Organization of the *hrp* Gene Cluster and Nucleotide Sequence of the *hrpL* Gene from *Pseudomonas syringae* pv. *morsprunorum*

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ABSTRACT

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Pseudomonas syringae pv. *morsprunorum* PM7 is pathogenic to cherry and induces the hypersensitive response (HR) in tobacco. Six of 1,300 Tn*5* mutants from strain PM7 neither elicit the HR in tobacco nor produce necrotic lesions in cherry plantlets. Plasmid pPM419, isolated from a genomic DNA library of wild-type strain PM7, restored the ability to cause a HR in five of the mutants. Restriction enzyme analysis of pPM419 revealed a 37-kilobase (kb) insert of genomic DNA from *P. s. morsprunorum* PM7. Tn*3*-spice mutagenesis of pPM419 and a second plasmid clone, pPM41, followed by marker exchange into the genome of *P. s. morsprunorum* PM7, indicated a 22-kb DNA fragment from *P. s. morsprunorum* PM7 has genes for elicitation of necrotic lesions in cherry plantlets and HR in tobacco. Complementation studies revealed that the *hrp* region of *P. s. morsprunorum* PM7 is organized into eight putative transcriptional units. Units II, VI, and VII exhibit DNA homology with the *hrpI, hrpH,* and *hrpZ* genes, respectively, of *P. s. syringae* 61. The nucleotide sequence of *hrpL,* the first transcriptional unit in the *hrp* cluster of *P. s. morsprunorum* PM7, encodes a polypeptide of 185 amino acids that exhibits 92% identity and 96% similarity with HrpL of *P. s. syringae* 61. Two transcriptional start sites, P1 and P2, located 63 and 25 bp upstream of the *hrpL* start codon, were identified by primer extension analysis. The −12 and −24 regions of the putative P2 promoter resemble a σ^{54} consensus sequence.

Pseudomonas syringae pv. *morsprunorum* (Wormald) Young et al is a pathogen of cherry and, like other pathovars of *P. syringae,* induces the hypersensitive response (HR) in tobacco (18). Genes controlling pathogenicity and elicitation of the HR are called *hrp* genes (21). Very little is known about the *hrp* cluster in *P. s. morsprunorum,* but some of the basic genetics of HR and pathogenicity have been determined for other gram-negative plant-pathogenic bacteria including *Erwinia amylovora* (30), *Pseudomonas solanacearum* (3), pathovars of *P. syringae* (11,13,21), and *Xanthomonas campestris* (2). *hrpZ* encodes for the protein harpin$_{pss}$, which functions as an elicitor of HR (8), *hrpI* a family of proteins represented by *Yersinia pestes* LcrD (12), *hrpH* a family of proteins represented by *Y. enterocolitica* YscC (10), and *hrpL* regulatory proteins in *P. s. tomato* (14,25), *P. s. glycinea* (13), and *P. s. phaseolicola* (4,23). Recently, Xiao et al (34) reported that the HrpL protein of *P. s. syringae* 61 exhibits 25% identity with AlgU, a putative alternate sigma factor of *P. aeruginosa.* Our study provides evidence for similarities between transcriptional units in the *hrp* cluster of *P. s. morsprunorum* and the *hrpI, hrpH, hrpL,* and *hrpZ* genes of *P. s. syringae.* In addition, we provide evidence for two transcriptional initiation sites for the *hrpL* gene in *P. s. morsprunorum.*

MATERIALS AND METHODS

Bacterial strains, plasmids, and cultural conditions. Bacterial strains and plasmids are described in Table 1. Strains of *E. coli* were grown at 37 C in Luria-Bertani broth (LB) (22) and strains of *P. s. morsprunorum* were grown at 25 C in King's medium B (KB) (15) containing the appropriate antibiotics at the following concentrations (μg/ml): ampicillin (100); chloramphenicol (20); kanamycin (20); nalidixic acid (15); rifampicin (100); spectinomycin (100); and tetracycline (20).

DNA manipulations. Plasmid DNA was extracted by alkaline lysis followed by cesium chloride gradient centrifugation (22). Genomic DNA was prepared by the method of Wilson (33). Restriction enzymes, T4 DNA ligase, and a random primed DNA labeling kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) were used as specified by the manufacturer. Southern and colony hybridizations were performed on GeneScreen Plus membranes as recommended by the manufacturer (New England Nuclear, Boston, MA).

