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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

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The *Pseudomonas syringae hrp* and *hrmA* genes controlling pathogenicity and elicitation of the hypersensitive response and the *avr* genes controlling host range have been shown previously to be regulated by carbon, nitrogen, pH, osmolarity, and hypothetical plant factors. In *P. syringae* pv. syringae Pss61, inactivation of *hrp* complementation groups II and XIII reduced expression of a plasmid-borne *hrmA'-lacZ* fusion. The *hrp* regions II and XIII were cloned on separate plasmids and shown to enhance the activity of the *hrmA* promoter in *Escherichia coli* MC4100 transformants at least 100-fold. The nucleotide sequence of region XIII revealed two open reading frames (*hrpR* and *hrpS*) whose deduced products share homology with *P. syringae* pv. phaseolicola NPS3121 HrpS and are both related to the NtrC family of two-component signal transduction systems. HrpR and HrpS differ from most members of the protein family by lacking an amino-terminal domain which modulates the regulatory activity. A single open reading frame, *hrpL*, whose product shares homology with AlgU, a putative alternate sigma factor of *P. aeruginosa*, as well as with the related alternate sigma factors was identified within region II. Key domains are partially conserved. Inactivation of *hrpS* in Pss61 repressed expression of a plasmid-borne *hrpL'-lacZ* fusion carried by pYXPL1R, and transformation of MC4100(pYXPL1R) with a plasmid carrying *hrpRS* increased *hrpL* promoter activity at least 200-fold. Neither *hrpS* nor *hrpR*, when cloned on separate plasmids, activated the *hrpL* promoter activity individually. The expression of *hrpL* when directed by a *lac* promoter was sufficient to express a set of plasmid-borne *hrmA'-*, *hrpJ'-*, and *hrpZ'-lacZ* fusions independently of other *hrp* genes. The results indicate that *hrpRS* and *hrpL* are part of a regulatory cascade in which HrpR and HrpS activate expression of *hrpL* and HrpL, a putative sigma factor, induces expression of HrpL-responsive genes.

Pseudomonas syringae strains are opportunistic pathogens of a wide variety of plant species. Individual strains usually cause disease in a limited subset of plant species, and strains sharing common host ranges are given pathovar designations (13, 47). During pathogenesis, the bacteria invade the tissue through natural openings, such as stomata or wounds, and colonize the surfaces of cells internal to the susceptible plant tissue (28). The inability of a strain to colonize tissue of other plants (non-host plant species or resistant varieties of susceptible plant species) is attributed, in part, to localized defense responses induced in nearby plant cells which limit further colonization of the tissue (35). Plant cells appear to respond to a plant response elicitor released by a plant pathogenic bacterium to initiate a defense response which is typically manifest as the hypersensitive response (HR), a rapid localized tissue necrosis (21, 35, 37).

A 25-kb *hrp* gene cluster has been shown to control the pathogenicity of *P. syringae* strains in susceptible plant hosts and the initiation of the HR in non-host or resistant plant tissue (30, 41, 60). An apparently complete *hrp* cluster, isolated from *P. syringae* pv. syringae strain Pss61, enables *Escherichia coli* transformants to elicit the HR in plant tissue (25, 26, 29, 30). The genetic dissection of this cluster has identified at least 16 *hrp* genes and the *hrmA* gene, which are organized into eight apparent transcriptional units (23, 25, 30, 39, 61). One of the *hrp* genes, *hrpZ*, produces a glycine-rich plant response elicitor, $harpin_{Pss}$, which has been proposed to be responsible for the Hrp^+ phenotype (21). The remaining genes of the cluster appear to form an autonomous system for the Sec-independent secretion of $harpin_{Pss}$ similar to that used by several enteric bacteria pathogenic to humans and other mammals to secrete virulence proteins (24, 27, 30, 39). A similar system has been identified in *Xanthomonas campestris* (15) and *Pseudomonas solanacearum* (18).

Expression of *hrp* genes has been shown to be environmentally regulated in *P. syringae* strains in response to carbon and nitrogen sources, pH, and osmotic conditions (40, 50, 51, 61). At least one *hrp* gene product, HrpS, shares partial homology with the response regulator (effector) component of two-component signal transduction systems (9, 19, 48, 57). Genetic analyses have suggested that a second *hrp* gene, designated *hrpL*, may also have regulatory activity (14, 23, 31, 50). Both *hrpS* and *hrpL* behave as positive-acting regulatory determinants (19, 23, 32, 50). The nutritional regulation of several *hrp* and *avr* (negative host range determinants [35]) genes in *P. syringae* appears to be mediated by the *hrpS/hrpL* system (14, 23, 31, 32, 51). The properties of *hrpL* and its interaction with *hrpS* have not been elucidated.

In *P. syringae* pv. syringae Pss61, the expression of most *hrp*

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genes and the *hrmA* gene is repressed in media containing broad-spectrum amino acid sources or high osmotic conditions and is also subject to a form of catabolite repression in which fructose is inductive and glucose is repressive (23, 61). Heu and Hutcheson (23) reported that activation of a *hrmA'-lacZ* fusion, carried by the plasmid pSGAMS1, in *E. coli* MC4100 was dependent in *trans* upon pHIR11, a cosmid clone carrying the Pss61 *hrp/hrmA* gene cluster. Insertional inactivation of *hrp* complementation group II or XIII in pHIR11 reduced the expression of the *hrmA'-lacZ* fusion in MC4100 to the basal levels observed in the absence of the *hrp* cluster. The other insertions screened had little effect on *hrmA* expression. The complementation groups originally defined by Huang et al. (25) by Tn*phoA* mutagenesis appear to represent translational units (23, 24, 27, 39, 61). The regulatory activity of *hrp* genes can thus be investigated through loss-of-function experiments in *P. syringae* mutants and gain-of-function experiments in *E. coli* transformants. Here we demonstrate that complementation groups II (*hrpL*) and XIII (*hrpRS*) of the Pss61 *hrp* cluster produce positive-acting transcriptional factors which function independently of other *hrp* genes and report their nucleotide sequences, characterization of the gene products, homology of HrpL with an alternate sigma factor, and elucidation of a HrpR-, HrpS-, and HrpL-dependent regulatory cascade.

(A preliminary report of this work has been presented previously [60a].)

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacteria were routinely grown in KB medium (36). Prior to the assay of reporter gene activity, bacteria were harvested, washed twice in M63 minimal salts medium (55) containing 0.2% mannitol (M63M medium), and cultured in M63M medium for 6 h. Strains and plasmids are described in Table 1. Media were supplemented with antibiotics as needed at the following concentrations (in micrograms per milliliter): nalidixic acid, 50; tetracycline, 100 (in KB medium) or 20 (in M63M medium); kanamycin, 50; ampicillin, 100; and spectinomycin, 20.

General DNA manipulations. Plasmid DNA was isolated and manipulated by using standard techniques (53). Restriction enzymes and related reagents were purchased from Bethesda Research Laboratories (Gaithersburg, Md.) and used as described in the manufacturer's instructions. Restriction fragments were purified after agarose gel electrophoresis by using Geneclean (Bio 101, Vista, Calif.). Electroporation-mediated transformation of *E. coli* strains was performed as described by Li et al. (38). Triparental matings into *P. syringae* strains were performed as described by Huang et al. (25).

Nucleotide sequence analysis. The 2.3-kb *Eco*RI fragment carrying complementation group II was cloned into pBluescriptII SK$^+$ (Stratagene, La Jolla, Calif.), and nested deletion derivatives were created by using exonuclease III after *Kpn*I and *Xho*I digestion of the isolated plasmid (Stratagene). For complementation group XIII, the inclusive 3-kb *Bam*HI-*Hin*dIII fragment as well as a set of *Pst*I, *Sst*I, and *Eco*RV fragments internal to the *Bam*HI-*Hin*dIII fragment were also cloned into pBluescriptII SK$^+$. For both regions, the constructed plasmids were transformed into *E. coli* DH5α, and plasmids from randomly selected colonies were isolated by using acid-phenol (59a) or a commercial plasmid isolation kit (QIAGEN, Chatsworth, Calif.). The nucleotide sequence was obtained by using Sequenase 2.0 (United States Biochemical Corp., Cleveland, Ohio), double-stranded templates, and, in most cases, vector primer sites. Gaps apparent in the sequence of either strand after compilation of the data were filled by using synthetic oligonucleotide primers. Compressions were resolved by using dITP in the reaction mixtures. Sequence data were analyzed by using the algorithms of the University of Wisconsin Genetics Computer Group package 7.2 (10).

