Research Note

A Race-Specific Insertion of Transposable Element IS801 in *Pseudomonas syringae* pv. *phaseolicola*

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The isolation and cloning of a random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) band specific for *Pseudomonas syringae* pv. *phaseolicola* race 1 allowed us to design a pair of primers that amplify 1.2-kb race 1–specific and 2.7-kb race 2–specific fragments, providing a rapid method for the identification of races by standard PCR methods. Restriction analysis revealed identical endonuclease sites in both fragments but the race 2 fragment contains a 1.5-kb insertion, identified as transposable element IS801 by sequence comparison. One complete and one partial open reading frame (ORF), each with a high probability of encoding a protein, have been identified in the 1.2-kb fragment common to both race 1 and race 2 sequences. As IS801 disrupts the partial ORF in the race 2 fragment, the complete sequence of this ORF has been obtained as well as its promoter region. The possibility that it may encode an avirulence gene is discussed as well as the role of transposable elements in pathogen evolution.

Additional keywords: halo-blight disease, pathogenicity, *Phaseolus vulgaris*.

Pseudomonas syringae pv. *phaseolicola* is the causal agent of halo-blight in common bean (*Phaseolus vulgaris* L.) and shows natural variability in its reaction to bean cultivars, which differentiates it into several races. Race 1 and race 2 were described by Walker and Patel (1964) and recognized by, respectively, their ability or inability to induce hypersensitive reaction (HR) in the cultivar Red Mexican. Taylor and Teverson (1985) described the identification of race 3 from African isolations of *P. syringae* pv. *phaseolicola*, which was recognized by the induction of HR in cv. Tendergreen, highly susceptible to races 1 and 2. These race-specific interactions are proposed to be governed by a pair of dominant corresponding genes: an avirulence (*avr*) gene in the pathogen and a resistance (*R*) gene in the host. Based on the presence of five putative *avr* genes in *P. syringae* pv. *phaseolicola* and five matching *R* genes in *P. vulgaris*, the existence of at least eight races

has been suggested (Jenner et al. 1991; Mansfield et al. 1994). Molecular cloning of avirulence genes *avrPphA.R1*, *avrPphE*, and *avrPphB.R3*, which correspond to resistance genes R1, R2, and R3, respectively, has strengthened the gene-for-gene relationship proposed in bean halo-blight disease (Shintaku et al. 1989; Mansfield et al. 1994; Jenner et al. 1991).

Methods used to identify *P. syringae* pv. *phaseolicola* races include pathogenicity tests in which pods or cotyledons of several bean cultivars are inoculated to express compatible (water-soaked lesions) or incompatible (HR) interactions after several days. Nucleotide sequences of avirulence genes cannot be used to develop rapid identification methods based on the polymerase chain reaction (PCR), as apparently nonfunctional homologues of *avrPphE* are present within all races of *P. syringae* pv. *phaseolicola* (Mansfield et al. 1994). Here we report two oligonucleotides that amplify a genomic region with different sizes for race 1 and race 2 due to a specific insertion of transposable element IS801, allowing a rapid race identification by PCR analysis of heat-killed bacteria taken directly from infected bean plants. The insertion of IS801 in race 2 disrupting an open reading frame (ORF) with a high probability to be expressed raises the possibility that this ORF encodes an avirulence gene, as it shows promoter region features described and shared for avirulence genes.

Principal bacterial strains and plasmids used or constructed in this study are shown in Table 1. *Escherichia coli* was grown at 37°C on Luria-Bertani (LB) medium (Maniatis et al. 1982), in liquid culture or on agar plates. Isolates of *P. syringae* pv. *phaseolicola* were grown routinely on King's medium B (KB) at 25°C in liquid cultures or on agar plates (King et al. 1954). Ampicillin was used in LB medium at 50 µg/ml.

A race 1–specific band was identified with the random amplified polymorphic (RAPD)–PCR technique in a study of genetic polymorphisms in 23 isolates of *P. syringae* pv. *phaseolicola* collected from Spanish bean fields (Table 1). This band of approximately 1.2 kb was obtained in all PCR reactions set up with 10mer primer OPB-7 (Operon Technologies, Alameda, CA) when genomic DNA from race 1 isolates was used. The method of Zhu et al. (1993) was used to extract genomic DNA of *P. syringae* pv. *phaseolicola* strains and standard recombinant DNA methods were generally performed as described by Sambrook et al. (1989).