General experimental methods. Tn*5* mutants of *P. s. morsprunorum* PM7 were generated by using pSUP1011 (27). Saturation mutagenesis with Tn*3*-spice and ice nucleation tests were conducted according to Lindgren et al (20). Genomic libraries were constructed by ligating *Sau*3A partially digested DNA into *Bam*HI-digested cosmid vector pLAFR3 (29). The ligation mixture was packaged in vitro with the Packagene lamda DNA packaging system (Promega, Madison, WI). Marker exchange mutagenesis was conducted according to the method of Lindgren et al (21). The genetic organization of the *hrp* region was determined by complementation analysis of plasmid-borne Tn*3*-spice insertions and chromosomal Tn*3*-spice insertions. Transposon Tn*3*-spice–induced nonpathogenic *P. s. morsprunorum* PM7 mutants were conjugated with different *E. coli* strains harboring pPM419::Tn*3*-spice or pPM41::Tn*3*-spice in which the transposon insertions were in a region flanking the site of insertion in the recipient. The transconjugants were tested on tobacco for HR production. Complementation analysis was confirmed by further complementing each transcriptional unit from both directions and with subclones in some cases. For each transcriptional unit the direction of transcription was based on at least two independent Tn*3*-spice insertions. Double-stranded templates were sequenced using a Model 373A sequencer (Applied Biosystems Inc., Foster

City, CA). Deletion derivatives of the 1.6-kb *Bam*HI-*Sac*I fragment from transcriptional unit I were generated with the Erase-a-Base system (Promega). The nucleotide sequence of *hrpL* has been deposited in GenBank under accession number L36536. To identify the *hrpL* gene product, plasmids pT516 and pT616 were constructed by cloning the 1.6-kb *Bam*HI-*Sac*I fragment into pT7-5 and pT7-6 in opposite orientations relative to the T7 promoter. Expression of pT516 and pT616 in *E. coli* K38(pGP1-2) was performed as described by Tambor (31). The HR was tested in tobacco (17) and pathogenicity was tested on cherry plantlets (19).

Primer extension and S1 analysis. To isolate RNA from *P. s. morsprunorum* cells, strain PM7 was grown overnight in M9 medium supplemented with 10 mM glutamate at 22 C. Total RNA was isolated using the method of Gilman (6). Five independent RNA extractions were surveyed in the experiments. For primer extension, a ^{32}P-end-labeled 24-mer oligonucleotide (5′-TGATCAGCCGTCAGTTGACGGATA-3′), complementary to the coding strand starting 63 bases downstream from the *hrpL* translational start site (Fig. 1), was prepared using T4 polynucleotide kinase (Promega) (16). S1 analysis was conducted with

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics[a]	Reference
Strains of *Pseudomonas syringae* pv. *morsprunorum*		
PM7	From sour cherry in Michigan, spontaneous Rif[r]	This study
PM7.1 to PM7.6	PM7::Tn*5* Path[−b] HR[−c] Km[r] Rif[r]	This study
PMM72, 205, 297, 291, 317, 47, 681, and 273	Tn*3*-spice fusion in *hrp* groups I to VIII, respectively, of PM7	This study
Strains of *Escherichia coli*		
HB101	F[−] *recA* Sm[r]	22
SM10	C 600 *thi thr leu recA*, ,Muc[+] chromosomally integrated RP$_4$-2-Tc::Mu contains pSUP1011	27
C2110	Nal[r] *polA*[−]	28
K38	HfrC (λ)	24
Plasmids		
pSUP1011	Cm[r] Km[r] 12.1 kb	27
Tn*3*-spice	Sp[r] *inaZ*[+] pMB8 replicon	20
pRK2013	Km[r] *Tra*[+] ColE1 replicon	5
pLAFR3	IncP Tc[r] *cos*[+] *rlx*[+]	29
pPM419	27-kb fragment of PM7 containing *hrp* cluster in pLAFR3	This study
pPM41	30-kb fragment of PM7 with *hrp* cluster except group VII in pLAFR3	This study
pPM1	2.2-kb *Bam*HI fragment with *hrpL* from pPM419 in pLAFR3	This study
pT7-5	Derivative of pT7-1, T7 ϕ10 in opposite orientation as *bla*; Ap[r]	31
pT7-6	Same as pT7-5 except containing an opposite cloning cassette	31
pT516	1.6-kb *Bam*HI-*Sac*I fragment from pPM1 subcloned in pT7-5; T7 and *hrpL* promoters in same orientation	This study
pT616	1.6-kb *Bam*HI-*Sac*I fragment from pPM1 subcloned in pT7-6; T7 and *hrpL* promoters in opposite orientation	This study
pGP1-2	λ*Ic875* T7 RNA polymerase; Km[r]	32
pCPP2151	Containing *hrpH* of *P. s. syringae* 61	10
pKS5	Containing *hrpI* of *P. s. syringae* 61	Gift from H.-C. Huang
pSYH10	Containing *hrpZ* of *P. s. syringae* 61	8

[a]Ap[r], Cm[r], Km[r], Rif[r], Sm[r], Sp[r], Tc[r], resistant to ampicillin, chloramphenicol, kanamycin, rifampicin, streptomycin, spectinomycin, and tetracycline, respectively.
[b]No pathogenicity on cherry plantlets.
[c]No hypersensitive response in tobacco.