T7 RNA polymerase-directed gene expression. The 1-kb *Ssp*I-*Hin*cII fragment carrying the *hrpL* open reading frame (ORF) was ligated into *Xba*I- and *Sma*I-digested pVEX11. The resulting plasmid was transformed into *E. coli* BL21(DE3). Protein analysis was performed as described by Huang et al. (27).

PCR amplification of the *hrpL-hrpJ* intergenic region. Synthetic oligonucleotide primers that incorporated a 25-nucleotide sequence derived from the Pss61 *hrpL* sequence modified to include a *Bam*HI restriction site (5′-CCGGATCC GTCAATTGACGAATACC) or an equivalent sequence from the *hrpJ* sequence engineered to contain a *Sma*I site (5′-GACCCCGGGCGACGATTTTCATAG) were designed. To clone the opposite orientation, primers were designed by using the *hrpL* sequence to generate a *Sma*I site (5′-CAACC CGGGTCAATTGACGAATAC) or by using the *hrpJ* sequence modified to create a *Bam*HI site (5′-GAGGATCC GACGATTTTCATAG). PCR was performed by using pHIR11 as the template and *Taq* polymerase as recommended by the manufacturer (Perkin-Elmer, Norwalk, Conn.). Templates were melted at 94°C and annealed at 50°C, and the PCR was run at 72°C for 2 min each. After 30 cycles, the fragments were digested with *Bam*HI and *Sma*I, gel purified, and ligated into *Bam*HI- and *Sma*I-cleaved pRG970 to create pYXPL1R (*hrpL'-lacZ hrpJ'-gusA*) and pYXPJ1R (*hrpJ'-lacZ hrpL'-gusA*). The nucleotide sequence of the cloned fragment was checked to confirm the validity of the amplification procedure.

Assays of β-galactosidase activity. Cells from overnight cultures in KB medium were harvested, washed twice in M63M medium, and resuspended in M63 medium to an optical density at 600 nm of 0.2. After 6 h of induction, β-galactosidase activity was determined as described by Miller (44) but modified to reduce the reaction volume to 1 ml. Neither MC4100 nor Pss61 exhibited detectable β-galactosidase activity unless transformed with the indicated plasmids. Data are presented as the means of at least three replicates derived from separate cultures.

Nucleotide sequence accession numbers. The nucleotide sequences of *hrpL*, *hrpR*, *hrpS* and *hrpK* have been deposited in GenBank under accession numbers U03854, U03853, U03852, and U03855, respectively.

RESULTS

Demonstration that complementation groups II and XIII carry positive-acting regulatory factors. To determine whether *P. syringae* pv. syringae Pss61 *hrp* complementation groups II and XIII (sensu [25]) are sufficient to form an independent regulatory system in *E. coli* MC4100, complementation group II was subcloned as a 2.3-kb EcoRI fragment into either pDSK519 or pLAFR3 to create pSGL1D and pSGL1L, respectively, and complementation group XIII was cloned as a 5.4-kb *Eco*RI-*Bam*HI fragment into pDSK519 (to create pSGRS3D) or as a 3.0-kb *Hin*dIII-*Bam*HI fragment into pLAFR3 (to create pSGRS1L; Fig. 1). Each fragment, when cloned into pLAFR3, complemented a representative Pss61 chromosomal mutation in the corresponding complementation group to produce a HR$^+$ phenotype. The resultant plasmids were transformed into MC4100(pSGAMS1) singly or in combination. MC4100 was used in these experiments because it is a Δ*argF-lac* mutant (5) and was shown previously to be a good host for expression of the Pss61 *hrp* genes (23, 38). When the

TABLE 1. Strains and plasmids

Bacterium or plasmid	Genotype	Source or reference[a]
Escherichia coli		
MC4100	F′ *araD139* Δ(*argF-lacZYA*)U169 *rpsL150 relA1 flb-5301 ptsF25 deoC1*	5
BL21(DE3)	B strain; F^- *ompT* r_B^- m_B^- *hsdS gal* (λDE3 c*Its*857 *int-1 Sam7 nin-5 lacUV5*-T7 gene 1)	58
DH5α	*endA1 hsdR17* (r_K^- m_K^-) *supE44 thi-1 recA1 gyrA96 relA1* Δ(*argF-lacZYA*)U169 ϕ80d*lacZDM15*	BRL
Pseudomonas syringae pv. syringae		
Pss61	Nx^r HR^+	4
Pss61-2074	Pss61 *hrpL*::Tn*phoA* mutant	25
Pss61-2089	Pss61 *hrpH*::Tn*phoA* mutant	25
Pss61-2094	Pss61 *hrpS*::Tn*phoA* mutant	25
Pss61-2095	Pss61 *hrpR*::Tn*phoA* mutant	25
Pss61-5134	Pss61 *hrpL*::Tn*5-gusA1* mutant	61
Plasmids		
pLAFR3	IncP-1; Tc^r *lacZ′*	56
pHIR11	31-kb *P. syringae* pv. syringae Pss61 fragment containing the *hrp/hrmA* cluster cloned into pLAFR3	26
pHIR11-2074	pHIR11 derivative carrying *hrpL*::Tn*phoA* insertion	25
pHIR11-2089	pHIR11 derivative carrying *hrpH*::Tn*phoA* mutation	25
pHIR11-2094	pHIR11 derivative carrying *hrpS*::Tn*phoA* mutation	25
pHIR11-2095	pHIR11 derivative carrying *hrpR*::Tn*phoA* mutation	25
pHIR11-2096	pHIR11::Tn*phoA* derivative; Hrp^+ $HrmA^+$	25
pHIR11-5134	pHIR11 derivative carrying *hrpL*::Tn*5-gusA1* mutation	61
pHIR12	*P. syringae* pv. syringae *hrp* I to XIII cloned into pLAFR3	26
pBluescriptII SK^+	ColE1, Ap^r mcs-*lacZ*	Stratagene
pVEX11	pET3a derivative; P_{T7}-mcs, *bla*	23
pMLB1034	pBR322 derivative carrying promoterless *lacZ*	55
pRG970	IncP; Sp^r promoterless *lacZ* and *gusA* in opposite orientation	59
pDSK519	IncQ; Kn^r *lacZ′*	34
pSPORT 1	ColE1; Ap^r *lacI lacZ′OP*	BRL
pSGL1D	2.3-kb *Eco*RI fragment carrying *hrpL* ligated into pDSK519	This report
pSGL1L	2.3-kb *Eco*RI fragment carrying *hrpL* ligated into pLAFR3	This report
pSGRS3D	5.4-kb *Eco*RI-*Bam*HI fragment carrying *hrpRS* ligated into pDSK519	This report
pSGRS1L	3.0-kb *Bam*HI-*Hin*dIII fragment carrying *hrpRS* ligated into pLAFR3	This report
pYXRS1D	2.2 kb *Bam*HI-*Bgl*II fragment carrying *hrpRS* ligated into pDSK519	This report
pJSS1L	1.1-kb *Bgl*II-*Bfa*I fragment carrying *hrpS* ligated into pLAFR3	This report
pJSS1D	1.1-kb *Bam*HI-*Bfa*I fragment carrying *hrpS* ligated into pDSK519	This report
pSGR1B	1.67-kb *Bam*HI-*Eco*RV fragment carrying *hrpR* ligated into pBluescriptII SK^+	This report
pYXL2B	1-kb *Ssp*I-*Hin*cII fragment carrying the *hrpL* ORF downstream of the *lac* promoter of pBluescriptII SK^+	This report
pYXL2SP	1-kb *Ssp*I-*Hin*cII fragment carrying the *hrpL* ORF downstream of the *lac* promoter of pSPORT 1	This report
pSGAMS1	0.8-kb *Bam*HI-*Hin*cII fragment cloned into pMLB1034 to create a *hrmA′-lacZ* translational fusion	23
pYXPL1R	0.3-kb PCR amplification product of the *hrpL-hrpJ* intergenic region cloned as a *Bam*HI-*Sma*I fragment into pRG970 to create a transcriptional fusion between the *hrpL* promoter and *lacZ*	This report
pYXPZ1R	0.84-kb *Bgl*II-*Hin*dIII fragment ligated into pRG970 to create a transcriptional fusion between the *hrpZ* promoter and *lacZ*	This report
pXYPJ1R	0.3-kb PCR amplification product of the *hrpJ-hrpL* intergenic region cloned as a *Bam*HI-*Sma*I fragment into pRG970 to create a transcriptional fusion between the *hrpJ* promoter and *lacZ*	This report
pSGR7	0.8-kb *Bam*HI-*Hin*cII fragment cloned into pRG970 to create *hrmA′-lacZ* transcriptional fusion	23

[a] BRL, Bethesda Research Laboratories.

