited 71.6% overall identity to the 3′ end of IS*1090* from *Ralstonia eutropha* (accession no. AJ010060). The corresponding transposase genes were fused in phase, which resulted in a 281-amino-acid (aa) chimeric transposase whose N-terminal 64 aa exhibited 92.2% identity with the TnpA1 transposase of IS*1492* and whose C-terminal 217 aa exhibited 78.3% identity with the transposase of IS*1090* (Fig. 2B). The deletion border was located 61 nt after the stop codon of this chimeric transposase and immediately after a 28-nt sequence that was very similar to the IS*1090* right terminal repeat (Fig. 2). Analysis of the closed genome sequence of strain 1448A showed that it contained five full copies of IS*Psy16*, three in its 132-kb plasmid and two in the chromosome, and 47 complete copies of IS*Psy17*, one of which was located in the 52-kb plasmid and the rest of which were chromosomal. In all the insertions of IS*Psy17*, the sequence of the 28-nt right repeat was fully conserved, and the similarity among the different copies of the insertion sequence ended 61 nt after the stop codon of the putative transposase coding region. Additionally, the 28-nt sequence always appeared in association with IS*Psy17* sequences. These data indicate that the 28-nt sequence truly corresponds

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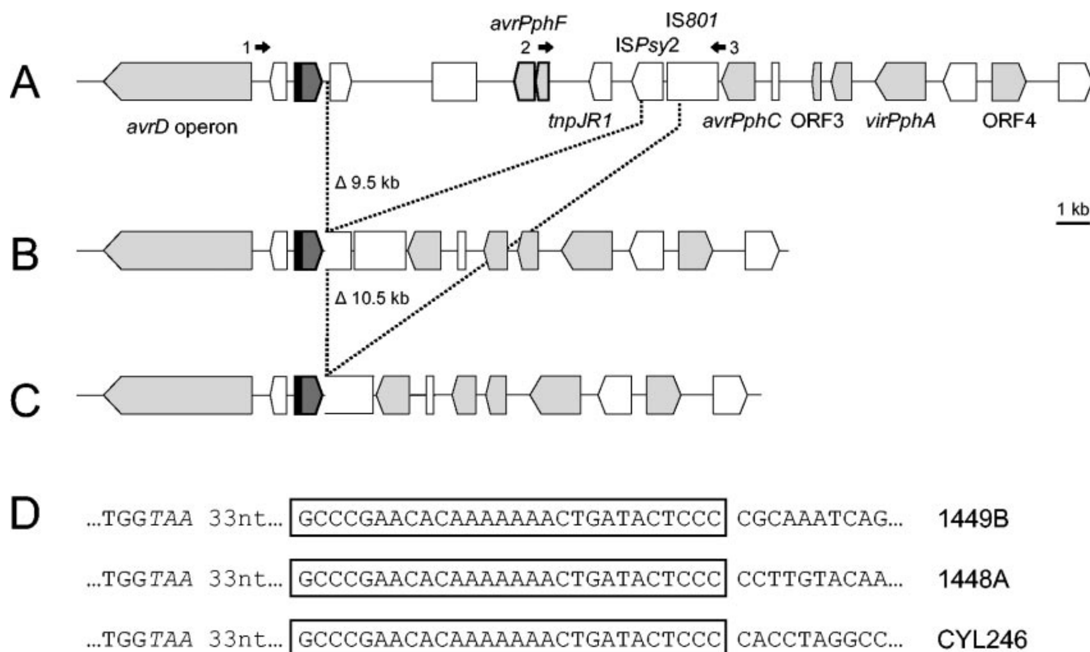