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                      .............................................
AAGCTGTAGTGATAAAAACGGCGTTGCGCAAAAAAATGTATTACAAAGAATTACAAATTTTAAAATAATCATATAAATCAGTAACTTAAATAATTATTTT 100
                                       ********  ********                                  P1

GGCTGGCACGGTTATCGCTATAGGGCTTGCACACCATTAAATTAAGGTAAGCCCATGTTTCCGAACCTAGTGATCCTTGATGTAACCCAGCCACGCCAGG 200
    -24         -12          P2            rbs         M  F  P  N  L  V  I  L  D  V  T  Q  P  R  Q  D  16

ACTCTTCGTCTGCCGGTATCCGTCAACTGACGGCTGATCAGATACAGATGCTCAGAGCGTTCATTCAGAAGCGCGTAATGAACCCGGATGATGTCGATGA 300
  S  S  S  A  G  I  R  Q  L  T  A  D  Q  I  Q  M  L  R  A  F  I  Q  K  R  V  M  N  P  D  D  V  D  D  49

CATCTTGCAATGCGTATTTCTGGAGGCCCTGCGCAACGAGCACAAGTTTCAACATGCCAGCAAGCCGCAGACCTGGTTGTGTGGTATTGCGTTGAACCTG 400
 I  L  Q  C  V  F  L  E  A  L  R  N  E  H  K  F  Q  H  A  S  K  P  Q  T  W  L  C  G  I  A  L  N  L   82

ATCCGTAATCACTTCCGCAAAATGTATCGCCAGCCCTATCAGGAAAGCTGGGAAGACGACGTTCATTCAGAGCTGGAATGGAATGGCGATATCACTCATC 500
I  R  N  H  F  R  K  M  Y  R  Q  P  Y  Q  E  S  W  E  D  D  V  H  S  E  L  E  W  N  G  D  I  T  H  Q 116

AGGTAGACGGGCACAGGCAATTGGCACGCGTCATCGCAGCCATTGATTGCTTGCCGTCGAACATGCAAAAAAGGGTCCTGGAAGTGTCGCTGGAGATGGA 600
  V  D  G  H  R  Q  L  A  R  V  I  A  A  I  D  C  L  P  S  N  M  Q  K  R  V  L  E  V  S  L  E  M  D 149

TGGCAATTATCAGGATACGGCGAACACGCTCGGCGTTCCGATTGGCACTGTGCGCTCGCGACTGTCCCGGGCACGGGTGCAATTGAAGCAGCAGATTGAC 700
 G  N  Y  Q  D  T  A  N  T  L  G  V  P  I  G  T  V  R  S  R  L  S  R  A  R  V  Q  L  K  Q  Q  I  D  182

CCGTTCGCCTGAGTGGATATCTGTCTGGAACCAACTCGCACGCAAAACCACACAGTTGCCATCCTTCACCACTTGGATGGCAACCATGCGTATATCCAGT 800
P  F  A  END                                                                                         185
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Fig. 1. Nucleotide sequence of first transcriptional unit (*hrpL*) in *hrp* gene cluster of *Pseudomonas syringae* pv. *morsprunorum* PM7. Deduced amino acid sequence is shown in one-letter code under first nucleotide of each codon. Transcriptional start sites, designated P1 and P2, are underlined. Putative promoter −12 and −24 regions of P2, and potential ribosome binding sequence (rbs), are underlined. An 8-bp repeated sequence in the −35 region of P1 is indicated with a line of asterisks. The highly conserved putative promoter sequence of *hrpL* in *P. s.* pv. *morsprunorum* PM7 and *P. s.* pv. *syringae* 61 is indicated using dots. Amino acids that form a potential helix-turn-helix motif are double-underlined.

the same primer and RNA as used for primer extension analysis (7).

RESULTS

Isolation of Tn*5*-induced HR⁻ mutants and clones complementing *hrp*⁻ mutants. After Tn*5* mutagenesis of *P. s. morsprunorum* PM7, 6 out of 1,300 (ca. 0.5%) transconjugants did not elicit the HR in tobacco leaves. One HR⁻ mutant (PM7.5) elicited necrosis in 13 of 16 cherry plantlets and five HR⁻ mutants (PM7.1, PM7.2, PM7.3, PM7.4, PM7.6) did not elicit necrosis in any of the cherry plantlets. Five mutants (PM7.1, PM7.2, PM7.4, PM7.5, PM7.6) were prototrophic and one (PM7.3) was auxotrophic. Southern analysis of genomic DNA indicated that each mutant contained a single Tn*5* insertion. Twenty cosmid clones that hybridized to Tn*5*-flanking DNA in mutant PM7.4 were mated with each of the six PM7 *hrp*⁻ mutants. Cosmids pPM45 and pPM420 restored the HR in mutants PM7.1, PM7.2, and PM7.4; cosmid pPM41 restored the HR in PM7.2 and PM7.4; and cosmid pPM419 restored the HR in all mutants except PM7.3.

Restriction map and organization of the *hrp* cluster. Cosmid clones pPM419 and pPM41 were selected for restriction mapping and saturation mutagenesis based upon their ability to complement *hrp*⁻ mutants. Both clones were mutagenized with the reporter transposon Tn*3*-spice, and 230 insertions were generated. Marker exchange of these insersions into the genome of *P. s. morsprunorum* PM7, indicated that a 22-kb region was involved in elicitation of necrotic lesions in cherry plantlets and the hypersensitive response in tobacco (Fig. 2). Restriction enzyme analysis of the insert DNA revealed that pPM419 contained a 27-kb insert of genomic DNA from *P. s. morsprunorum,* including the entire 22-kb region of the *hrp* cluster. A minimum of eight putative transcriptional units were defined within this gene cluster based on the position of plasmid-borne Tn*3*-spice insertions within the gene cluster and the subclones that restored wild-type phenotype. The Tn*5* insertion sites were mapped within the defined *hrp* cluster of mutants PM7.1, PM7.2, and PM7.4 (Fig. 2), and a different region of the chromosome of mutant PM7.3 (data not shown). The Tn*5* insertion sites for mutants PM7.5 and PM7.6 were not determined.