MC4100(pSGAMS1) transformants were screened for β-galactosidase activity after induction in a minimal salts medium for 6 h (23, 61), only transformants carrying both complementation groups II and XIII, irrespective of the host vector, exhibited expression of the *hrmA′-lacZ* construct (Table 2). Apparent expression from the *hrmA′-lacZ* fusion increased at least 200-fold in transformants carrying one each of the *hrp* complementation group II- and *hrp* complementation group XIII-bearing plasmids.

Nucleotide sequence and properties of the complementation group XIII gene products. To further characterize the complementation group XIII gene(s) responsible for the regulatory activity, the nucleotide sequence for the 3.03-kb *Bam*HI-*Hin*dIII fragment was obtained. The *Bam*HI-*Hin*dIII fragment

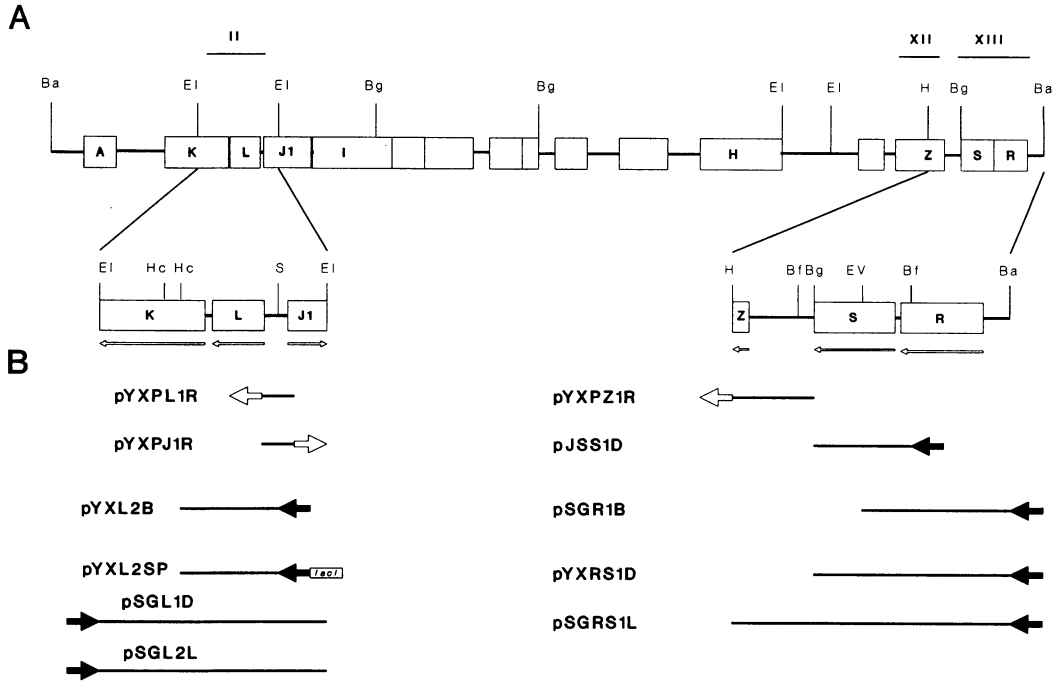

FIG. 1. Restriction map, organization, and subclones of the *P. syringae* pv. syringae Pss61 *hrp/hrmA* gene cluster. (A) Translational organization of the cluster deduced from the nucleotide sequence (23, 24, 27, 39) and complementation analyses (25, 61). Boxes indicate ORFs. The 2.3-kb *Eco*RI fragment carrying complementation group II and the 3.03-kb *Bam*HI-*Hin*dIII fragment containing complementation group XIII are expanded. The translational orientation of the ORFs is indicated by the unfilled arrows. Abbreviations: Ba, *Bam*HI; EI, *Eco*RI; Bg, *Bgl*II; H, *Hin*dIII; Hc, *Hin*cII; S, *Ssp*I; Bf, *Bfa*I; EV, *Eco*RV. (B) Subclones of *hrpL* and *hrpRS*. Bars indicate the region cloned. Filled arrows indicate the orientation of the *lac* promoter of the vector. Open arrows indicate the *lacZ* coding sequence.

contained two complete ORFs whose orientation was in agreement with the deduced direction of transcription for complementation group XIII (Fig. 2) (61). A potential σ^{70} promoter (20) is located 103 nucleotides upstream of the translational initiation codon of the first ORF. No consensus pseudomonad-type σ^{54} promoter sequences (9) are apparent. The *hrpZ* ORF is located 471 nucleotides downstream of the ORF2 termination codon (21). ORF1 was subcloned as a 1.67-kb *Bam*HI-*Eco*RV fragment into pSF6 or pBluescriptII SK$^+$ to construct pSGR1W or pSGR1B, respectively (Fig. 1). ORF2 was subcloned as a 1.09-kb *Bfa*I-*Bgl*II fragment into pLAFR3 and pDSK519 to create pJSS1L and pJSS1D, respectively (Fig. 1).

TABLE 2. Effect of *hrp* complementation groups II and XIII on *hrmA* promoter activity in *E. coli* MC4100(pSGAMS1)

Plasmid[a]	Region[b]	Promoter activity[c]
None		12 ± 6
pLAFR3	Vector	12 ± 8
pDSK519	Vector	20 ± 5
pLAFR3; pDSK519		17 ± 4
pHIR12	*hrp* I–XIII	2,210 ± 230
pSGL1D	*hrp* II	9 ± 7
pSGL1L	*hrp* II	10 ± 5
pSGRS3D	*hrp* XII–XIII	8 ± 7
pSGRS1L	*hrp* XIII	8 ± 5
pSGL1D; pSGRS1L	*hrp* II; *hrp* XIII	3,281 ± 425
pSGL1L; pSGRS3D	*hrp* II; *hrp* XII–XIII	2,778 ± 253

[a] *E. coli* MC4100(pSGAMS1) transformant carrying the indicated plasmid(s).
[b] Complementation groups carried by the plasmids as defined by Huang et al. (25).
[c] As indicated by expression of a *hrmA'-lacZ* fusion carried by pSGAMS1. Data are presented as β-galactosidase activity in Miller units after induction for 6 h. The activities detected in parallel experiments in which the vector pMLB1034 was substituted for pSGAMS1 were lower than 10 Miller units.

Homology of complementation group XIII products with response regulators of two-component signal transduction systems. The deduced product of the initial ORF (314 amino acids [aa]; M_r, 34,800) was found to share homology with the response regulator (effector-receiver) (48) component of two-component signal transduction systems but was distinct from the previously characterized HrpS (Fig. 3) (19). Only 72% similarity and 57% identity were detected with the *P. syringae* pv. phaseolicola NPS3121 HrpS (see below). Homologies with the central domain of the effector-receiver component necessary for transcriptional activation and the helix-turn-helix (HTH) motif which functions in DNA binding are apparent (45, 48, 57). The strongest homology was observed within the central domain, and all three domains of the σ^{54} interaction motif typical of this protein family (1) are retained. This locus, henceforth designated *hrpR*, appears to be a homolog of the *P. syringae* pv. phaseolicola NPS3121 *hrpR* locus identified by the genetic analysis of Rahme et al. (50), but its sequence has not yet been reported. Hybridization analysis had predicted that *P. syringae* pv. syringae Pss61 complementation group XIII carries *hrpR* (23).

The product of the second ORF (302 aa; M_r, 33,309) exhibited 88% identity with the *P. syringae* pv. phaseolicola NPS3121 HrpS described previously (19). Production of the NPS3121 HrpS has been verified by in vitro translation (19). Most of the substitutions were conservative (Fig. 3). As originally reported for the NPS3121 HrpS, the Pss61 HrpS

