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HrpXv, an AraC-Type Regulator, Activates Expression of Five of the Six Loci in the *hrp* Cluster of *Xanthomonas campestris* pv. vesicatoria

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***hrp* genes, basic pathogenicity genes of the pepper and tomato pathogen *Xanthomonas campestris* pv. vesicatoria, are regulated dependent on environmental conditions. We isolated the *hrpXv* gene, which was found to be outside the large *hrp* cluster comprising the six loci *hrpA* to *hrpF*. The predicted HrpXv protein is 476 amino acids long and has a molecular mass of 52.5 kDa. HrpX is highly conserved among xanthomonads and is a member of the AraC family of regulatory proteins. An *hrpXv* insertion mutant has a typical *hrp* phenotype and no longer allows induction of the five *hrp* loci *hrpB* to *hrpF* in the new *hrp* induction medium XVM2, indicating that HrpXv is the positive regulator of these loci. An *hrpXv* mutant could be partially complemented by the related *hrpB* gene of *Burkholderia solanacearum*, the protein product of which shows 40 and 58% amino acid identity and similarity, respectively, to HrpXv. The *hrpXv* gene itself has a low basal level of expression that is enhanced in XVM2. Expression of *hrpXv* as well as that of the *hrpA* locus is independent of the *hrpXv* gene. The transcription start site of *hrpXv* was mapped. Comparison between the *hrpXv* promoter and the corresponding region of the *hrpXc* gene from *X. campestris* pv. campestris revealed sequence conservation up to position −84. A putative helix-turn-helix motif in the C-terminal region of HrpXv and its possible interaction with a conserved *hrp* promoter element, the plant-inducible promoter box, are discussed.**

The species *Xanthomonas campestris* has been described as being exclusively plant pathogenic and shows a high degree of host specificity. This finding has led to division of this species into many pathovars, according to the host ranges of strains. *X. campestris* pv. vesicatoria is the causal agent of bacterial spot disease of pepper and tomato plants and has served as a model system with which to study the molecular basis of pathogenesis. In a compatible interaction, the bacteria multiply in the intercellular spaces of the plant tissue, while in resistant plants, bacterial growth is inhibited and often accompanied by the hypersensitive reaction (HR), a rapid cell death (necrosis) at the site of infection (10, 11). The 23-kb chromosomal *hrp* (hypersensitive reaction and pathogenicity) gene cluster (6, 38) is indispensable for pathogenesis in susceptible host plants and for the elicitation of the HR in resistant host and nonhost plants (8). The cluster contains six *hrp* loci, *hrpA* to *hrpF*, which have been sequenced (7, 21, 22, 54). Transposon mutagenesis with Tn*3-gus* that carries a promoterless β-glucuronidase (GUS) gene allowed expression studies of five out of six *hrp* loci (45). *hrpA*, *hrpB*, *hrpC*, *hrpD*, and *hrpF* are suppressed during growth in complex medium and are induced in planta and in TCM (tomato conditioned medium) (45). The *hrp* induction medium TCM, the quality of which varied, has recently been replaced by the synthetic medium XVM2, which has been shown to induce the *hrpA* locus (54). *hrpE* expression has not been studied yet because of lack of Tn*3-gus* insertions with the *gusA* gene in the direction of *hrpE* transcription.

An additional *hrp* locus, *hrpX*, has been identified in the cabbage pathogen *X. campestris* pv. campestris (32) (*hrpXc*) and in the rice pathogen *X. oryzae* pv. oryzae (31) (*hrpXo*). It was stated in a recent publication (40) that the corrected sequences of the predicted HrpX proteins have significant sequence similarity with the *hrp* regulatory protein HrpB of *Burkholderia solanacearum* (formerly *Pseudomonas solanacearum*) (27), but a regulatory function of HrpX has not been demonstrated.