Hybridization of pPM419 to *hrp* genes from *P. s. syringae* 61 and expression of *P. s. morsprunorum* hrp::Tn*3*-spice insertions in vitro. When restriction digests of pPM419 were probed with ^{32}P-labeled sequences from *hrpI, hrpH,* and *hrpZ* of *P. s. syringae* 61, strong hybridization was observed between DNA from transcriptional unit II and *hrpI,* unit VI and *hrpH,* and unit VII and *hrpZ* (Fig. 2). Assay of ice nucleation activity of eight

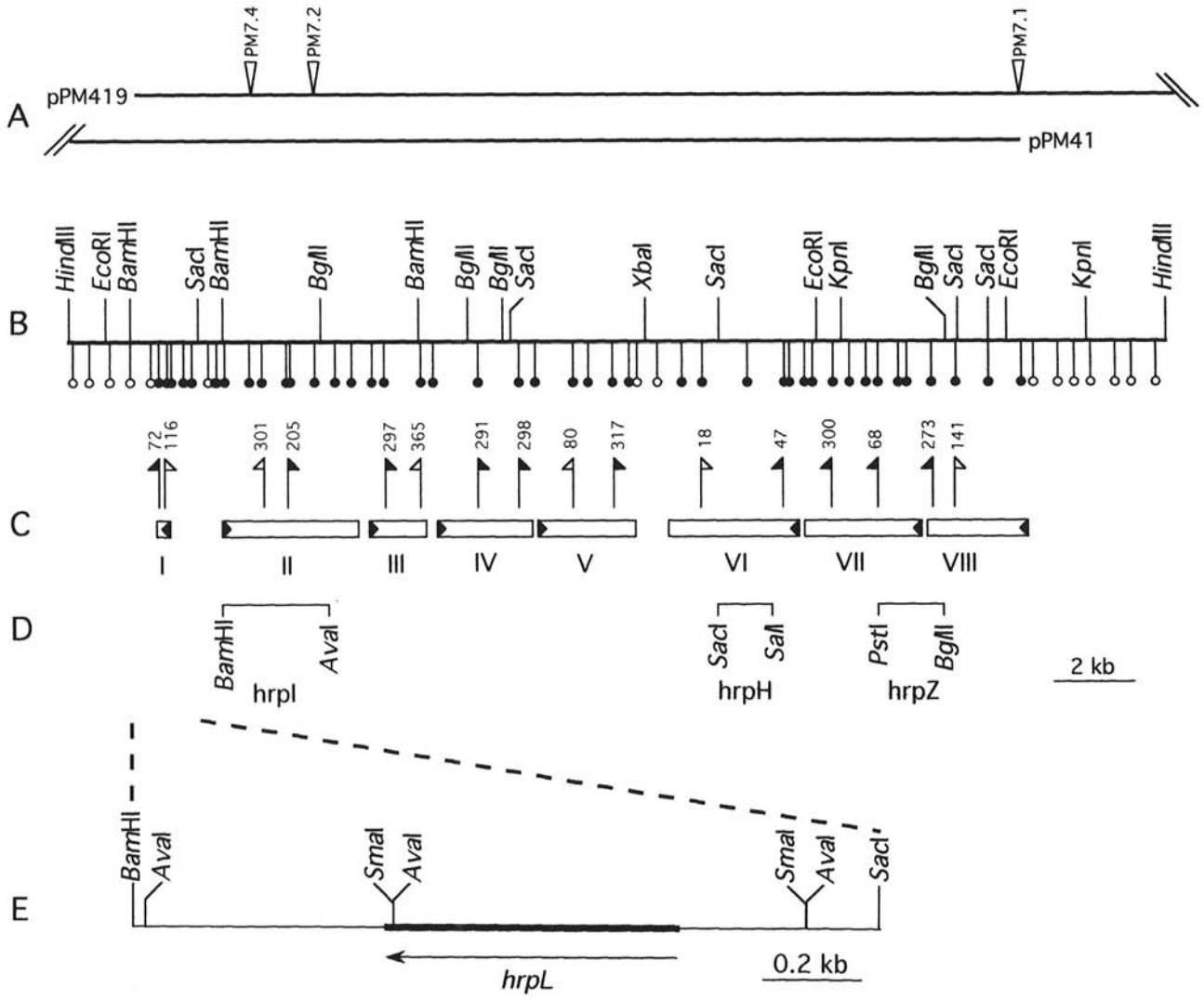

Fig 2. Genetic and physical map of *hrp* gene cluster of *Pseudomonas syringae* pv. *morsprunorum* PM7. **A,** Chromosomal DNA of *P. s. morsprunorum* PM7 ligated into plasmid pLAFR3. Open triangles indicate sites of Tn*5* insertions. **B,** Restriction sites and Tn*3*-spice insertion sites are indicated. Open circles indicate that Tn*3*-spice insertions had no effect on hypersensitive response (HR) elicited by *P. s. morsprunorum* PM7. Solid circles indicate that Tn*3*-spice insertions resulted in a loss of HR activity in *P. s. morsprunorum* PM7. **C,** Proposed transcriptional organization of the *hrp* gene cluster. Flags indicate direction of transcription for *inaZ* gene of Tn*3*-spice. Filled flags indicate detectable ice nucleation activity of corresponding strain, open flags indicate no detectable ice nucleation activity. **D,** Restriction fragments of pPM419 that hybridized with *hrpI, hrpH,* and *hrpZ* of *P. s. syringae* 61. **E,** Enlarged *Bam*HI-*Sac*I 1.6-kb fragment containing *hrpL*.

hrp::Tn*3*-spice insertions (PMM72, PMM205, PMM297, PMM291, PMM317, PMM47, PMM68, and PMM273), one mutant per each transcriptional unit, indicated that the *hrp* genes were not expressed well in rich medium (KB). High levels of expression were observed in minimum medium (M9 supplemented with glutamate) (data not shown). We did not detect ice nucleation activity in strains HB101 and JM109 of *E. coli* containing plasmid pPM419::Tn*3*-spice, whereas activity was detected when pPM419::Tn*3*-spice was present in wild-type or *hrp* mutants of *P. s. morsprunorum* PM7.

Nucleotide sequence of *hrpL*. The 1.6-kb *Bam*HI-*Sac*I fragment contained a single open reading frame (ORF) with an ATG initiation codon and a TGA stop codon (Figs. 1 and 2). No functional ORFs were identified on the opposite strand. A typical ribosome binding site (TAAGG) was located 7 bp upstream from the initiation codon. The deduced protein product contains 185 amino acid residues with a predicted molecular weight of 20 kDa. A putative helix-turn-helix domain was found within the C-terminal end of the predicted amino acid sequence. HrpL of *P. s. morsprunorum* and of *P. s. syringae* (34) contained 185 and 184 amino acids, respectively (Fig. 3), and shared 96% similarity and 92% identity. A single amino acid deletion occured at position 139 in *P. s. syringae* 61.