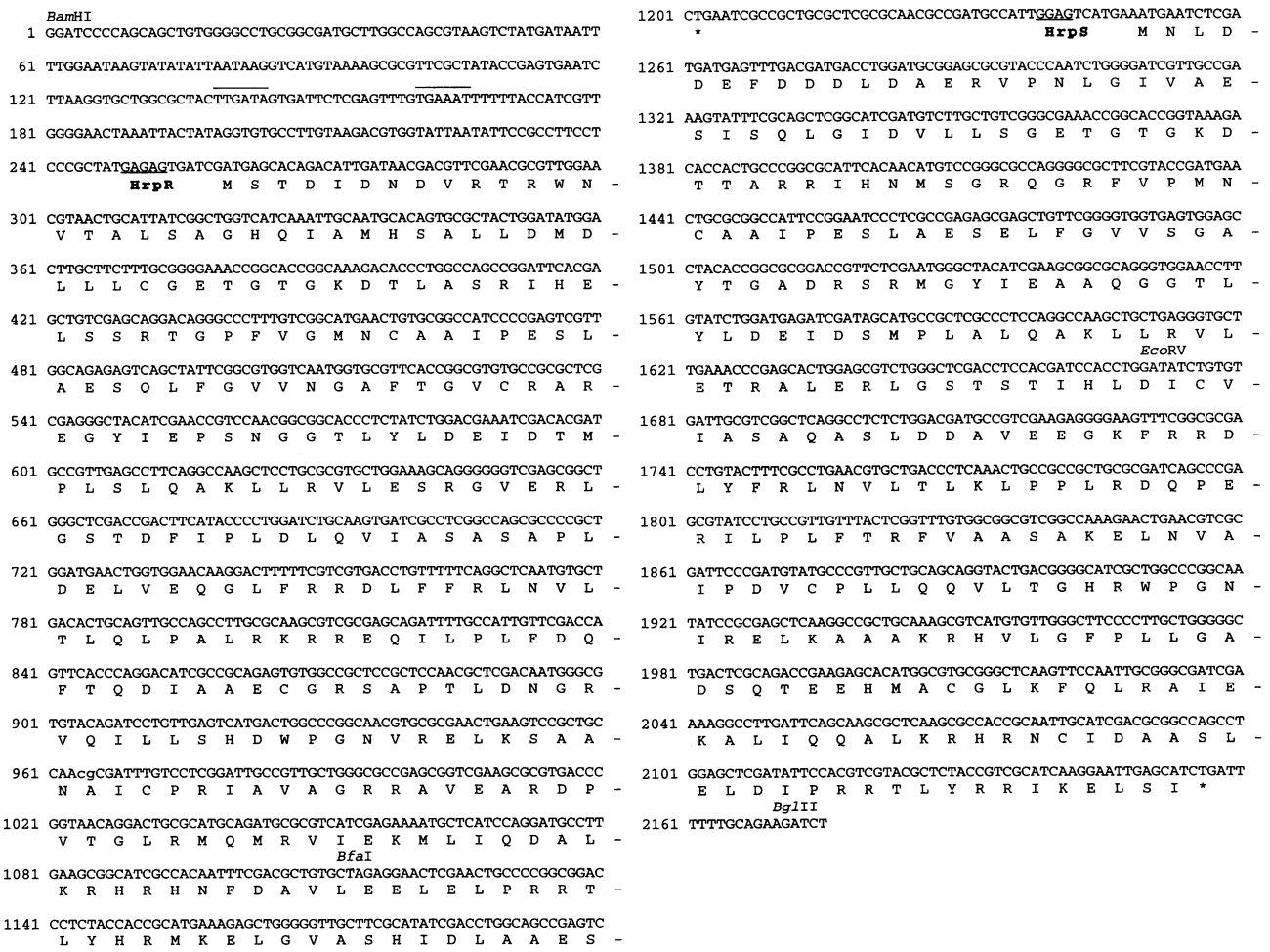

FIG. 2. Nucleotide sequence and deduced gene products of *hrpRS*. The nucleotide sequence for the *Bam*HI to *Bgl*II sites is shown. The sequencing strategy is described in the text. Deduced translation products are shown by using single-letter amino acid residue codes. Potential ribosome binding sites are underlined. A potential σ^{70} promoter (20) is overlined.

shares strong homology with the central domain of the receiver-effector component of two-component signal transduction systems (Fig. 3). The σ^{54} interaction motif is conserved. An HTH motif (residues 276 to 299) is present. Like HrpR, there is no apparent amino-terminal modulation domain. When the HTH motifs of the two deduced *P. syringae* HrpS products are compared, the only difference is a conservative methionine substitution for isoleucine at position 288. By using alignment predicted by the procedure of Dodd and Egan (11), this residue is likely to be part of the turn within this motif. The Pss61 HrpS HTH motif exhibits only 55% identity with the HrpR equivalent. Most of the differences are located in the first deduced helix. Conservative substitutions are present in the second deduced helix. Overall, the Pss61 HrpS shared 71% similarity and 57% identity with the Pss61 HrpR.

Nucleotide sequence of complementation group II and identification of the *hrpL* ORF. The nucleotide sequence of the 2.3-kb *Eco*RI fragment revealed an ORF (ORF1) encoding 184 aa positioned as indicated by previously mapped complementation group II Tn*phoA* and Tn*5-gusA1* insertions but located on the strand opposite that predicted by Xiao et al. (61) (Fig. 4). The location of the complementation group II::Tn*5-gusA1*insertion 5134 (61) was confirmed by sequence analysis employing a Tn*5-gusA1*-specific primer. The basis of the low-level expression detected from this fusion has not been established. Since ORF1 is the only complete ORF within the fragment and coincides with the mapped mutations, it was designated *hrpL*. The predicted gene product of *hrpL* is 21.2 kDa with a pI of 6.87. A potential σ^{54} promoter exhibiting high homology to the consensus σ^{54} promoter for *Pseudomonas aeruginosa* (9) is located 33 bp upstream of the *hrpL* translational start codon. Fourteen of the 17 conserved nucleotides are present. Seventy-three base pairs downstream of *hrpL* is another ORF, designated *hrpK*, which continues in frame into the contiguous *Bam*HI-*Eco*RI fragment carrying *hrmA*. The Tn*5-gusA1* mutation 5165 (61) maps to this locus. This deduced ORF is preceded by a potential ribosome binding site. No transcriptional termination signals are apparent in this region, but other genetic evidence suggests that this locus is part of a separate transcriptional unit (60b).

No fortuitous restriction sites that were suitable for subcloning the *hrpL-hrpJ* intergenic region for analysis of the *hrpL* promoter were identified. To confirm the transcriptional orientation of *hrpL*, the 336-bp fragment extending from 78 nucleotides internal to *hrpL* ORF to 16 nucleotides internal to *hrpJ* ORF (Fig. 1) was amplified from pHIR11 by PCR employing synthetic oligonucleotide primers and cloned into pRG970 to create a *hrpL'-lacZ* fusion (designated pYXPL1R).

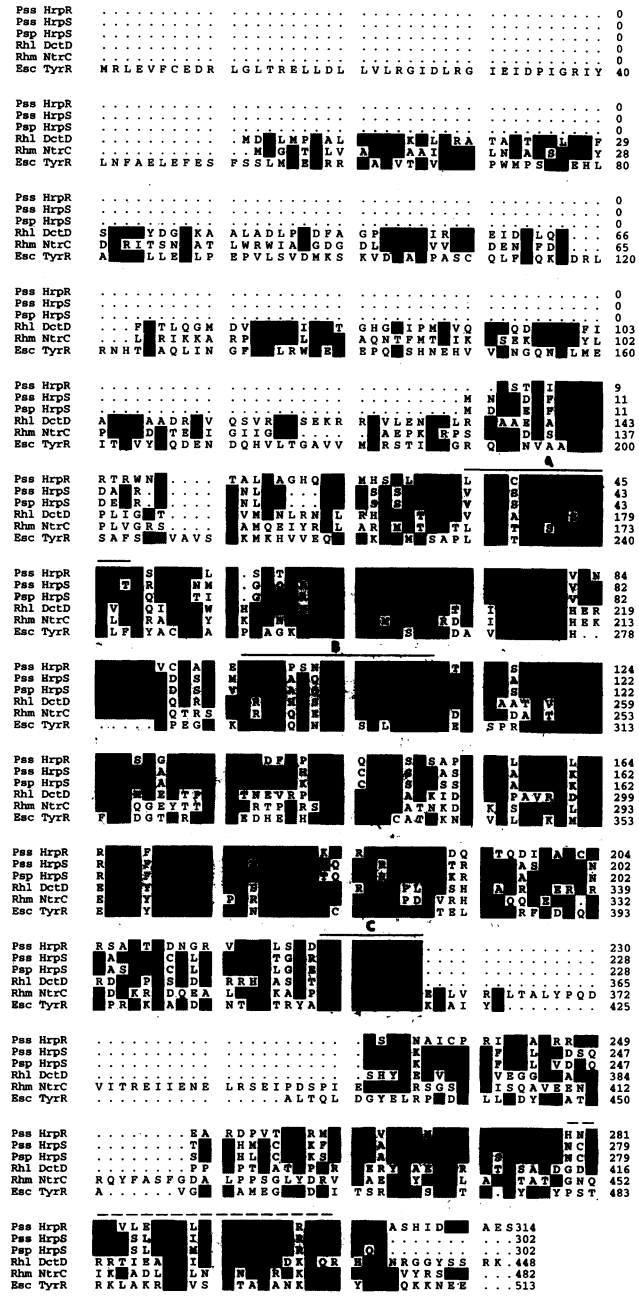

FIG. 3. Similarities between HrpR, HrpS, and receiver-effector components of two-component signal transduction systems. Sequence alignments were aligned by using the algorithm PILEUP (10). Residues found in a majority of the sequences shown are highlighted in black. Conservative substitutions are indicated by the shaded boxes. The signature motifs for interaction with σ^{54} (1) are overlined and labeled A, B, and C. The region exhibiting homology to an HTH by the criteria of Dodd and Egan (11) are indicated by the dashed line. Abbreviations: Pss, *P. syringae* pv. syringae Pss61; Psp, *P. syringae* pv. phaseolicola NPS3121, Rhl, *Rhizobium leguminosarum*; Rhm, *Rhizobium meliloti*; Esc, *E. coli*.