The band was gel extracted (Bovie and Lew 1995), labeled with digoxigenin, and used as a probe against Southern blots

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Nucleotide and/or amino acid sequence data have been submitted to EMBL as accession number Y09452.

Table 1. Bacterial strains, plasmids, and oligonucleotides used in this study

Designation	Relevant characteristics	Source or reference ^a
<i>Escherichia coli</i> JM101	<i>supE</i> , <i>thi</i> , <i>D(lac-proAB)</i> , F' [<i>TraD36</i> , <i>proAB</i> ⁺ , <i>lacI</i> ^q , <i>lacZDM15</i>]	Messing 1979
<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> Spanish strains		
Ca1	Race 1	This study
Ca2	Race 1	This study
Ca3	Race 2	This study
Ca4	Race 1	S.I.A.
Ca5	Race 2	This study
Cv1	Race 1	S.I.A.
Cv2	Race 2	S.I.A.
Fa1	Race 1	This study
Fa2	Race 2	This study
Go1	Race 1	This study
Gr1	Race 1	S.I.A.
Ma1	Race 1	This study
Ma2	Race 1	This study
Mo1	Race 2	S.I.A.
Pi1	Race 2	This study
Ri1	Race 1	This study
Ri2	Race 1	This study
Ri3	Race 1	This study
Ri4	Race 1	This study
Ri5	Race 2	S.I.A.
Ri6	Race 2	S.I.A.
Ri7	Race 2	This study
Rv1	Race 1	This study
U.S. strains		
0285-1	Race 2	N. T. Keen
G50	Race 2	N. T. Keen
British strain 882	Race 2	S.I.A.
Plasmids		
pUC119	Ap ^r cloning and sequencing vector	Sambrook et al. 1989
pGEM-T	Ap ^r PCR cloning vector	Promega
pRA1-4, 5, 6, and 8	1.2-kb PCR product from race 1 cloned in pGEM-T	This study
pR1EE-4, 5, 6, and 8	0.5-kb <i>EcoRI-EcoRI</i> frag- ment from pRA1-# cloned in pUC119	This study
pRA2-1, 2, 4, and 5	2.7-kb PCR product from race 2 cloned in pGEM-T	This study
pR2EE-1, 2, 4, and 5	0.5-kb <i>EcoRI-EcoRI</i> frag- ment from pRA2-# cloned in pUC119	This study
pINS-1, 2, 4, and 5	1.7-kb <i>EcoRI-PstI</i> fragment from pRA2-# cloned in pUC119	This study
pRK415	Tc ^r broad host range vector, mob ⁺	Keen et al. 1988
pRK2013	Km ^r , Tra ⁺ , helper plasmid	Ditta et al. 1980
pORF-25	1.5-kb fragment containing the putative <i>avr</i> gene cloned in pRK415	This study
Oligonucleotides		
OPB-7	5'-GGT GAC GCA G-3'	Operon
RP-1	5'-GGT GAC GCA GCA ATT GA-3'	This study
RP-2	5'-GGT GAC GCA GGA AAC GA-3'	This study

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containing RAPD-PCR reactions from race 1 and race 2 isolates. Hybridization results confirmed that the 1.2-kb band was not present in race 2 isolates (data not shown). The gel-extracted, 1.2-kb band was cloned into pGEM-T (Promega, Madison, WI) following the manufacturer's protocol and was partially sequenced in order to design longer primers for standard PCR reactions. These primers, designated as RP-1 and RP-2 (Table 1), were designed with OLIGOTEST software (Beroud et al. 1990) and synthesized by the PNACL facility at the University of Leicester.

PCR reactions were carried out in a Perkin-Elmer (Norwalk, CT) 480 thermocycler with *Taq* polymerase (Promega, Madison, WI) in final volumes of 25 µl containing 2.5 µl of 10× PCR buffer (100 mM Tris-HCl; 500 mM KCl; 1% Triton X-100; pH 9.0), 1.5 µl of MgCl₂ (25 mM), 2.5 µl of dNTPs (25 mM each), 1 µl of RP-1 oligonucleotide (5 pmol/µl), 1 µl of RP-2 oligonucleotide (5 pmol/µl), 50 ng of template DNA, and 0.1 µl of enzyme (5 U/µl). PCR reactions were incubated for 30 cycles at the following: 94°C, 1 min; 60°C, 1 min; 72°C, 3 min. Amplified products were electrophoresed in 1% agarose gels and visualized by ethidium bromide staining.