FIG. 1. Loss of the *avrPphF* avirulence gene is associated with large deletions in the pathogenicity island of *P. syringae* pv. phaseolicola. The grey boxes represent avirulence (effector) genes, and relevant potential mobile elements are indicated by open boxes. Deletions in the pathogenicity island are indicated by dotted lines, and a chimeric mobile element that defines the left border of the deletions is represented by a box that is black and dark grey. (A) Organization of the insert of cosmid pAV520, containing the pathogenicity island of *P. syringae* pv. phaseolicola 1449B. The arrows indicate primers used for PCR amplification (arrow 1, primer avrD3For; arrow 2, primer avrPphFRev; arrow 3, primer avrPphC2Rev). (B and C) Organization the pathogenicity island and extent of the deletion spanning *avrPphF* in *P. syringae* pv. phaseolicola 1448A and 65 other strains (B) and in six strains from Spain (C). (D) DNA sequences bordering the deletion in strains 1448A and CYL246 lacking *avrPphF* and strain 1449B containing *avrPphF*. The 28-nt right border of the chimeric mobile element is enclosed in a box, and the stop codon of the putative transposase is indicated by italics.

to the right border of the transposable element and that it marks the end of a transposition event.

The right border of the discontinuity in strain 1448A, which lacks *avrPphF*, was located within a truncated homolog of IS*Psy2* (accession no. AB063175) (25) and 209 nt upstream of the stop codon of the transposase coding sequence (Fig. 1) compared to the wild-type element. However, the PAI of strain 1449B contained in the corresponding place a contiguous sequence of IS*Psy2* that included the complete 3′ end of the transposase coding sequence. This strongly suggests that *avrPphF* was lost from strain 1448A due to deletion of an approximately 9.5-kb DNA fragment from a pathogenicity island that already contained this gene.

Deletion of *avrPphF* has occurred more than once. In addition to race 6, *avrPphF* is also absent from strains of races 2, 3, 4, and 8 (38, 51). Therefore, we tested if the position of *avrPphF* is conserved among strains containing this gene and if it was also eliminated by similar deletion events from strains of races 2, 3, 4, 6, and 8.

None of the 24 strains containing *avrPphF* examined in this work produced a PCR product when we used a primer pair anchoring in the *avrD* and *avrPphC* genes (primers avrD3For and avrPphC2Rev), as expected because of the physical (14 kb) separation of these genes in pAV520 (Fig. 1). PCR amplification with primers avrPphFRev and avrPphC2Rev yielded the expected 5.6-kb amplicon for 10 of these strains but only a 4.4-kb amplicon for the remaining 14 strains, revealing length variations in the DNA region between the *avrPphF* and *avrPphC* genes. By using restriction digests obtained with BglI and SalI (data not shown) and by DNA sequencing of appropriate PCR products, we determined that the 14 strains lacked a 1,180-nt putative transposable element homologous to a transposase from *Pseudomonas* sp. strain JR1 (ORFE; accession no. AAF80262), which is present between coordinates 15532 and 16712 in pAV520 (Fig. 1). These results suggest that this mobile element inserted between the *avrPphF* and *avrPphC* genes following the formation of the PAI. This mobile element, previously designated *tnpJR1*, has been found to accompany alleles of *virPphA* (21).

Likewise, 66 of the 72 strains lacking *avrPphF* examined produced the expected 4.6-kb amplicon with primers avrD3For and avrPphC2Rev, which in all cases showed an identical restriction pattern with AatII and AccI (Fig. 3). Partial sequencing of this amplicon from representative strains 882 (race 2), 1301A (race 3), 1302A (race 4), and 2656A (race 8) also showed that the deletion point was identical to that of strain 1448A. These results suggest that the loss of *avrPphF* from these 66 strains is the result of a single genetic deletion event. On the other hand, the remaining six *P. syringae* pv. phaseolicola strains, all of which were isolated in Spain, produced a 3.6-kb amplicon instead of the expected 4.6-kb product, indicating that there was deletion of a 10.5-kb fragment instead of the 9.5-kb fragment. Partial sequences of the amplicons from strains CYL246 and CYL376, which were identical, showed that the left border of the mobilization site was identical to that found in the 4.6-kb amplicon. The right border, however, was