The resulting construct was promoter active in Pss61 transformants (see below). Production of the predicted *hrpL* gene product was verified by cloning *hrpL* as a 1-kb *Ssp*I-*Hin*cII fragment into pVEX11, and its expression was driven by T7 RNA polymerase in BL21(DE3) transformants. A 21-kDa protein was detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown). No products were detected when the fragment was cloned in the opposite direction or in uninduced cells.

Homology of HrpL with group III alternate sigma factors. A survey of the data bases by using the BLAST algorithm (2, 17) revealed homology of HrpL with AlgU, a putative alternate sigma factor of *P. aeruginosa* controlling the expression of *algD* (Fig. 5) (43). AlgU is most closely related to *Bacillus subtilis* Spo0H (σ^{30}) (12). HrpL and AlgU were of similar size and exhibited 48% similarity and 25% identity. Low homologies between sigma factors are typical (22, 42). HrpL retained many of the features of group III sigma factors (42). Regions corresponding to sigma factor domains 2 and 4 (22) exhibited the highest homology. Subdomain 2.2 has been proposed to interact with the core RNA polymerase and exhibits the highest conservation. Subdomain 4.2 has been postulated to recognize the −35 analog of promoter sequences (22), and a potential HTH motif was detected. The amino acid sequence within the second deduced α-helix of the HrpL HTH is nearly identical to that of AlgU. Little homology was detected in region 2.4, which is thought to interact with the −10 region of promoters.

Regulation of the *hrpJ* and *hrpZ* promoters by *hrpL* and *hrpRS*. The *hrpZ* locus has recently been shown to produce a plant response elicitor which may control the pathogenicity and host range of *P. syringae* strains (21). The *hrpJ* operon has been proposed to produce an inner membrane complex for protein translocation (27, 39). To determine whether the *hrpZ* promoter controlling the *hrpZ* operon is regulated by the *hrpLRS* system, the 0.8-kb *Bgl*II-*Hin*dIII fragment beginning 10 nucleotides downstream of *hrpS* ORF and extending to the *Hin*dIII site internal to *hrpZ* was cloned into pRG970 to create a *hrpZ'-lacZ* transcriptional fusion (pXYPZ1R). A 0.3-kb PCR fragment containing the *hrpL-hrpJ* intergenic region amplified as described above was ligated into pRG970 to construct a *hrpJ'-lacZ* (pXYPJ1R) fusion. Both constructs were expressed in Pss61 transconjugants carrying the wild-type *hrp* cluster and in Pss61-2089 containing the *hrpH*::Tn*phoA* insertion (Table 3), indicating that they are promoter active. The *hrpH* mutations were used as a control in these experiments because HrpH is thought to be an outer membrane protein lacking regulatory activity (24). Like that observed previously with the *hrmA'-lacZ* fusion (23), expression from both fusions is reduced more than 90% in the Pss61-2074 (*hrpL*::Tn*phoA*) and Pss61-2094 (*hrpS*::Tn*phoA*) mutants. Similar results were obtained when MC4100(pYXPJ1R) and MC4100(pYXPZ1R) were transformed with the pHIR11::Tn*phoA* derivatives (Table 4). The plasmid pRG970 has been reported to stably coexist with pRK290 family plasmids (e.g., pLAFR3) in the presence of antibiotic selection (59). MC4100 transformants carrying pHIR11-2096 exhibited a 30-fold increase in the apparent activity of the *hrpJ* and *hrpZ* promoters which was not observed when pHIR11-2074 and pHIR11-2094 derivatives were substituted. Only basal expression of the *hrpJ* and *hrpZ* promoters was detected in the *hrpS*::Tn*phoA* and *hrpL*::Tn*phoA* mutants. These observations indicate that HrpL and HrpRS control the expression of these promoters in addition to that of *hrmA*.

Expression of *hrpL* is dependent upon *hrpRS*. Expression of the *hrpL'-lacZ* fusion carried by pYXPL1R was also screened in the Pss61::Tn*phoA* mutants for dependence upon other *hrp* genes. The *hrpL'-lacZ* fusion was expressed in wild-type Pss61 and in the *hrpH* mutant Pss61-2089 (Table 3). Much lower expression was detected in the *hrpS* mutant Pss61-2094. The β-galactosidase levels were 6% of that detected in Pss61.

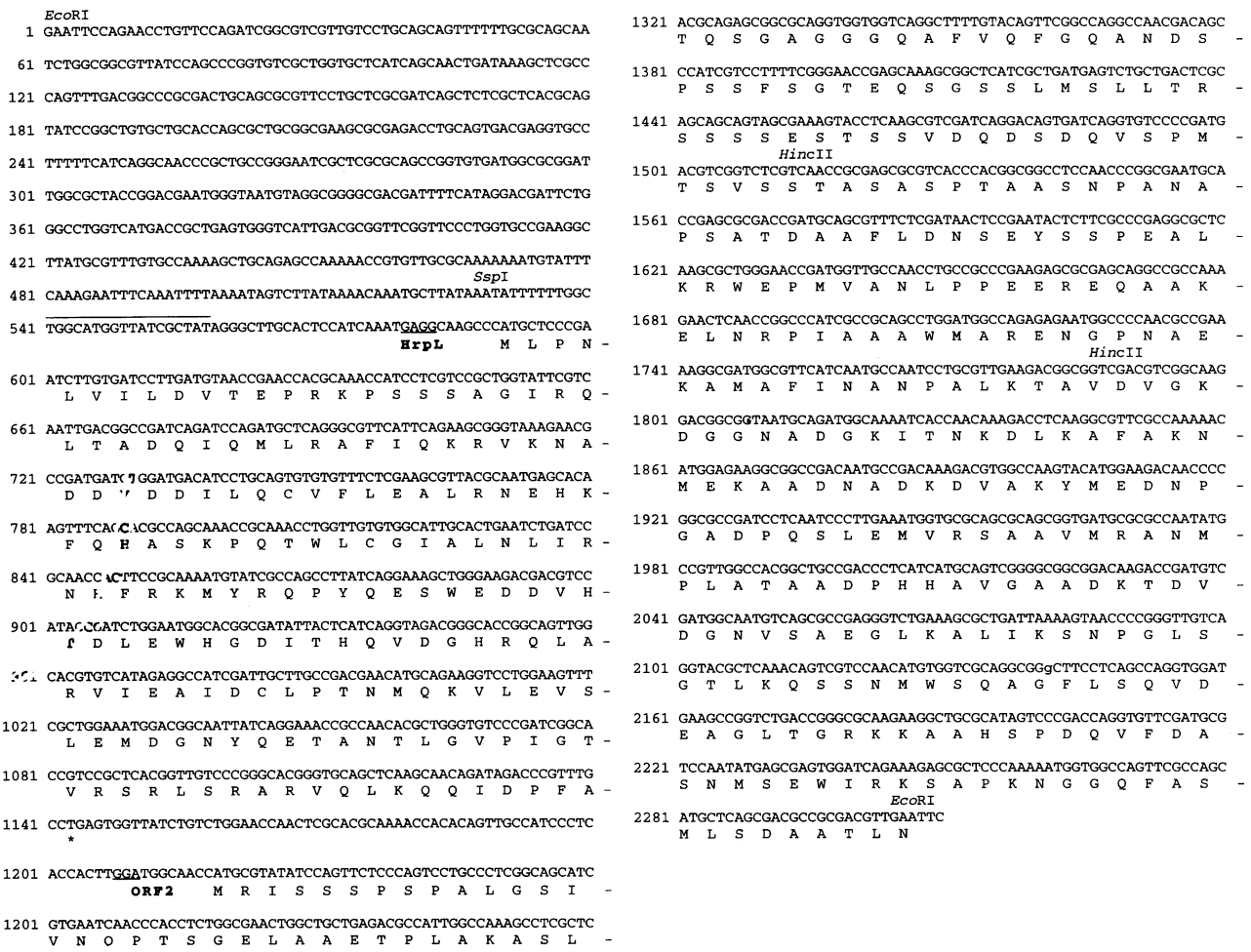

FIG. 4. Nucleotide sequence and deduced gene product of *hrpL*. The nucleotide sequence for the *Eco*RI fragment is shown. The sequencing strategy is described in the text. Deduced translation products are shown by using single-letter amino acid residue codes. Potential ribosome binding sites are underlined. A potential σ^{54} promoter (9) is overlined. Tn*5-gusA1* insertion 5134 was mapped to nucleotide 737. The *hrpJ1* ORF (not shown) is located on the reverse strand (27) and begins at nucleotide 348.