When primers RP-1 and RP-2 were used in PCR reactions with extension times of 3 min and with genomic DNA from races 1 and 2, the expected 1.2-kb band was obtained for all race 1 isolates but a 2.7-kb band was amplified for all race 2 isolates (Fig. 1). The same bands were obtained when genomic DNA in PCR reactions was substituted by heat-killed bacteria cultures or heat-killed bacteria suspensions taken from infected plants. In this case, 50 µl of overnight cultures or bacterial suspensions directly taken from infected plants was incubated at 95°C for 15 min and then 1 µl was incorporated into PCR reactions. Bacterial suspensions were obtained by soaking silver-colored halo-blight lesions of bean leaves with 0.5 ml of sterile water and recovering as much volume as possible with a micropipet.

A restriction map was made for both the 1.2-kb race 1 fragment and the 2.7-kb race 2 fragment with the restriction endonucleases *ApaI*, *EcoRI*, *PstI*, and *SphI* in single and double digestions (Fig. 2A). No variability of restriction sites was found between different isolates of races 1 and 2. Location of restriction sites was similar for both race-specific fragments but revealed the presence of a 1.5-kb insertion in the race 2 fragment. The insertion was located in an *EcoRI-PstI* fragment of approximately 0.2 kb in race 1 while the matching fragment in race 2 showed a size of 1.7 kb.

PCR-amplified, race-specific bands were subcloned with *EcoRI* and *PstI* restriction sites and their nucleotide sequences were obtained, except the central 900 bases of the 1.5-kb insert. DNA sequences were determined by the dideoxynucleotide method (Sanger et al. 1977), usually with Sequenase 2.0 (USB, Cleveland, OH) incorporating deoxyadenosine 5'-([α-³⁵S] thio) triphosphate (1,200 Ci/mmol; Amersham, Little Chalfont, UK) according to the manufacturer's protocol. Polyacrylamide gels contained 6% acrylamide and 7 M urea. Compressions were resolved with dITP to replace dGTP and four independent clones were sequenced to avoid *Taq* polymerase errors.

Apart from the 1.5-kb insertion, the sequences of both fragments were identical. Three hundred bases at each end of the 1.5-kb insertion were sequenced, and found to match the

corresponding ends of the 1,517-bp published sequence for insertion element IS801. Restriction sites for *Ava*I, *Pvu*II, *Sst*II, and *Acc*I were identified in IS801 sequence by computer analysis and were also found by restriction analysis in the 1.5-kb, race 2-specific insertion (Fig. 2A).

Sequences of 3' and 5' ends from the race 2-specific, 1.5-kb insert were identical to corresponding IS801 sequences except for two base substitutions. Each of these is located in one of the 5-base direct repeats present at each end of the transposon as described by Romantschuk et al. (1991): Cgaac instead of Tgaac in the left terminus and tgCac instead of tgAac in the right terminus. The same modified repeat elements were found in the sequence of the race 1 fragment but separated by 6 bases instead of the IS801 sequence.

ORFs present in the 1,195-bp sequence obtained for the race 1 fragment amplified with primers RP-1 and RP-2 were analyzed with GeneMark software (Borodovsky et al. 1994) for their probability to encode proteins. The GeneMark method has shown a high accuracy for gene prediction, as 303 out of 354 ORFs of *E. coli* predicted to encode proteins have been confirmed so far (Borodovsky et al. 1994, 1995). Two regions in the same frame showed an average $P = >0.5$, the threshold used. One of these included a complete ORF en-

coding a 208 amino acid protein of 23.3 kDa (ORF-2; Fig. 2B). Data base comparison with the BLAST algorithm revealed significant homology with two proteins, the hypothetical 23.1-kDa protein ypgQ (accession no. P54168) located in the *Bacillus subtilis* DNA region between the *serA* and *kdg* loci and the hypothetical 25.9-kDa protein Yedj (accession no. P76333) located in the Seru-dcm intergenic region of *E. coli* (Borodovsky et al. 1995). The region from primer RP-1 to the next in-frame stop codon showed a GeneMark average probability to encode a protein of 0.71 and includes the location where IS801 is inserted in race 2 (Fig. 2B), but the start codon of this highly probable ORF would be located upstream of RP-1.