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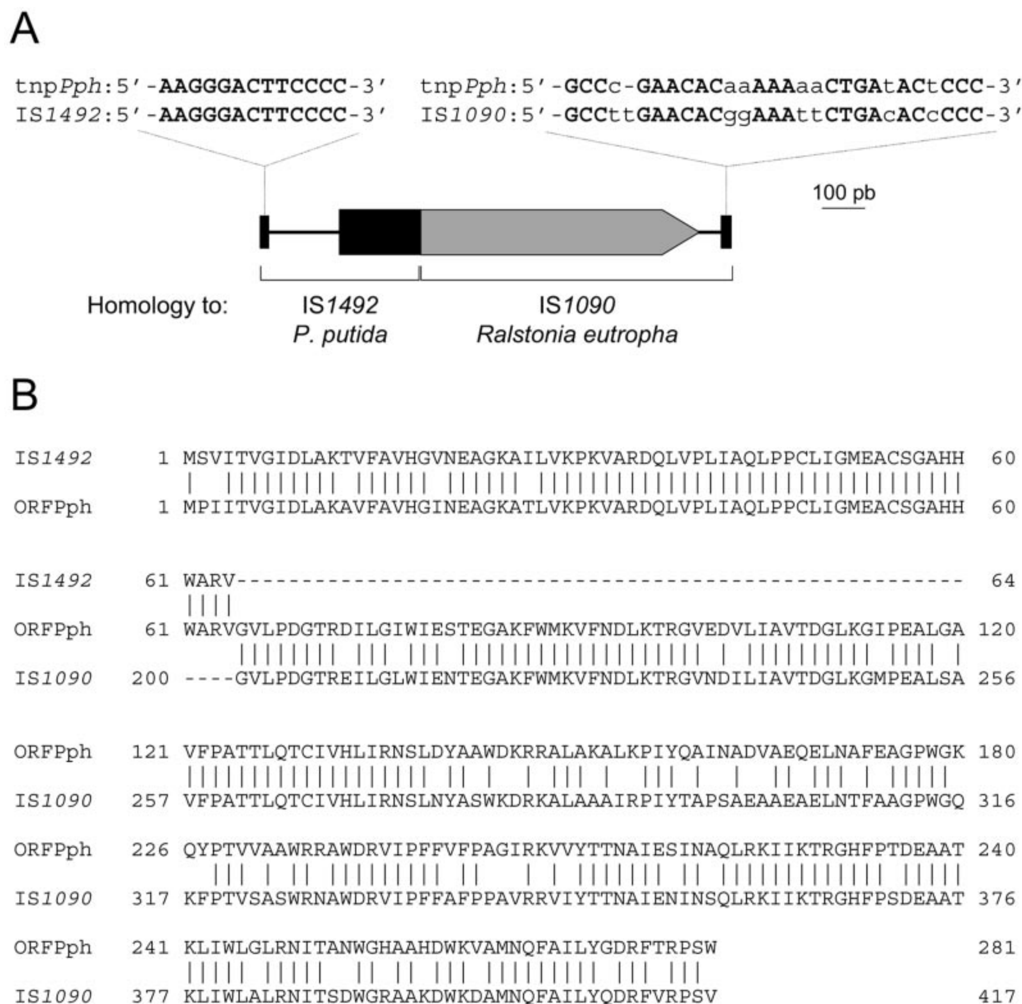

FIG. 2. (A) Organization of the chimeric mobile element from the *P. syringae* pv. phaseolicola pathogenicity island associated with deletion of *avrPphF*. The coding region for the putative transposase and the direction of transcription are indicated by an arrow. Terminal repeats are indicated by solid boxes, and their sequences are compared to the left repeat of IS*1492* (accession no. AJ288910) and the right repeat of IS*1090* (accession no. AJ010060). (B) Comparison of the amino acid sequences of the putative transposase of the chimeric mobile element (ORFPph), transposase TnpA1 of IS*1492*, and the transposase of IS*1090*; only relevant residues are shown for the latter two elements. Dashes indicate amino acids that were excluded to simplify the alignment, and identical amino acids are indicated by vertical lines.

located inside a reorganized copy of IS*801* (Fig. 1), truncating an otherwise contiguous sequence present in the PAI of strain 1449B. These results suggest that the deletion of *avrPphF* in the six Spanish strains might have occurred through an independent deletion event mediated by the chimeric mobile element.