T7 RNA polymerase expression of *hrpL*. A ^{35}S-labeled 26-kDa protein band was detected from *E. coli* K38(pGP1-2) cells containing pT516, but not from cells containing plasmids pT616 or pT7-5 (Fig. 4). The predicted transcriptional direction of *hrpL* was the same as that of the T7 promoter in pT516 (Fig. 2). As predicted from the nucleotide sequence, *hrpL* only encoded a single protein. However, the polypeptide detected by sodium dodecyl sulfate–polyacrylamide gel electrophoresis was slightly larger than the predicted gene product of HrpL of 20 kDa.

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PsmHrpL MFPNLVILDVTQPRQDSSSAGIRQLTADQIQMLRAFIQKRVMNPDDV  47
        : ::::::::::.::  :::::::::::::::::::::::::: :.:::
PssHrpL MLPNLVILDVTEPRKPSSSAGIRQLTADQIQMLRAFIQKRVKNADDV  47

PsmHrpL DDILQCVFLEALRNEHKFQHASKPQTWLCGIALNLIRNHFRKMYRQP  94
        ::::::::::::::::::::::::::::::::::::::::::::::::
PssHrpL DDILQCVFLEALRNEHKFQHASKPQTWLCGIALNLIRNHFRKMYRQP  94

PsmHrpL YQESWEDDVHSELEWNGDITHQVDGHRQLARVIAAIDCLPSNMQKRV 141
        ::::::::::..::: :::::::::::::::::: ::::::.:::: :
PssHrpL YQESWEDDVHTDLEWHGDITHQVDGHRQLARVIEAIDCLPTNMQK-V 140

PsmHrpL LEVSLEMDGNYQDTANTLGVPIGTVRSRLSRARVQLKQQIDPFA    185
        ::::::::::::.:::::::::::::::::::::::::::::::
PssHrpL LEVSLEMDGNYQETANTLGVPIGTVRSRLSRARVQLKQQIDPFA    184
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Fig. 3. Amino acid sequence alignment of predicted protein products of *Pseudomonas syringae* pv. *morsprunorum* PM7 *hrpL* (PsmHrpL) and *P. s.* pv. *syringae* 61 *hrpL* (PssHrpL). Double dots indicate identity; a single dot indicates similarity. There is 92% identity and 96% similarity between the gene products.

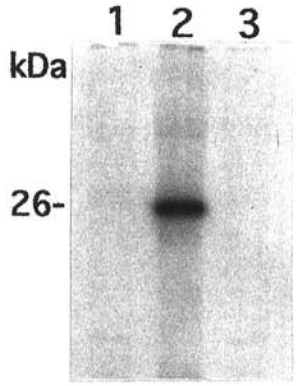

Fig 4. Autoradiograph of protein products of *hrpL* from *P. s. morsprunorum* following T7 RNA polymerase-dependent expression in *E. coli* K38(pGP1-2). Lanes 1-3, proteins from cells containing pT7-5, pT516, and pT616, respectively. Transcriptional direction of T7 promoter in pT516 is same as predicted direction for *hrpL*. Transcriptional direction in pT616 is opposite predicted direction for *hrpL*.