A plasmid minilibrary of *Eco*RI-digested genomic DNA was screened with a 312-bp fragment from RP-1 to *Eco*RI site in position 1116 (Fig. 2B) as the radioactive-labeled probe and a single positive clone containing a 1.1-kb insert was isolated and sequenced. The insert included the 312-bp probe used, as well as the initiation codon and the promoter region of the ORF that is disrupted by the insertion of IS801 in race 2 (Fig. 2B), allowing completion of the sequence of ORF-1, of which the probability to encode a protein according to the GeneMark method was 0.71.

The completed ORF nucleotide sequence and the translated protein sequences were compared against the EMBL and SWISS-PROT data bases, respectively, with FASTA and BLAST methods, but no significant homology was found. Protein motif searches were also unsuccessful. Analysis of the promoter region of ORF-1 showed the presence of the GG-10 bp-GC motif that is recognized by the σ^{54} RNA polymerase holoenzyme and the presence of a sequence homologous to the original version of the "harp box" motif that is found in the promoter region of *hrp* and avirulence genes (Fig. 2B).

A fragment including the complete ORF-1 (from position 1 to *Eco*RI site in 1566; Fig. 2B) was cloned in the broad-host-range plasmid pRK415 (plasmid pORF-25) and transformed into *P. syringae* pv. *phaseolicola* G50 strain (race 2) by triparental mating with *E. coli* HB101 carrying pRK2013 as the mobilizing strain, following the method described by Keen et al. (1992). G50 transconjugants as well as the original G50 strain were used to inoculate Red Mexican (cultivar resistant to race 1) cotyledons. Four-day-old cotyledons attached to the plant were injected with suspensions of bacteria (5×10^8 ml⁻¹) in sterile water, using a hypodermic syringe and fine-gauge needle. Water-soaked symptoms at inoculation sites in cotyledons were assessed 3 days after injection. Cotyledons infected by original G50 strain as well as cotyledons infected by G50 transformants containing pORF-25 developed water-soaking lesions.

Restriction and sequence analysis of the specific bands amplified for race 1 and race 2 *P. syringae* pv. *phaseolicola* with primers RP-1 and RP-2 revealed that both bands represent the same genomic DNA fragment with the presence of transposable element IS801 inserted in the race 2 band. This IS801 insertion has been found not only in Spanish isolates of race 2 but also in two U.S. (0285-1 and G50) and one British (882) strains of *P. syringae* pv. *phaseolicola* race 2. Therefore, it seems to be a characteristic of race 2 and it would be in concordance with the proposed origin of race 2 in the U.S. and its later spread to other countries (Patel and Walker 1965; Guthrie and Fenwick 1967; Wallen 1968).

Fig. 1. Race-specific products obtained by polymerase chain reaction (PCR) with primers RP-1 and RP-2, and genomic DNA extracted from *Pseudomonas syringae* pv. *phaseolicola* strains. After amplification, PCR reactions were loaded into a 1% agarose gel and electrophoresed at 70V, and products were visualized by ethidium bromide staining. First and last lanes in upper and lower rows correspond to molecular markers. Race 1 strains (from left to right): upper row, Ca1, Ca2, Ca4, Cv1, Fa1, Go1, and Gr1; lower row, Ma1, Ma2, Ri1, Ri2, Ri3, Ri4, and Rv1. Race 2 strains (from left to right): upper row, Ca3, Ca5, Cv2, Fa2, Mo1, and Pi1; lower row, Ri5, Ri6, Ri7, 0285-1, G50, and 882.

IS801 was originally characterized in LR700 strain of *P. syringae* pv. *phaseolicola* and two sequences, TGAAC and TGGAC, were described as targets with IS801 inserting immediately preceding these sequences. It was indicated that no

duplication of target sequences would occur in the transposition event if the motif TGAAC was also present in the left end of IS801 (Romantschuk et al. 1991). Based on homology between IS801 and IS91, Mendiola et al. (1992) proposed that