DISCUSSION

The host ranges of *P. syringae* and other phytopathogenic bacteria appear to be determined by the synergistic activity of effector genes. These genes often confer distinct phenotypes, depending on the plant host, leading either to an increase in virulence or to reduction of the host range. The effector gene *avrPphF*, which is located in the well-characterized PAI of the *P. syringae* pv. phaseolicola race 7 strain 1449B, is a paradigm of this multiplicity of phenotypes, inducing a defense response in resistant plants or conferring pathogenicity or virulence, depending on the plant species and cultivar (51). However, in spite of its contribution to virulence, it is absent from five of the nine races described for this pathogen (51). Our results show that the effector gene *avrPphF* is lost from the PAI due to the occurrence of large deletions. Also, we present evidence that most of the strains belonging to races lacking *avrPphF* were derived from a common *P. syringae* pv. phaseolicola strain after a unique deletion event in the PAI. It is remarkable that all the strains lacking the avirulence phenotype conferred by *avrPphF* do indeed lack the gene (38, 51), and our analysis of 72 *P. syringae* pv. phaseolicola strains showed that the lack of the *avrPphF* phenotype was the result of only large deletions in the PAI (Fig. 1). This is in sharp contrast to the different inactivation events and mechanisms described for other avirulence genes (12, 28, 44, 46). For instance, the *avrPphE* avirulence phenotype has been eliminated from five of the nine described races of *P. syringae* pv. phaseolicola by the occur-

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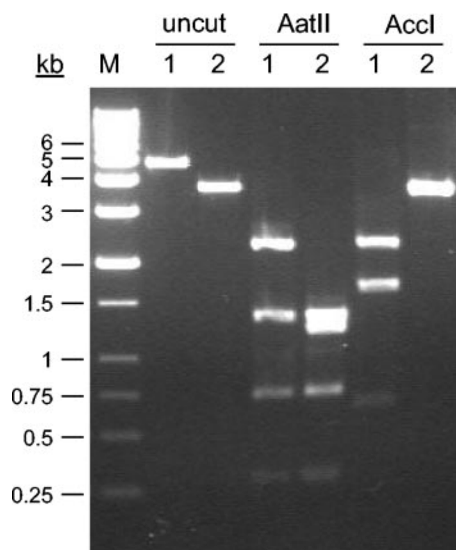

FIG. 3. PCR analysis of the DNA bordering the deletion associated with *avrPphF*. DNA was amplified with primers avrD3For and avrPphC2Rev from *P. syringae* pv. phaseolicola strains 1448A (lanes 1) and CYL246 (lanes 2) and electrophoresed uncut or after digestion with AatII or AccI, as indicated at the top. Lane M contained a 1-kb molecular weight marker.

rence of at least five independent mutation events that appear to be characteristic of each race (38, 44); these events include point mutations, as well as the insertion of a 104-bp sequence. Also, the restriction profiles of an amplicon spanning the deletion site were identical for 91.7% of the strains of *P. syringae* pv. phaseolicola lacking *avrPphF*, and the sequences of the deletion site were identical in six of these strains. Similarly, Marques et al. (33) showed that the results of randomly amplified polymorphic DNA analysis separated *P. syringae* pv. phaseolicola strains containing or lacking *avrPphF* into two well-defined groups indicative of two separate evolutionary lines. A highly unlikely alternative is that the deletions removing *avrPphF* occurred independently and ended in the same sequence; additionally, these deletions must occur with a higher frequency than any other gene inactivation mechanism, and the deleted variants must be positively selected from the pathogen populations. The occurrence of such directed deletions and a constant selective force favoring variants without *avrPphF* are currently difficult to explain. Therefore, the limited number of different deletion events removing *avrPphF* and the fact that the lack of the avirulence phenotype determined by *avrPphF* has been associated exclusively with these deletions and not with any other type of genetic mutation strongly support our hypothesis of the clonal origin of the *P. syringae* pv. phaseolicola races lacking *avrPphF*. The strains analyzed here represent all the known races of *P. syringae* pv. phaseolicola that lack *avrPphF* (races 2, 3, 4, 6, and 8) and were isolated in different countries, suggesting that the majority of isolates belonging to these races were derived from a common ancestor. Given that races lacking *avrPphF* are found worldwide (Table 2), our results imply that the pathogen was very effectively disseminated once the *avrPphF* deletion event occurred. This is highly likely, because *P. syringae* pv. phaseolicola is a seedborne pathogen and is therefore easily dispersed worldwide on contaminated seeds (39).