In this study, we report on the isolation and sequence analysis of the *hrpX* gene of *X. campestris* pv. vesicatoria (*hrpXv*) and demonstrate that *hrpXv* is essential for the transcriptional activation of the *hrp* gene cluster. The transcription start site of *hrpXv* was mapped, and the in vitro medium XVM2 was shown to induce expression of all seven *hrp* loci.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used are described in Table 1. Plasmids were introduced into *Escherichia coli* by electroporation and into *Xanthomonas* strains by conjugation using pRK2013 as the helper plasmid in triparental matings (18, 24). *E. coli* cells were cultivated at 37°C in Luria-Bertani medium, and *Xanthomonas* strains were grown at 28°C in NYG (16) or XVM2 (54). Antibiotics were added to the media in the following final concentrations: ampicillin, 100 μg/ml; cycloheximide, 50 μg/ml; kanamycin, 25 μg/ml; rifampin, 100 μg/ml; spectinomycin, 100 μg/ml; and tetracycline, 10 μg/ml.

Plant material and plant inoculations. Inoculations of tomato Moneymaker and the near-isogenic pepper lines ECW and ECW-10R were performed as described previously (8). Bacterial strains were tested for their phenotypes by inoculation of a suspension of 5×10^8 bacteria per ml in 1 mM $MgCl_2$ into the intercellular space of the plant leaf, and symptom development was scored over a period of 5 days. To analyze bacterial growth in planta, suspensions of 10^5 bacteria per ml in 1 mM $MgCl_2$ were inoculated into leaves of the susceptible pepper cultivar ECW. Leaf discs were cut out at different time points after inoculation and macerated in 1 mM $MgCl_2$, and dilutions were plated on selective media.

Construction of insertion mutants. To increase the density of transposon insertions in the right part of the *hrp* cluster, pXV4, a cosmid from the genomic library of *X. campestris* pv. vesicatoria 75-3 (39) containing the region from *hrpC2* to downstream of *hrpF*, was mutagenized with Tn*3-gus*. Thirty-two insertion derivatives were introduced into the genome of strain 85-10 by marker exchange as described previously (8). For mutagenesis of the *hrpXv* gene, the 7-kb internal *Bam*HI fragment of pBX1 was subcloned into pUC118, resulting in pUX4. The omega cassette (42), conferring resistance to spectinomycin and streptomycin, was inserted into the unique *Eco*RV site of pUX4, and the insert of this plasmid, pUXO4, was cloned into pLAFR6, giving pSXO4. pSXO4 was conjugated into *X.*

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or source
X. campestris pv. vesicatoria		
85-10	Pepper race 2; wild type; Rif^r	9
85E	*gumG*::Tn*3-gus* insertion mutant of 85-10; *hrp*$^+$ EPS$^-$ Rif^r Km^r	54
75-3	Tomato pathogen; wild type; Rif^r	39
85-10::*hrpA*14, 85-10::*hrpB*35, 85-10::*hrpC*17, 85-10::*hrpD*54, 85-10::*hrpF*312	Tn*3-gus* insertion mutants of 85-10; *hrp* Rif^r Km^r	45
85-10::*hrpE*525	Tn*3-gus* insertion mutant of 85-10; *hrpE* Rif^r Km^r	This study
X. campestris pv. campestris		
2D520	Wild type; Rif^r	46
JS111	Tn*4431* mutant of 2D520; *hrpXc* Rif^r Tc^r	46
E. coli DH5α	F$^-$ *recA* f80*dlacZ* Δ*M15*	Bethesda Research Laboratories, Gaithersburg, Md.
Plasmids		
pLAFR3	RK2 replicon Mob$^+$ Tra$^-$; Tc^r; contains p*lac*	48
pLAFR6	pLAFR3 derivative; multiple cloning site flanked by transcriptional terminators; Tc^r	9
pBluescript-KS/SK II	Phagemid, pUC derivative; Ap^r	Stratagene, La Jolla, Calif.
pUC118	ColE1 replicon; Ap^r	51
pUFR043	Mob$^+$ Tra$^-$; contains p*lac*; Gm^r Nm^r	D. Gabriel
pL6GUSB	Promoterless *gusA* gene in pLAFR6; Tc^r	36
pXV74	pLAFR3 clone from *X. campestris* pv. vesicatoria 75-3 genomic library, containing *hrpA* to *hrpF*	54
pXV4	pLAFR3 clone from *X. campestris* pv. vesicatoria 75-3 genomic library, containing *hrpC2* to *hrpF*	This study
pXV9::A14, pXV9::B35 pXV9::C17, pXV9::D54	*hrp*-Tn*3-gus* insertion derivatives of pXV9	45
pXV4::E525	*hrpE*-Tn*3-gus* insertion derivative of pXV4	This study
pXV2::F312	*hrpF*-Tn*3-gus* insertion derivative of pXV2	45
pHK1a	4.4-kb *Eco*RI-*Bgl*I fragment of *X. campestris* pv. campestris in pTZ19R; contains *hrpXc*; Ap^r	C. I. Kado
pAM5	2-kb *Nsi*I-*Pst*I fragment containing *hrpB* of *B. solanacearum* in pLAFR3 under control of p*lac*; Tc^r	C. Boucher
pRK2013	ColE1 replicon TraRK$^+$ Mob$^+$; Km^r	24