Fig. 2. A, Restriction maps of polymerase chain reaction (PCR)-amplified products for race 1 and race 2 strains. The 1.5-kb insert present in race 2 is indicated by a dotted line. After homology was discovered by sequence comparison of 3' and 5' ends of this insert with insertion element IS801, the 1.7-kb *Pst*I-*Eco*RI fragment from race 2 was digested with a different set of endonucleases to compare with targets identified by computer analysis in the IS801 sequence. **B,** Location of open reading frames ORF-1 and ORF-2 within the 1,999-bp sequence obtained after integration of the 1.2-kb sequence of race 1 PCR-amplified product with RP-1 and RP-2 primers (striped and gray areas) and the 1.1-kb sequence of a positive genomic clone (black and striped areas) selected with the fragment from RP-1 to *Eco*RI site in 1116 (striped area) as labeled probe. Features marked include region where IS801 is inserted in race 2 disrupting ORF-1, consensus motif for the original "harp box," and potential σ^{54} promoter. Sequence data have been submitted to EMBL as accession number Y09452.

the first T derives from the target DNA, and that GAAC was present at the left end of IS801 as well as being a target sequence, together with GGAC. Thus, there is no duplication of target sequences after IS801 transposition. Our results confirmed GAAC as the left end of IS801, but the target sequence in this case was not GAAC or GGAC but GCAC, with a T preceding the tetramer as in the three insertions described by Romantschuk et al. (1991). With the information of all four described insertions, it seems that IS801 inserts between T and G in the target sequence TGVAC (V = G, A or C). Probably when a higher number of insertions are available the target sequence will be defined as TGNAC (N = G, A, T or C). Comparison between race 1-specific and race 2-specific band sequences has indicated that the IS801 insertion involved a deletion of 11 bp. No deletions were reported by Romantschuk et al. (1991) for the three transposition events they analyzed. Presence of the motif GAAC, identical to the left end of IS801, only 6 bases away from the target sequence, could have caused some kind of error during the transposition process.

IS801 is located in race 2, interrupting an ORF with high probability to encode a protein according to the GeneMark method. The possibility exists that this ORF encodes an avirulence gene of race 1 that allows race 2 pathogens to infect race 1-resistant bean cultivars as this avirulence gene is nonfunctional in race 2. According to Jenner et al. (1991), *P. syringae* pv. *phaseolicola* race 1 strains possess only one avirulence gene, *A1*. Shintaku et al. (1989) reported the cloning and partial characterization of an avirulence gene for race 1 located in a 21.2-kb genomic DNA fragment but no sequence data are available to compare with the above ORF. Lack of homology between this ORF and identified avirulence genes is not significant and these genes themselves are generally nonhomologous (Keen 1990; Long and Staskawicz 1993; Mansfield et al. 1994). However, conserved motifs are present in their promoter regions, indicating common regulation (Shen and Keen 1993; Xiao and Hutcheson 1994).

The promoter region of the ORF interrupted by IS801 contains an appropriately positioned consensus sequence (GG-10bp-GC) that interacts with the σ^{54} RNA polymerase holoenzyme. This consensus sequence is present in the promoter region of some but not all the *P. syringae* avirulence genes analyzed (Shen and Keen 1993). Another motif located in the promoter region of avirulence genes is the "harp box" consensus sequence GGAACC-15N-CCAC, which is a revised version of the original motif described by Fellay et al. (1991). The promoter of the above ORF contains the original version of the "harp box" as it does the *P. syringae*

pv. *phaseolicola* avirulence gene *avrPphE* (Mansfield et al. 1994). Those two features located in the promoter region of the IS801 interrupted ORF support that it may encode an avirulence gene.

However, transconjugants of *P. syringae* pv. *phaseolicola* race 2 carrying this ORF did not induce HR in Red Mexican cotyledons, as would be expected if it encoded and expressed the avirulence gene *A1*. It may be that the ORF is not being expressed in the transconjugants, as the clone insert only included the ORF and its expression may be regulated by proximal genes. Expression experiments as well as mutagenesis of the ORF in race 1 strains are needed to completely rule out its function as *A1*. A second possibility is that this ORF encodes the product of an avirulence gene that did not match the *R1* gene for resistance to halo-blight disease in *P. vulgaris*. *P. syringae* pv. *phaseolicola* race 2 and transconjugants expressing the ORF need to be tested for alteration in their virulence toward several cultivars and lines of *P. vulgaris* in order to investigate this option. Ultimately, the ORF analyzed here may be involved in the expression of *A1* and this avirulence gene would also be non-functional in race 2 strains.