Transcriptional initiation site of *hrpL*. Primer extension terminated at A and C bases located 63 and 25 bp upstream from the ATG start codon of *hrpL*. Apparent transcriptional start sites for *hrpL* were designated as P1 and P2 (Figs. 1 and 5). The intensity of the P1 band was greater than the intensity of the P2 band (Fig. 5, lane 5), and the P2 band was sometimes below the sensitivity of the assay. At the −35 region of promoter P1, an 8-bp sequence repeat ATTACAAA was found. A 45 bp sequence at the −35 region of promoter P1 was highly conserved in both *P. s. morsprunorum* PM7 *hrpL* (Fig. 1) and *P. s. syringae* 61 *hrpL* genes (34). The promoter sequence did not exhibit sequence homology with known sigma factors. A characteristic σ^{54} promoter with −12(GC) and −24(GG) regions was located 11 bp upstream of the P2 initiation site. These results were confirmed by running RNase protection assays (data not shown).

DISCUSSION

The protein expressed by the first transcriptional unit in the *P. s. morsprunorum* PM7 *hrp* cluster was nearly identical to the HrpL protein reported for *P. s. syringae* 61 (34). Accordingly, we adopt the designation HrpL for the predicted 20-kDa protein and *hrpL* for this gene in *P. s. morsprunorum* PM7. This nomenclature is consistent with our determination that portions of transcriptional units II, VI, and VII of *P. s. morsprunorum* PM7 were homologous to sequences from *hrpI, hrpH,* and *hrpZ,* respectively, of *P. s. syringae* 61.

Our primer extension studies support the prediction by Xiao et al (34) that *hrpL* is driven by a sigma 54–like promoter (P2 promoter). In addition, we demonstrate that *hrpL* is driven by a second promoter (P1 promoter). The P1 promoter of *P. s. morsprunorum* PM7 showed no homology with known sigma factors and appeared to be a stronger promoter than the P2 promoter in this experiment. The possible involvement of HrpL as an alternate sigma factor was proposed by Xiao et al (34) based on homology of HrpL with AlgU and group III alternate sigma factors. A three component regulatory cascade, consisting of HrpR, HrpS, and HrpL, was shown to mediate the environ-

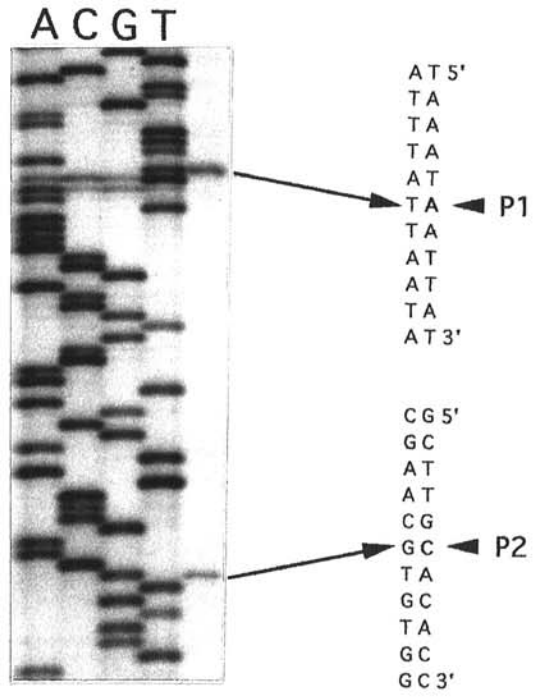

Fig. 5. Primer extension analysis using RNA from *Pseudomonas syringae* pv. *morsprunorum* PM7 cells grown on M9 medium with 10 mM glutamate. Lanes 1–4, ACGT DNA tracks produced by described primer. Lane 5, primer extension products made by using a 24-mer primer complementary to coding strand starting 63 bases downstream from *hrpL* translational start site.

mental regulation of *hrp* genes in *P. s. syringae* Pss61 (9,34). HrpR and HrpS regulate *hrpL* expression positively, and HrpL activates the expression of *hrpJ, hrpZ,* and *hrmA* and, likely, *hrpK, hrpU,* and *hrpH* as well (34). Recently, a homolog to the *avr* conserved sequence motif was identified in the promoter-active regions of *hrmA, hrpJ,* and *hrpZ* and in the deduced promoter regions of *hrpK, hrpU,* and *hrpH* (35). A 34-bp fragment carrying this motif from the *hrpZ* promoter region was shown to confer HrpL-dependent promoter activity (35). This conserved sequence motif was identified in the upstream regions of 10 *avr* genes (14,25,26). These data suggest that the expression of pathogenicity and host range determinants in *P. syringae* strains or pathovars including *P. s. morsprunorum* is directed by the alternate sigma factor HrpL.