Unlike the *hrpJ* and *hrpZ* promoters, however, an intermediate level of expression was detected in the *hrpL* mutant Pss61-2074. Expression levels were consistently at least threefold higher than that detected in Pss61-2094. The higher expression detected in the *hrpL* mutant suggested that transcription of *hrpL* may not be directly dependent upon itself but could be affected indirectly by other factors.

The effect of pHIR11 derivatives on *hrpL* promoter activity in MC4100 supported this deduction. Transformation of MC4100(pYXPL1R) with pHIR11-2096 carrying the wild-type *hrp* cluster caused at least a 15-fold enhancement in the activity of the *hrpL'-lacZ* fusion (Table 4). In contrast to the other *hrp* and *hrmA* promoters screened, however, a pHIR11 derivative carrying a *hrpL*::Tn*phoA* insertion (pHIR11-2074) was as effective as the wild-type *hrp* cluster in stimulating expression of the *hrpL'-lacZ* fusion in MC4100. Inactivation of *hrpS* (pHIR11-2094), as described before, reduced expression of the fusion to undetectable levels. This observation suggests that HrpR and HrpS control the activity of the *hrpL* promoter independently of HrpL.

Activation of the *hrpL* promoter by HrpR and HrpS was demonstrated by transforming MC4100(pYXPL1R) with pYXRS1D carrying *hrpRS* cloned as a 2.2-kb *Bam*HI-*Bgl*II fragment into pDSK519 so that expression of *hrpRS* is directed by the *lacZ* promoter of the vector. In MC4100, the *lac* promoter is constitutively expressed because of the *lacI* deletion. Expression of the plasmid-borne *hrpL'-lacZ* fusion increased at least 300-fold in the presence of pYXRS1D (Table 5). Neither pSGR1B carrying *hrpR* nor pJSS1D containing *hrpS* alone could substitute for pYXRS1D. Transformation of MC4100(pYXPL1R) with both pSGR1B and pJSS1L activated expression of the *hrpL'-lacZ* fusion at least 100-fold.

HrpL functions independently of HrpR and HrpS to direct expression of *hrp*-responsive genes. Since *hrpL* expression is dependent upon HrpR and HrpS, it is a possibility that HrpL, as a putative alternate sigma factor, could control the expression of *hrp*-responsive genes independently of HrpR and HrpS. In the previous experiments, the plasmids carrying *hrpL* (pSGL1D and pSGL1L) had the locus cloned in the orientation opposite that of the *lac* promoter of the vector. To drive the expression of *hrpL* independently of *hrpRS*, the 1-kb *Ssp*I-*Hinc*II fragment beginning 60 bp upstream of the *hrpL* ORF and extending 372 bp downstream of the *hrpL* ORF was cloned into pBluescriptII SK$^+$ such that expression could be driven by the *lac* promoter of the vector. The resulting plasmid, pYXL2B, or the pBluescript vector was transformed

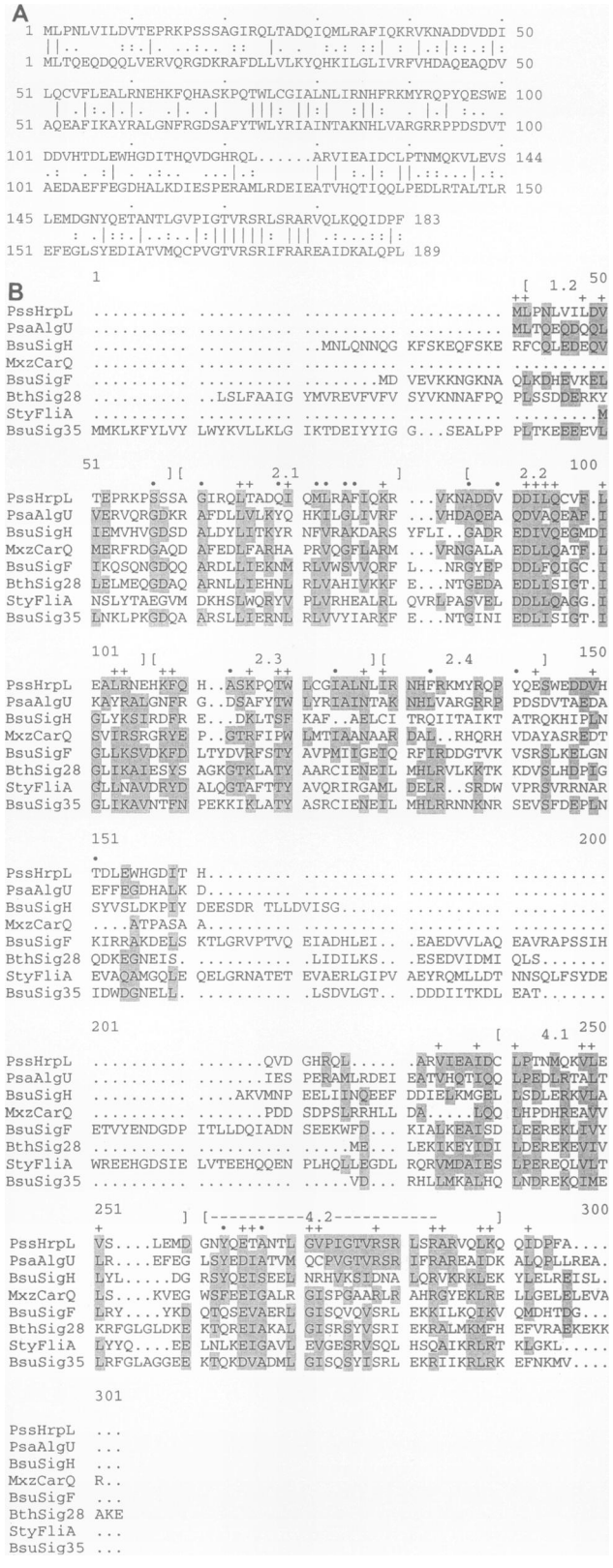

FIG. 5. Similarity of HrpL to group III sigma factors. (A) Similarity between HrpL and AlgU as determined by the BESTFIT algorithm (10). Vertical lines indicate identity, two dots indicate a conservative substitution, and one dot indicates a less-conservative substitution. (B) Similarity to selected group III sigma factors. Sequences were aligned by using the algorithm PILEUP (10). Brackets delimit sigma factor domains 1, 2, 2.1, 2.2, 2.3, 2.4, 4.1, and 4.2 identified in the SigH sequence. HrpL amino acid residues with identity with the σ^{70} consensus sequence (42) are indicated by a plus, and those that are similar are marked with a boldface period. Similar or identical residues in HrpL and AlgU or in a majority of the group III sigma factor sequences shown are shaded. The similarity matrix of Lonetto et al. (42) was employed here. A potential HTH identified by the procedures of Dodd and Egan (11) is indicated by a dashed line. Accession numbers for the sequences are listed in reference 42. Abbreviations: Pss, *P. syringae* pv. syringae Pss61; Psa, *P. aeruginosa*; Bsu, *B. subtilis*; Mxc, *Myxococcus xanthus*; Bth, *Bacillus thuringiensis*; Sty, *Salmonella typhimurium*.