The transposase gene of IS801 has been identified by sequence homology with the IS91 transposase (Mendiola et al. 1992). We have compared the IS801 transposase promoter region with the consensus sequence of the *E. coli* σ^{32} promoter, also known as the heat-shock promoter, and with the IS53 transposase promoter that has been described in *P. syringae* pv. *savastanoi* as homologous to the *E. coli* heat-shock promoter (Soby et al. 1993) (Fig. 3). Homology with both the -10 and -35 regions indicated the possibility that the IS801 transposase could be heat-shock-inducible or stress-inducible.

Insertions of IS801 will be permanent, as it uses a replicative transposition mechanism in which a copy of the element remains in the old position (Mendiola et al. 1994). Transpositions of such transposons into avirulence genes will generate new pathogen races as these genes are inactivated. This kind of transposon mutagenesis for avirulence genes has been reported previously in *Xanthomonas campestris* pv. *vesicatoria*, whose race 2 strains carry the avirulence gene *avrBs1* that matches the pepper resistance gene *Bs1*. Four spontaneous race 2 mutants that had become virulent and able to overcome *Bs1* were found to be mutated by the insertion of transposon IS476 into *avrBs1* (Kearney et al. 1988; Kearney and Staskawicz 1990). This mechanism allows plant pathogens to evolve to avoid recognition and response by hosts overcoming their resistance genes.

	-35	spacing (bp)	-10
<i>E. coli</i> σ^{32}	TnnCnCnCTTGAA	13-15	CCCC-ATnT
	+ + + ++		+ + ++ +
IS801	TCGCCC-GTTCTT	21	CATCGATT
	+ +++ ++		+ + +
IS53	TGACCCAGTAGAG	20	GCCC-AGGT

Fig. 3. Comparison of the *Escherichia coli* σ^{32} heat-shock consensus promoter with the 5' regions of transposases from insertion elements IS53 and IS801. Bases identical to the consensus sequence are in bold. Identity between IS801 and either IS53 or the consensus sequence is indicated by + symbols. The -10 and -35 regions are indicated, as is the spacing between the regions.