The *hrp* gene clusters cloned from *E. amylovora* and *P. s. syringae* were shown to confer on nonpathogenic bacteria the ability to elicit HR after infiltration of bacterial suspensions into the intercellular spaces of leaves of tobacco and other plants (1,11). Unlike the *hrp* genes of *P. s. syringae* and *E. amylovora, P. s. morsprunorum hrp* genes did not enable *E. coli* to elicit the HR in tobacco. No ice nucleation activity was detected in *E. coli* containing plasmid pPM419::Tn*3*-spice, while activity was detected when pPM419::Tn*3*-spice was present in wild-type or *hrp* mutants of *P. s. morsprunorum* PM7. Therefore, we cannot eliminate the possibility that additional genes are necessary for eliciting HR in nonhost plants. Mutant PM7.3 was the only mutant we evaluated that was auxotrophic on M9 medium. The Tn*5* insertion site for mutant PM7.3 was mapped into a different region of the chromosome, but it behaved like other mutants in HR and pathogenicity tests. This suggests that strain PM7.3 carries a mutation in a gene involved with basic metabolism, rather than a gene encoding a *hrp* product. No Tn*3*-spice insertions that resulted in a hrp^- phenotype were detected between transcriptional units II, III, IV, and V and between units VI, VII, and VIII. Because different plasmid-borne Tn*3*-spice mutants and subclones complemented chromosomal Tn*3*-spice mutants for each transcriptional unit, it suggests that transcriptional units II, III, IV, and V, and units VI, VII, and VIII are separate functional units.