TABLE 3. Effect of *P. syringae* pv. syringae Pss61 *hrpL* and *hrpS* mutations on the activity of *hrpJ*, *hrpZ*, and *hrpL* promoters

Strain[a]	Mutation[b]	Promoter activity[c]		
		hrpJ[d]	*hrpZ*	*hrpL*
Pss61	None (wild type)	19 ± 1	78 ± 5	116 ± 3
Pss61-2074	*hrpL*	1 ± 1	2 ± 1	16 ± 1
Pss61-2094	*hrpS*	2 ± 1	2 ± 1	5 ± 1
Pss61-2089	*hrpH*	55 ± 9	125 ± 19	105 ± 25

[a] Transformant carrying plasmid-borne *hrp-lacZ* fusions.
[b] Chromosomal Tn*phoA* mutation.
[c] As indicated by expression of a *lacZ* fusion. Data are presented as β-galactosidase activity in Miller units after induction for 6 h.
[d] Plasmids employed: for *hrpJ*, pYXPJ1R; for *hrpZ*, pYXPZ1R; for *hrpL*, pYXPL1R.

into MC4100(pYXPL1R), MC4100(pYXPJ1R), MC4100 (pYXPZ1R), and MC4100(pSGAMS1). Activity of the *hrpJ*, *hrpZ*, and *hrmA* promoters increased 70- to 150-fold after transformation (Table 6). Consistent with the previous observations, P_{lac}-directed expression of *hrpL* did not affect the activity of the *hrpL* promoter. In transformants carrying the constitutively expressed *hrpL* construct, the presence of pYXRS1D carrying *hrpRS* had little or no effect on the activity of the *hrpJ*, *hrpZ*, and *hrmA* promoters. Expression of the *hrpL'-lacZ* fusion was stimulated as before but the activity of the other promoters was equivalent to that observed in the absence of *hrpRS*.

It was not possible to regulate the activity of the *lac* promoter in the previous experiments because of the *argF-lac* deletion in MC4100 and the absence of *lacI* on the vectors employed. To confirm that the observed increase in *lacZ* activity observed in the MC4100(pYXL2B) transformants is due to *hrpL* expression, the 1-kb *Ssp*I-*Hin*cII fragment carrying *hrpL* was cloned into the pSPORT 1 plasmid which carries *lacI* and an inducible *lac* promoter upstream of a multicloning site. As described before, in the absence of *hrpL* expression, the *hrpJ*, *hrpZ*, and *hrmA* promoters exhibited low basal expression. Upon isopropyl-β-D-thiogalactopyranoside (IPTG) induction of *hrpL* expression, activity of the assayed promoters increased at least sevenfold after 6 h of induction (Table 7). Transformation with pYXRS1D had no effect on the basal expression observed in the absence of IPTG induction (data not shown).

DISCUSSION

Multiple components were shown to mediate the environmental regulation of *P. syringae* pv. syringae Pss61 *hrp* and *hrmA* genes. Similar to the results reported previously for other *P. syringae* strains (14, 19, 31, 32, 52), inactivation of

TABLE 4. Effect of *hrpL* and *hrpS* mutations carried by a plasmid-borne *P. syringae* pv. syringae Pss61 *hrp/hrmA* gene cluster on the activity of *hrpJ*, *hrpZ*, and *hrpL* promoters in *E. coli* MC4100

Plasmid[a]	Mutation[b]	Promoter activity[c]		
		hrpJ[d]	*hrpZ*	*hrpL*
None		1 ± 1	3 ± 1	5 ± 1
pHIR11-2096	None	34 ± 3	115 ± 3	84 ± 5
pHIR11-2074	*hrpL*	1 ± 1	2 ± 1	128 ± 9
pHIR11-2094	*hrpS*	2 ± 1	3 ± 1	4 ± 1

[a] *E. coli* MC4100 transformant carrying the indicated plasmid.
[b] *hrp*::Tn*phoA* mutation.
[c] As indicated by expression of the indicated *lacZ* fusion. Data are presented as β-galactosidase activity in Miller units after induction for 6 h.
[d] Plasmids employed: for *hrpJ*, pYXPJ1R; for *hrpZ*, pYXPZ1R; and for *hrpL*, pYXPL1R.

complementation groups II and XIII (sensu [25]) reduced expression of *hrp*-regulated promoters such as the *hrpJ*, *hrpZ*, and *hrmA* promoters. A novel observation has been the ability to reassemble the *hrp* regulatory system in *E. coli* MC4100. By using subclones carrying each complementation group individually, *hrp* regions II and XIII were shown here to be sufficient to activate expression of the assayed promoters in MC4100 independently of other *hrp* genes. The loss-of-function data from the Pss61 *hrp*::Tn*phoA* mutants together with the gain of activity observed in MC4100 transformants carrying promoter-*lacZ* fusions provides conclusive evidence that these regions carry positive-acting transcriptional factors.

The nucleotide sequences of both regions revealed ORFs whose deduced gene products have features of known transcriptional factors. The deduced complementation group II product, HrpL, appears to be an alternate sigma factor. A key observation is the homology with *P. aeruginosa* AlgU. AlgU is a putative alternate sigma factor controlling the expression of the *algD* operon in *P. aeruginosa* (43), a bacterium closely related to *P. syringae* (47). The *algD* operon encodes the enzymes for extracellular polysaccharide biosynthesis (7, 43). AlgU and HrpL are of similar size, and the homology extends over the length of the deduced products. The homology with AlgU enabled the alignment of HrpL with a subset of group III sigma factors related to the *B. subtilis* SigH (σ^{30}) (12, 42, 46). Group III sigma factors are highly divergent from primary sigma factors (42). The sequence alignments revealed that regions 2 and 4 common to this family of sigma factors (22, 42) are partially conserved within the HrpL sequence. The retention of these regions coupled with the demonstrated regulatory activity provides a strong indication that HrpL could function as an alternate sigma factor. If HrpL proves to be an alternate sigma factor, it will be the smallest known member of the protein family. HrpL is deduced to consist of 184 aa residues with an M_r of 21,236. The next larger member of the family is AlgU (193 aa; M_r, 22,194).

TABLE 5. Effect of plasmids carrying *hrpR* and *hrpS* on the activity of *hrpL* promoter activity in *E. coli* MC4100(pYXPL1R)

Plasmid[a]	Gene[b]	Promoter activity[c]
None		5 ± 4
pDSK519	Vector	19 ± 5
pBluescriptII SK$^+$	Vector	7 ± 4
pDSK519; pBluescriptII SK$^+$		13 ± 5
pYXRS1D	*hrpRS*	1,620 ± 650
pSGR1B	*hrpR*	8 ± 7
pJSS1D	*hrpS*	24 ± 8
pSGR1B; pJSS1D	*hrpR*; *hrpS*	613 ± 248

[a] *E. coli* MC4100(pYXPL1R) transformant carrying the indicated plasmids.
[b] *hrp* gene(s) carried by the plasmid(s).
[c] As indicated by expression of the *hrpL'-lacZ* fusion. Data are presented as β-galactosidase activity in Miller units after induction for 6 h. In parallel experiments in which pRG970 was substituted for pYXPL1R, activities detected were lower than 10 Miller units.

Consistent with the interpretation that HrpL may function as an alternate sigma factor is the observation that HrpL, once it is expressed, can stimulate expression of several *hrp* or *hrmA* genes independently of other *hrp* genes. Although factors conserved among diverse bacteria cannot be fully excluded, expression of *hrmA*, *hrpJ*, and *hrpZ* operons appears to be solely dependent upon HrpL. The *hrpH* and *hrpK* genes as well as complementation groups VIII and IX are also likely to be regulated by HrpL. These genes are coregulated with *hrmA*, *hrpJ*, and *hrpZ* (14, 23, 50, 61). The *avr* genes also appear to be regulated by HrpL (31, 32, 52). The promoter regions of most *P. syringae hrp*, *hrmA*, and *avr* genes lack strong homology to known promoter sequences (19, 23, 24, 27, 32). For the *P. syringae avr* genes, a conserved sequence motif has been identified 6 to 8 bp upstream of the transcription initiation site (32). The proximity of this motif to the known transcriptional initiation sites for *avr* genes is suggestive that this motif functions as a promoter. A similar motif is located upstream of several *hrp* genes within promoter-active fragments (39, 60b) as well as upstream of *hrmA* (23). It is interesting that the second deduced helices of the HTH motifs of HrpL and AlgU are nearly identical, suggesting that these putative sigma factors may recognize similar promoter sequences (16, 22, 42, 54). The deduced *algD* promoter sequence (8) shares 66% identity with conserved residues of the *avr* motif. We are presently investigating whether the *avr*-derived motif forms the HrpL-responsive promoter sequence.

HrpR and HrpS identified in complementation group XIII are members of a large family of regulatory proteins associated with signal transduction. Both deduced gene products share homology with the NtrC family of regulatory proteins, and the central and C-terminal domains are conserved. The C-terminal domain is thought to function in DNA binding (45), and a potential HTH motif (11) is present in both deduced gene products. HrpR and HrpS were shown to specifically activate the *hrpL* promoter. Inactivation of *hrpS* in Pss61 suppressed the expression of a plasmid-borne *hrpL'-lacZ* fusion, and a clone carrying *hrpRS* activated the *hrpL* promoter in MC4100. Both *hrpR* and *hrpS* are required for this activity. It therefore appears likely that these proteins interact with unique regulatory sequences upstream of *hrpL*, but regulatory factors broadly conserved among bacterial species could also mediate in this process. The regulatory sequences recognized by HrpR and HrpS appear to be located in the 242-bp *hrpL-hrpJ* intergenic region. Since transformation of *hrpRS* into strains carrying the *hrpL* ORF cloned downstream of the *lac* promoter had little effect on the expression of *hrpL*-responsive promoters irrespective of the activity of the *lac* promoter (with or without IPTG), it appears likely that the HrpR- and HrpS-linked regulatory region is at least 60 bp upstream of the *hrpL* initiation codon.