From our results, it appeared that the deletions that eliminated *avrPphF* were directed by a chimeric mobile element composed of two novel insertion sequences that were designated IS*Psy16* and IS*Psy17* (http://www-is.biotoul.fr/). The left one-third of this element corresponds to IS*Psy16*, a homolog of IS*1492* from *P. putida*, and the other two-thirds corresponds to IS*Psy17*, a homolog of IS*1090* from *R. eutropha*. The 28-nt right repeat of this chimeric element is identical to the right repeat of the 47 full copies of IS*Psy17* found in the genome of *P. syringae* pv. phaseolicola 1448A, and it marks the left border of the deletions involving *avrPphF*. The striking preservation of this 28-nt sequence in the two deletion genotypes that we have found is difficult to explain unless we assume that the deletions were mediated by the activity of the mobile element. It has been widely documented that mobile elements can induce adjacent deletions by a variety of mechanisms (31). In this case, an obvious possibility is the formation of adjacent deletions from duplicative intramolecular transposition of the chimeric element. Indeed, the fusion of the two insertion sequences occurred in the coding region for the respective transposases and in frame, so that the resulting element could potentially

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TABLE 2. Percentages of strains of *P. syringae* pv. phaseolicola from different parts of the world belonging to the different races

Year	Place[a]	% of strains										% of strains with *avrPphF*[c]	% of strains without *avrPphF*[c]	Reference
		Race 1	Race 2	Race 3	Race 4	Race 5	Race 6	Race 7	Race 8	Race 9	NT[b]			
1965[d]	New York (n = 212)	75	25									75	25	37
1973[d]	New Zealand (n = 37)	67.6	32.4									67.6	32.4	16
1992[e]	Tanzania (n = 118)	45	52.5	2.5								45	55	30
1996	Africa (n = 118)	5.9	8.5	5.1	28.1	4.2	27.1	7.6	11	0.8	1.7	19	81	47
1996	World (not Africa) (n = 57)	24.5	12.3	1.8		1.8	40.3	15.7		1.8	1.8	44.6	55.4	47
1998	South Africa (n = 255)	27	6.3		0.4		16.8	1.6	46.3	1.6		30.2	69.8	11
2001	Bulgaria (n = 42)	35.7	4.8				50	4.7		4.8		45.2	54.8	27
2002	North Dakota (n = 161)		1.9				91.9				6.2		100	29
2003[f]	Spain (n = 29)	3.4	6.9				65.5	20.7			3.4	27.6	72.4	38

[a] n is the number of isolates examined.
[b] NT, nontypeable (the isolates do not belong to any known race).
[c] *avrPphF* is present in races 1, 5, 7, and 9. Most of the nontypeable strains were not taken into account to calculate the percentages; the only exception was the nontypeable strain from Spain, which contained *avrPphF*.
[d] Race 2 was first described in 1964.
[e] Only three races of *P. syringae* pv. phaseolicola were distinguished in this work.
[f] Only toxigenic strains were taken into account.

produce a chimeric functional transposase. However, it is unlikely that this transposase could effectively recognize the dissimilar inverted repeats of the chimeric element. In support of this, we did not find any other copy of the chimeric mobile element in the genome of *P. syringae* pv. phaseolicola 1448A. Alternatively, the deletions could have been produced by recombination between the chimeric element and an adjacent copy of IS*Psy17* or through a failed transposition event mediated by the chimeric transposase. Independent of the mechanism, the deletions mediated by this element appear to occur or to be selected from the pathogen population only rarely because we found only two differentiable deletion genotypes among 72 *P. syringae* pv. phaseolicola strains (Fig. 1).