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LITERATURE CITED

- Beroud, C., Antignac, C., Jeanpierre, C., and Junien, C. 1990. Un programme informatique pour la recherche d'amorces pour l'amplification par PCR. *Medicine/Sciences* 6:901-903.
- Borodovsky, M., McIninch, J. D., Koonin, E. V., Rudd, K. E., Médigue, C., and Danchin, A. 1995. Detection of new genes in a bacterial genome using Markov models for three gene classes. *Nucleic Acids Res.* 23:3554-3562.
- Borodovsky, M., Rudd, K. E., and Koonin, E. V. 1994. Intrinsic and extrinsic approaches for detecting genes in a bacterial genome. *Nucleic Acids Res.* 22:4756-4767.
- Bovie, J. S., and Lew, A. M. 1995. An inexpensive alternative to glass-milk for DNA purification. *Trends Genet.* 11:8.
- Ditta, G., Stanfield, S., Corbin, D., and Helinski, D. R. 1980. Broad host range DNA cloning system for gram-negative bacteria: Construction of a gene bank of *Rhizobium meliloti*. *Proc. Natl. Acad. Sci. USA* 77:7347-7351.
- Fellay, R., Rahme, L. G., Mindrinos, M. N., Frederick, R. D., Pisi, A., and Panopoulos, N. J. 1991. Genes and signals controlling the *Pseudomonas syringae* pv. *phaseolicola*-plant interaction. Pages 45-52 in: *Advances in Molecular Genetics of Plant-Microbe Interactions*. Vol. 1. H. Hennecke and D. P. S. Verma, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Guthrie, J. W., and Fenwick, H. S. 1967. Pathogenicity of Idaho isolates of *Pseudomonas phaseolicola*. *Plant Dis. Rep.* 51:591-593.
- Jenner, C., Hitchin, E., Mansfield, J., Walters, K., Betteridge, P., Teverson, D., and Taylor, J. 1991. Gene-for-gene interactions between *Pseudomonas syringae* pv. *phaseolicola* and *Phaseolus*. *Mol. Plant-Microbe Interact.* 4:553-562.
- Kearney, B., Ronald, P. C., Dahlbeck, D. and Staskawicz, B. J. 1988. Molecular basis for evasion of plant host defence in bacterial spot disease of pepper. *Nature* 332:541-543.
- Kearney, B., and Staskawicz, B. J. 1990. Characterization of IS476 and its role in bacterial spot disease of tomato and pepper. *J. Bacteriol.* 172:143-148.
- Keen, N. T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. *Annu. Rev. Genet.* 24:247-263.
- Keen, N. T., Shen, H., and Cooksey, D. A. 1992. Introduction of cloned DNA into plant pathogenic bacteria. Pages 45-50 in: *Molecular Plant Pathology: A Practical Approach*. Vol. 1. S. J. Gurr, M. J. McPherson, and D. J. Bowles, eds. Oxford University Press, New York.
- Keen, N. T., Tamaki, S., Kabayashi, D., and Trollinger, D. 1988. Improved broad host-range plasmids for cloning in Gram-negative bacteria. *Gene* 70:191-197.
- King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301-307.
- Long, S. R., and Staskawicz, B. J. 1993. Parasites at the table. *Cell* 73:921-935.
- Maniatis, T. A., Fritsch, E. F., and Sambrook, J. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Mansfield, J., Jenner, C., Hockenhull, R., Bennett, M. A., and Stewart, R. 1994. Characterization of *avrPphE*, a gene for cultivar-specific avirulence from *Pseudomonas syringae* pv. *phaseolicola* which is physically linked to *hrpY*, a new *hrp* gene identified in the halo-blight bacterium. *Mol. Plant-Microbe Interact.* 7:726-739.
- Mendiola, M. V., Bernales, I., and de la Cruz, F. 1994. Differential roles of the transposon termini in IS91 transposition. *Proc. Natl. Acad. Sci. USA* 91:1922-1926.
- Mendiola, M. V., Jubete, Y., and de la Cruz, F. 1992. DNA sequence of IS91 and identification of the transposase gene. *J. Bacteriol.* 174:1345-1351.
- Messing, J. 1979. A multipurpose cloning system based on single-stranded DNA bacteriophage M13. *Recomb. DNA Tech. Bull.* 2:43.
- Patel, P. N., and Walker, J. C. 1965. Resistance in *Phaseolus* to halo blight. *Phytopathology* 55:889-894.
- Romantschuk, M., Richter, G. Y., Mukhopadhyay, P., and Mills, D. 1991. IS801, an insertion sequence element isolated from *Pseudomonas syringae* pathovar *phaseolicola*. *Mol. Microbiol.* 5:617-622.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. A. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain termination inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463-5467.
- Shen, H., and Keen, N. T. 1993. Characterization of the promoter of avirulence gene *D* from *Pseudomonas syringae* pv. *tomato*. *J. Bacteriol.* 175:5916-5924.
- Shintaku, M. H., Kluepfel, D. A., Yacoub, A., and Patil, S. S. 1989. Cloning and partial characterization of an avirulence determinant from race 1 of *Pseudomonas syringae* pv. *phaseolicola*. *Physiol. Mol. Plant Pathol.* 35:313-322.
- Soby, S., Kirkpatrick, B., and Kosuge, T. 1993. Characterization of an insertion sequence (IS53) located within IS51 on the *iaa*-containing plasmid of *Pseudomonas syringae* pv. *savastanoi*. *Plasmid* 29:135-141.
- Taylor, J. D., and Teverson, D. M. 1985. Halo-blight of *Phaseolus* bean. Page 87 in: *Rep. Nat. Veg. Res. Sta. [for] 1984. NVRS*, Wellesbourne, UK.
- Walker, J. C., and Patel, P. N. 1964. Splash dispersal and wind as factors in epidemiology of halo-blight of bean. *Phytopathology* 54:140-141.
- Wallen, V. R. 1968. Identification of races of *Pseudomonas phaseolicola* from Quebec bean fields. *Can. Plant Dis. Surv.* 48:97-98.
- Xiao, Y. X., and Hutcheson, S. W. 1994. A single promoter sequence recognized by a newly identified alternate sigma factors directs expression of pathogenicity and host-range determinants in *Pseudomonas syringae*. *J. Bacteriol.* 176:3089-3091.
- Zhu, H., Feng, Q., and Zhu, L. H. 1993. Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride. *Nucleic Acids Res.* 21:5279-5280.