campestris pv. vesicatoria 85E and 75-3 to generate the mutants 85EX and 75X, respectively, by marker exchange. Correct genomic insertion of Tn*3-gus* and the omega cassette was verified by Southern blot analysis.

Sequence analysis. T3 and T7 primers and custom primers were used with the T7 DNA polymerase sequencing kit (Pharmacia, Uppsala, Sweden) to sequence a single strand of the *hrpXv* gene of *X. campestris* pv. vesicatoria 85E present in pBX1. Any ambiguities were clarified by sequencing the opposite strand. Both DNA strands of the region upstream of the *Bam*HI site were sequenced. To sequence the promoter region of the *hrpX* gene of *X. campestris* pv. campestris the 500-bp *Bam*HI fragment from pHK1a was cloned into pBluescript-KS II and partially sequenced by using the T3 and T7 primers.

Sequences were analyzed with the University of Wisconsin GCG 8.0 package (17). Sequence alignments were done with the BESTFIT and GAP programs, and their significance was determined by comparing them against 10 randomized sequences. Database searches were done by using TBLAST (3).

Primer extension analysis. Bacteria were grown for 16 h in NYG or XVM2 or recovered from susceptible pepper plants (ECW) 3 days after whole-plant infiltration (8). Bacterial RNA was extracted as described previously (2). Fifteen micrograms of RNA and ^{32}P-labeled oligonucleotide 137 (5′ TTCTGCGTAT GACAACGCAGAGATCGCTGC 3′) were annealed at 65°C for 90 min (5), and the extension reaction was performed in the presence of actinomycin D with 200 U of MMLV-Superscript II reverse transcriptase (Gibco-BRL) for 1 h at 42°C (5). Extension products were analyzed on 6% denaturating polyacrylamide gels.

Determination of GUS activities. GUS activities were measured after growth of *Xanthomonas* strains in NYG or XVM2 for 14 to 16 h or in susceptible pepper plants for 3 days as described previously (45) except that bacterial numbers in liquid cultures were determined by measuring the optical density at 600 nm.

Nucleotide sequence accession number. The 1,806-bp sequence of *hrpXv* shown in Fig. 2 can be retrieved from GenBank (accession number U45888).

RESULTS

Isolation of the *hrpXv* gene. Sequences homologous to the *hrpX* gene of *X. campestris* pv. campestris are present in several *Xanthomonas* species (31). To find out whether *X. campestris* pv. vesicatoria 75-3 and 85-10 contain a homolog, the 700-bp internal *Pst*I fragment of the *hrpXc* gene (33) present in plasmid pHK1a was used as a probe in Southern hybridizations under stringent conditions. A single 9.4-kb *Eco*RI fragment was identified in both strains 75-3 and 85-10 (data not shown). With the same probe, we isolated two cosmid clones (pXV30 and pXV751) from a genomic cosmid library of strain 75-3 (39). pXV30 and pXV751 contain about the same 24-kb insert, but in opposite orientation. The *hrpXv* gene is located at the extremity of the insert of pXV751 on a 2.5-kb *Eco*RI-*Hin*dIII (vector site) fragment that was subcloned in pBluescript-KS II (pBX10) and partially sequenced. Comparison of the obtained sequence with the published sequences of *hrpXo* (31) and *hrpXc* (40) indicated that the *hrpXv* gene was only partially present in the cosmid clones. We therefore cloned the genomic 9.4-kb *Eco*RI fragment described above from *X. campestris* pv. vesicatoria 85E in pBluescript-KS to generate pBX1 (Fig. 1). Restriction analysis revealed that the *hrpXv* region is located outside the large *X. campestris* pv. vesicatoria *hrp* cluster, including 20 kb of flanking regions.