LITERATURE CITED

1. Beer, S. V., Bauer, D. W., Jiang, X. H., Laby, R. J., Sneath, B. J., Wei, Z. M., Wilcox, D. A., and Zumoff, C. H. 1991. The *hrp* gene cluster of *Erwinia amylovora.* Pages 53-60 in: Proc. Int. Symp. Mol. Gen. Plant-Microbe Interact., 5th. H. Hennecke and D. P. Verma, eds. Kluwer Academic Publishers, Dordrecht.
2. Bonas, U., Schulte, R., Fenselau, S., Minsavage, G. V., Staskawicz, B. J., and Stall, R. E. 1991. Isolation of a gene cluster from *Xanthomonas campestris* pv. *vesicatoria* that determines pathogenicity and the hypersensitive response on pepper and tomato. Mol. Plant-Microbe Interact. 4:81-88.
3. Boucher, C. A., Van Gijsegem, F., Barberis, P. A., Arlat, M., and Zischek, C. 1987. *Pseudomonas solanacearum* genes controlling both pathogenicity and hypersensitivity on tobacco are clustered. J. Bacteriol. 169:5626-5632.
4. Felley, R., Rahme, L. G., Mindrinos, M. N., Frederick, R. D., Pisi, A., and Panopoulos, N. J. 1989. Genes and signals controlling the *Pseudomonas syringae* pv. *phaseolicola*-plant interaction. Pages 45-52 in: Advances in Molecular Genetics of Plant-Microbe Interactions. H. Hennecke and D. P. S. Verma, eds. Kluwer Academic Publishers, Dordrecht.
5. Figurski, D. H., and Helinski, D. R. 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. Proc. Natl. Acad. Sci. U.S.A. 76:1648-1652.
6. Gilman, M. 1989. Rapid isolation of RNA from gram-negative bacteria. Pages 4.4.2-4.4.4 in: Current Protocols in Molecular Biology. Vol. 1. F. A. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, eds. Greene Publishing Associates and Wiley-Interscience, New York.
7. Greene, J. M., and Struhl, K. 1989. S1 analysis of messenger RNA using single-stranded DNA probes. Pages 4.6.1-4.6.13 in Current Protocols in Molecular Biology. Vol. 1. F. A. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, eds. Greene Publishing Associates and Wiley-Interscience, New York.
8. He, S. H., Huang, H.-C., and Collmer, A. 1993. *Pseudomonas syringae* pv. *syringae* $harpin_{pss}$: A protein that is secreted via the *hrp* pathway and elicits the hypersensitive response in plants. Cell 73:1255-1266.
9. Heu, S., and Hutcheson, S. W. 1993. Nucleotide sequence and properties of the *hrmA* locus associated with the *P. syringae* pv. *syringae* 61 *hrp* gene cluster. Mol. Plant Microbe Interact. 6:553-564.
10. Huang, H.-C., He, S. Y., Bauer, D. Y., and Collmer, A. 1992. The *Pseudomonas syringae* pv. *syringae* 61 *hrpH* product, an envelope protein required for elicitation of the hypersensitive response in plants. J. Bacteriol. 174:6878-6885.
11. Huang, H.-C., Schuurink, R., Denny, T. P., Atkinson, M. M., Baker, C. J., Yucel, I., Hutcheson, S. W., and Collmer, A. 1988. Molecular cloning of a *Pseudomonas syringae* pv. *syringae* gene cluster that enables *Pseudomonas fluorescens* to elicit the hypersensitive response in tobacco plants. J. Bacteriol. 170:4748-4756.
12. Huang, H.-C., Xiao, Y., Lin, R.-H., Lu, Y., Hutcheson, S. W., and Collmer, A. 1993. Characterization of the *Pseudomonas syringae* pv. *syringae* 61 *hrpJ* and *hrpI* genes: Homology of *hrpI* to a superfamily of proteins associated with protein translocation. Mol. Plant-Microbe Interact. 6:515-520.
13. Huynh, T. V., Dahlbeck, D., and Staskawicz, B. J. 1989. Bacterial blight of soybean: Regulation of a pathogen gene determining host cultivar specificity. Science 345:1374-1377.
14. Innes, R. W., Bent, A. F., Kunkel, B. N., Bisgrove, S. R., and Staskawicz, B. J. 1993. Molecular analysis of avirulence gene *avr*Rpt2 and identification of a putative regulatory sequence common to all known *Pseudomonas syringae* avirulence genes. J. Bacteriol. 175:4859-4869.
15. 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. 44:301-313.
16. Kingston R. E. 1989. Primer extension. Pages 4.8.1-4.8.3 in Current Protocols in Molecular Biology. Vol. 1. F. A. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, eds. Greene Publishing Associates and Wiley-Interscience, New York.
17. Klement, Z., Farkas, G. L., and Lovrekovich, L. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54:474-477.
18. Latorre, B. A., and Jones, A. L. 1979. *Pseudomonas morsprunorum,* the cause of bacterial canker of sour cherry in Michigan, and its epiphytic association with *P. syringae.* Phytopathology 69:335-339.
19. Liang, L. Z., Sobiczewski, P., Paterson, J. M., and Jones, A. L. 1994. Variation in virulence, plasmid content, and genes for coronatine synthesis between *Pseudomonas syringae* pv. *morsprunorum* and *P. s. syringae* from *Prunus.* Plant Dis. 78:389-392.
20. Lindgren, P. B., Frederrick, R., Govindarajan, A. G., Panopoulos, N. J., Staskawicz, B. J., and Lindow, S. E. 1989. An ice nucleation reporter system: Identification of inducible pathogenicity genes in *Pseudomonas syringae* pv. *phaseolicola.* EMBO J. 8:1291-1301.
21. Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. 1986. Gene cluster of *Pseudomonas syringae* pv. *"phaseolicola"* controls pathogenicity of bean plants and hypersensitivity on nonhost plants. J. Bacteriol. 168:512-522.
22. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
23. Rahme, L. G., Mindronos, M. N., and Panopoulos, N. J. 1992. Plant and environmental sensory signals control the expression of *hrp* genes in *Pseudomonas syringae* pv. *phaseolicola.* J. Bacteriol. 174:3499-3507.
24. Russel, M., and Model, P. 1984. Replacement of the *flp* gene of *Escherichia coli* by an inactive gene cloned on a plasmid. J. Bacteriol. 159:1034-1039.
25. Salmeron, J. M., and Staskwicz, B. J. 1993. Molecular characterization and *hrp* dependence of the avirulence gene *avrPto* from *Pseudomonas syringae* pv. *tomato.* Mol. Gen. Genet. 239:6-16.
26. 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.
27. Simon, R., Preifer, U., and Puhler, P. 1983. A broad host range mobilization system for *in vivo* genetic engineering: Transposon mutagenesis. Biotechnology 1:784-791.
28. Stachel, S. E., An, G., Flores, C., and Nester, E. W. 1985. A Tn*3*-*lacZ* transposon for the random generation of β-galactosidase gene fusions: Application to the analysis of gene expression in *Agrobacterium.* EMBO J. 4:891-898.
29. Staskawicz, B., Dahlbeck, D., Keen, N., and Napoli, C. 1987. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea.* J. Bacteriol.

169:5789-5794.
30. Steinberger, E. M., and Beer, S. V. 1988. Creation and complementation of pathogenicity mutants of *Erwinia amylovora*. Mol. Plant-Microbe Interact. 1:135-144.
31. Tabor, S. 1990. Expression using the T7 polymerase/promoter system. Vol. 2. Pages 16.2.1-16.2.11 in: Current Protocols in Molecular Biology. F. A. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, eds. Greene Publishing Associates and Wiley-Interscience, New York.
32. Tambor, S., and Richardson, C. C. 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. U.S.A. 82:1074-1078.
33. Wilson, K. 1989. Preparation of genomic DNA from bacteria. Pages 2.4.1-2.4.5 in: Current Protocols in Molecular Biology. Vol. 1. F. A. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, eds. Greene Publishing Associates and Wiley-Interscience, New York.
34. Xiao, Y., Heu, S., Yi, J., Lu, Y., and Hutcheson, S. W. 1994. Identification of a putative alternate sigma factor and characterization of a multicomponent regulatory cascade controlling the expression of *Pseudomonas syringae* pv. *syringae* Pss61 *hrp* and *hrmA* genes. J. Bacteriol. 176:1025-1036.
35. Xiao, Y., and Hutcheson, S. W. 1994. A single promoter sequence recognized by a newly identified alternate sigma factor directs expression of pathogenicity and host range determinants in *Pseudomonas syringae*. J. Bacteriol. 176:3089-3091.