The use of resistant cultivars as the sole control method exerts a strong selective pressure on the populations of plant pathogens, leading in many cases to the breakdown of resistance and to dramatic changes in the race structure of the pathogen (34). In bacteria, race shifts as a result of cultivar resistance have been well documented for *Xanthomonas campestris* pv. *vesicatoria* and *X. campestris* pv. *oryzae* (12, 35), even within a single growing season (28). Likewise, the widespread deployment since the 1940s of dry bean cultivars displaying the *R1*-based resistance matching *avrPphF* (56) predictably led to the breakdown of resistance in the United States, resulting in the description in 1964 of a new strain classified as race 2, which lacks *avrPphF* (9, 54). It is tempting to speculate that the first identified strain of race 2 was the putative ancestor with the first deletion that eliminated *avrPphF*. Isolates belonging to race 2 were soon described in other parts of the world (8, 16, 37), and in subsequent years new races were described. So far, nine races of the pathogen have been characterized (24, 47), and there is evidence that further differentiation may be possible (14, 29, 33, 38, 47). The putative existence of a common ancestor for most of the *P. syringae* pv. phaseolicola strains lacking *avrPphF* implies that at least some of the further genetic changes that led to the emergence of the modern races 2, 3, 4, 6, and 8 necessarily occurred after the deletion of *avrPphF*. This is clear for *avrPphE*, because the abolition of its avirulence phenotype in races 1, 3, 6, 8, and 9 occurred by independent mutations, including mutations in strains 1301A (race 3), 1448A (race 6), and 2656A (race 8), which were also analyzed in this study and contained identical deletions removing *avrPphF*. The *avrPphB* gene, which is present only in races 3 and 4, also appears to be a recent acquisition (22). This gene is in a chromosomal region larger than 40 kb located within a tRNA locus, and it is in different areas of the genome in distinct isolates and races of *P. syringae* pv. phaseolicola. The other two putative genes defining race structure, avirulence genes 4 and 5, have not been cloned yet (51).

From the few inventories of the races of *P. syringae* pv. phaseolicola present in different production areas of the world (Table 2), it is apparent that the proportion of strains lacking *avrPphF* in the field has increased considerably in the last 40 years. It does not seem likely, however, that deletion of *avrPphF* by itself would confer a significant selective advantage that would justify this dominance, because not all the races lacking *avrPphF* are found with equal frequency (Table 2). In particular, race 6 is in general the race most frequently found worldwide (Table 2), suggesting that other genetic changes, besides or in addition to the deletion of *avrPphF*, could be responsible for its predominance. Nevertheless, the reasons for the preponderance of one race in given areas are currently elusive, and these reasons could include the use of local cultivars to the detriment of imported varieties or with genetic backgrounds that favor certain genotypes of the pathogen. Some unexplained examples are the unusual high frequency of race 8 in South Africa (11) and the presence of *avrPphF* in all the nontoxigenic isolates of *P. syringae* pv. phaseolicola, which are prevalent in Spain (38).

ACKNOWLEDGMENTS

We thank T. Goszczynska and B. Völksch for kindly supplying bacterial strains and Nerea Mateo for excellent technical assistance. We are very grateful to The Institute for Genomic Research for granting access to the draft sequence of the genome of *P. syringae* pv. phaseolicola 1448A, from which we greatly benefited.

This work was supported by grant AGL2001-1948-CO2-01 from the Spanish Ministerio de Ciencia y Tecnología to J.M. and by a grant from the UK Biotechnology and Biological Sciences Research Council to J.W.M.

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