Sequence analysis of *hrpXv*. The *hrpXv* gene in plasmid pBX1 was sequenced (Fig. 2). The ATG at position 240, which is preceded by a putative ribosome binding site, is most likely used as a translation start codon (see below), although the first ATG of the open reading frame is located 306 bp further upstream. A TAA stop codon is at position 1670, predicting a hydrophilic protein of 476 amino acids with a molecular mass

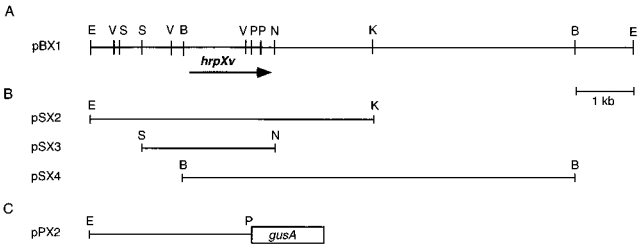

FIG. 1. The *hrpXv* region and subcloned fragments used in this study. (A) Restriction map of the genomic 9.4-kb *Eco*RI fragment in plasmid pBX1. Location and orientation of the *hrpXv* gene are indicated by the arrow. B, *Bam*HI; E, *Eco*RI; K, *Kpn*I; N, *Nhe*I; P, *Pst*I; S, *Sac*I; V, *Eco*RV. For *Sac*I and *Pst*I, only relevant restriction sites are shown. (B) Plasmids used for complementation studies. Inserts in pLAFR6 are indicated as bars. (C) *hrpXv* promoter-*gusA* fusion in plasmid pPX2.

of 52.5 kDa and an isoelectric point of 8.35. A similar-size protein has been expressed from the homologous *hrpXo* gene in *E. coli* (31), indicating that the predicted size is correct.

HrpXv is a member of the AraC-type family of regulatory proteins. Comparison of the HrpXv amino acid sequence with sequences in the databases revealed the following similarities. HrpXv is 97 and 91% identical to HrpXo and HrpXc (40), respectively. HrpXv and HrpXc are identical in size, while HrpXo is one amino acid shorter. The sequence identities of the coding regions of *hrpXv* to *hrpXo* and *hrpXc* on the DNA level are 91 and 85%, respectively. HrpXv is 40% identical and 58% similar to the predicted *hrp* regulatory protein HrpB of *B. solanacearum* (27), which is one amino acid longer than HrpXv (Fig. 3). The amino acid identity is much higher (62%) if only the C-terminal 115 amino acids of the two proteins are compared, while alignment of the N-terminal 360 amino acids (32% identity) requires introduction of several gaps. HrpXv is a member of the AraC- and XylS-type family of regulatory proteins and contains a C-terminal sequence motif (amino acids 443 to 470) that characterizes this protein family (26). XylS is the activator protein of the meta operon of the TOL plasmid in *Pseudomonas putida* (30), and AraC is the regulatory protein of the arabinose operon in *E. coli* (19). Two other members of this protein family are involved in pathogenicity of mammalian pathogenic bacteria: the VirF protein of *Yersinia enterocolitica* acts on the *yop* and *vir* operons (15), and the *Shigella Flexneri* VirF protein (44) regulates the *virB* gene (1). All four proteins are significantly shorter than HrpXv. Amino acid similarity of HrpXv to these and other members of this family is weak but significant (about 45% similarity and 20 to 25% identity) in the C-terminal region of 110 to 130 amino acids, while the N-terminal regions are not conserved (data not shown).

Genetic analysis of the *hrpXv* locus. A defined *hrpXv* mutant was constructed by inserting the omega cassette (42) into the unique *Eco*RV site of the gene (position 1335 in Fig. 2). This cassette carries the gene conferring resistance to spectinomycin and streptomycin, as well as translational stop codons in all three reading frames at both ends. The insertion is predicted to interrupt translation of *hrpXv* after amino acid 344 and was introduced into *X. campestris* pv. vesicatoria 85E and 75-3 by marker exchange mutagenesis. The resulting mutants, 85EX and 75X, showed a clear *hrp* phenotype, i.e., no watersoaking symptoms on susceptible pepper and tomato plants and no elicitation of the HR in a resistant plant. To determine the minimal size of the *hrpXv* locus, subfragments of pBX1 (Fig. 1B) in pLAFR6 were tested for complementation of mutant strains 85EX and 75X. Both mutants were complemented by plasmids pSX2 and pSX3, indicating that no sequences downstream of the *Nhe*I site are necessary for *hrpXv* function. The 7.0-kb *Bam*HI fragment in pSX4 did not complement the mutants.