The proteins most similar to HrpR and HrpS are members of two-component signal transduction systems involving a transmitter-sensor with protein kinase activity and a receiver-effector which is activated by phosphorylation. As noted before (19, 45) for HrpS and as reported here for HrpR, these proteins differ from other members of the family by the apparent absence of an amino-terminal domain that modulates

TABLE 6. Effect of *hrpRS* and *hrpL* on the activity of *hrmA*, *hrpJ*, and *hrpZ* promoters in *E. coli* MC4100

Plasmid[a]	Cloned gene(s)	Promoter activity[b]			
		hrmA[c]	*hrpJ*	*hrpZ*	*hrpL*
pBluescriptII SK$^+$	Vector	6 ± 3	1 ± 1	2 ± 1	5 ± 1
pYXL2B	*hrpL*	785 ± 23	101 ± 8	304 ± 33	4 ± 1
pDSK519	Vector	7 ± 1	1 ± 1	2 ± 1	4 ± 1
pYXRS1D	*hrpRS*	4 ± 1	1 ± 1	2 ± 1	694 ± 15
pYXL2B; pYXRS1D	*hrpL*; *hrpRS*	594 ± 30	101 ± 4	312 ± 6	700 ± 4

[a] *E. coli* MC4100 transformant carrying the indicated plasmids.
[b] As indicated by expression of a *lacZ* fusion. Data are presented as β-galactosidase activity in Miller units after induction for 6 h.
[c] Plasmids employed: for *hrmA*, pSGR7; for *hrpJ*, pYXPJ1R; for *hrpZ*, pYXPZ1R; and for *hrpL*, pYXPL1R.

the regulatory activity. Most members of the family carry an approximately 130-aa domain containing a highly conserved Asp residue which is phosphorylated by the transmitter/ kinase component (45, 48, 57). TyrR, NifA, and XylR also lack this domain but contain a different N-terminal domain necessary for the regulatory activity (3, 6, 9, 33, 49, 62). No such domain is apparent in the deduced gene product of either HrpR or HrpS. The apparent absence in HrpR and HrpS of an amino-terminal domain that modulates the regulatory activity argues that it is unlikely that these proteins are activated by phosphorylation. This may explain the activity observed in *E. coli* MC4100, but the involvement of additional conserved components cannot be excluded. One indication that additional components may function in the regulation of the *hrpL* promoter is the reduced expression of the plasmid-borne *hrpL* promoter in a Pss61 *hrpL*::Tn*phoA* mutant. Since the *hrpL* promoter does not appear to be directly regulated by itself, another locus in Pss61, whose activity or expression is dependent in part upon HrpL, affects the activity of the *hrpL* promoter. The absence of a similar effect in MC4100 transformants carrying pHIR11 argues that the postulated regulatory determinant lies outside of the *hrp* cluster.

Felley et al. (14) reported that expression of several *hrp* genes in *P. syringae* pv. phaseolicola NPS3121 is reduced in *rpoN* mutants. The deduced promoter regions for several *hrp* genes, however, exhibit weak (minimal GG n_{10} GC match; common in GC-rich DNA) or no similarity to the consensus σ^{54} promoter sequence (9). In contrast, the *hrpL* promoter region exhibits strong homology to the *P. aeruginosa* consensus σ^{54} promoter sequence. Fourteen of the 17 bases are conserved. With the exception of TyrR, all members of the family of regulatory proteins homologous to HrpR or HrpS interact with σ^{54} RNA polymerase holoenzyme (for examples, see references 45, 48, and 57). The deduced HrpR and HrpS carry signature features indicative of a potential interaction with σ^{54}. The central domain common to the protein family which functions in the activation of the σ^{54}-RNA polymerase holoenzyme (45) as well as the σ^{54} interaction motifs (1) are retained in both HrpR and HrpS. It therefore appears likely that transcription of *hrpL* is initiated by σ^{54}.

The mechanism by which HrpRS and HrpL perceive and transduce nutritional signals to alter expression of *hrp*, *hrmA*, and *avr* genes remains an interesting question. A possible model to explain the regulatory activity is presented in Fig. 6. There are three levels at which an environmental signal could affect expression of *hrp*-regulated genes: (i) regulation of *hrpRS* expression; (ii) modification of *hrpL* expression via HrpR or HrpS or indirect factors; and (iii) activity of the HrpL-responsive promoters. Xiao et al. (61) reported that, unlike the other *hrp* fusions screened, expression of a complementation group XIII::Tn*5-gusA1* (*hrpS*) insertion was unaffected by nutritional conditions. Expression of *hrpRS* therefore may be constitutive under these conditions. In other two-component signal transduction systems, the expression of the regulator-effector component is influenced by environmental stimuli through an autoregulatory circuit (1). Further analysis of level 1, 2, and 3 activity is needed to elucidate the mechanism by which environmental signals affect *hrp*, *hrmA*, and *avr* gene expression.

In conclusion, the environmental regulation of *hrp*-respon-

TABLE 7. Effect of induced expression of *hrpL* on the *hrmA*, *hrpJ*, and *hrpZ* promoter activity in *E. coli* MC4100

Promoter construct[a]	Promoter activity[b]	
	−IPTG	+IPTG
hrpL	5 ± 1	5 ± 1
hrpJ	1 ± 1	15 ± 1
hrpZ	5 ± 1	53 ± 4
hrmA	8 ± 1	58 ± 1

[a] Promoter-active fragment cloned into pRG970 to create a *lacZ* fusion. Plasmids employed: for *hrpL*, pYXPL1R; for *hrpJ*, pYXPJ1R; for *hrpZ*, pYXPZ1R; and for *hrmA*, pSGR7.
[b] As indicated by expression of the indicated fusion in *E. coli* MC4100(pYXL2SP) transformants. Data are presented as β-galactosidase activity in Miller units after growth in M63M medium for 6 h. IPTG (1 mM) was added as indicated to the M63M medium.

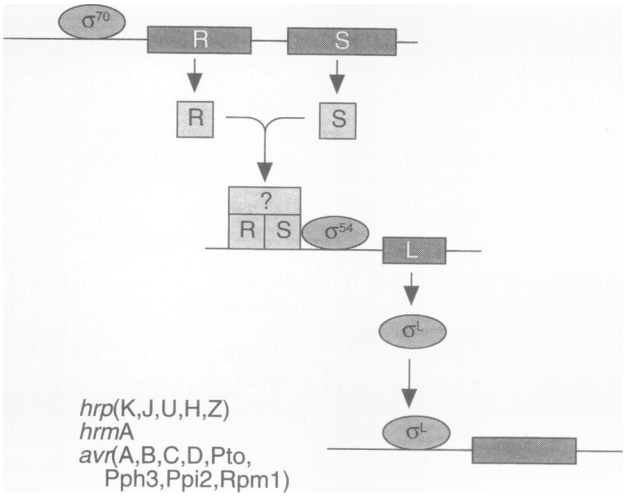

FIG. 6. Regulation cascade controlling *hrp*, *hrmA*, and *avr* expression. Postulated regulatory components functioning in the regulation of *hrpRS*, *hrpL*, and other *hrp*, *hrmA*, and *avr* genes.

sive genes is mediated by a multicomponent regulatory cascade involving HrpR, HrpS, and HrpL. The complexity of the adaptive process in *P. syringae* strains may reflect the need to coordinate multiple functions associated with the pathogenicity. The *hrp* cluster is organized as at least eight transcriptional units (61) and encodes inner and outer membrane components associated with protein secretion and a secreted plant response elicitor (21, 24, 27, 39). The production of these components appears to be genetically segregated as a regulon from other housekeeping activities in *P. syringae* strains. It remains to be determined whether other factors associated with the pathogenicity or virulence of *P. syringae* are regulated by HrpL or HrpR and HrpS. Since secretion of proteineous virulence factors in *Shigella*, *Yersinia*, and *Salmonella* spp. involve components similar to those identified in the *hrp* cluster (24, 27, 39) which are organized as multiple transcriptional units, a similar regulatory system may control the expression of these systems.

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