For strain 85EX, growth in planta was scored and was shown to be identical to that of a typical *hrp* mutant, i.e., a 10^5-fold-lower bacterial titer compared with the wild-type strain 85E 7 days after inoculation (data not shown). It has previously been reported that the *hrpXc* mutant could be complemented for growth in planta by coinoculation with the wild-type strain (32). This effect was not observed in *X. campestris* pv. vesicatoria when strains 85E and 85EX were coinoculated at a 1:1 ratio into leaves of the susceptible pepper cultivar ECW. Each strain behaved as when it was inoculated alone, indicating that the wild-type strain does not support growth of the *hrpXv* mutant (data not shown).

XVM2 induces expression of all *hrp* loci. Recently, the synthetic medium XVM2 was developed and shown to induce expression of the *hrpA* locus to levels comparable to those observed after growth in the plant (54). Here, induction of expression of the other *hrp* loci in XVM2 was tested. One Tn*3-gus* insertion in each *hrp* locus previously shown to be under control of the corresponding *hrp* promoter was chosen for this experiment. Transconjugants of wild-type strain 85-10 carrying the plasmid-borne Tn*3-gus* insertions B35, C17, D54, and F312 (45) were analyzed for their GUS activities after growth in complex medium NYG and in XVM2. XVM2 induced these *hrp* loci (Table 2) to activities comparable to those obtained after incubation in TCM (45).

Expression of the *hrpE* locus has not been studied because of the lack of suitable insertions. Recently, a Tn*3-gus* insertion derivative of pXV4, E525, carrying the *gusA* gene downstream of the *hrpE* promoter, was isolated and included in these studies. In contrast to the other *hrp* loci, the *hrpE* locus was expressed at a basal level in NYG. Nevertheless, expression was increased by a factor of about 20 after growth in the plant (data not shown). The same level of induction was observed in XVM2. In conclusion, these results prove that induction of *hrp* gene expression in *X. campestris* pv. vesicatoria does not depend on plant-derived molecules.

***hrpXv* controls expression of other *hrp* loci.** To analyze the predicted function of *hrpXv* in *hrp* gene regulation, the same plasmids as described above carrying Tn*3-gus* fusions to the promoters of the loci *hrpB* to *hrpF* were introduced into the *hrpXv* mutant 85EX. GUS activities were determined after growth in NYG and XVM2 under the same conditions as for the wild type. In mutant 85EX, the loci *hrpB*, *hrpC*, *hrpD*, and *hrpF* were not expressed in XVM2, indicating that the *hrpXv* gene is necessary for their induction in XVM2 (Table 2). Basal expression of the *hrpE* locus in NYG was not altered; however, induction in XVM2 was abolished in the *hrpXv* mutant. Expression of *hrpA*, which is located at the left border of the *hrp* cluster, has previously been shown to be independent of the *hrpXv* gene (54).

We tested whether additional copies of *hrpXv* would lead to elevated levels of *hrp* induction or even to expression in complex medium. The *hrpXv* gene on pSX2 was therefore introduced into *X. campestris* pv. vesicatoria marker exchange mutants carrying one of the transposon insertions described above. pSX2 contains the *hrpXv* gene under control of its own promoter in the low-copy-number plasmid pLAFR6. We found that none of the *hrp* loci was expressed in NYG in the presence of pSX2. Levels of induction in XVM2 were similar with and without additional copies of *hrpXv*, but the activities for *hrpA* and *hrpC* seemed to be slightly increased. Surprisingly, expression of *hrpF* was 10-fold decreased in the presence of pSX2.

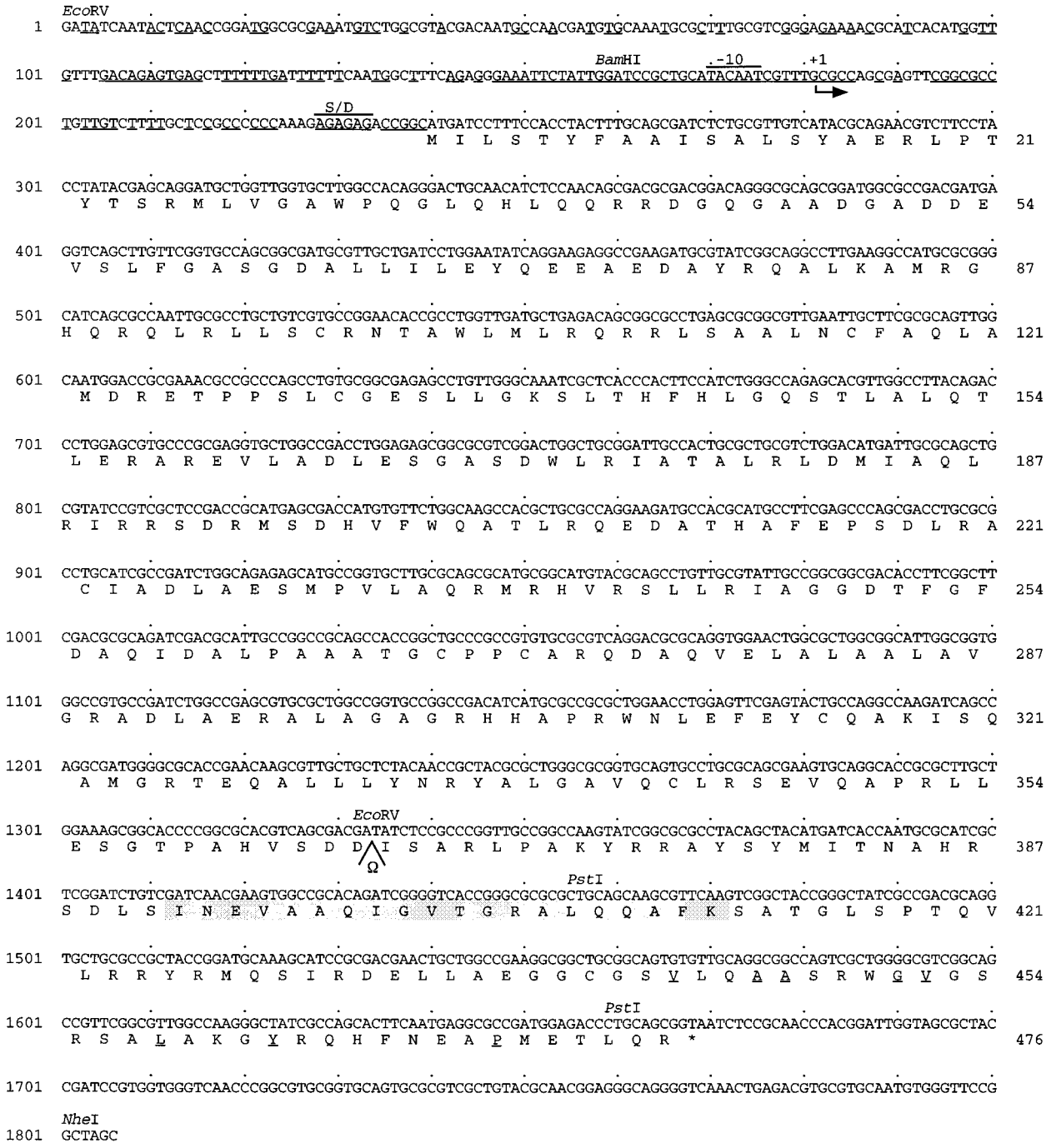

FIG. 2. Nucleotide sequence of the *hrpXv* gene. The deduced amino acid sequence of HrpXv is given in the one-letter code below and is numbered on the right. Relevant restriction sites shown in Fig. 1 are indicated. Sequences resembling the −10 box and a ribosome binding site (S/D) are marked with a bar above the sequence. The transcription start site (+1) is indicated by an arrow. Nucleotides that are identical in the region upstream of the translation start of the *hrpXc* gene are underlined. The insertion site of the omega cassette used to construct the *hrpXv* mutant is indicated. The predicted helix-turn-helix motif (see Fig. 5) is shaded, and highly conserved amino acids in the C-terminal region that characterize HrpXv as a member of the AraC family of regulatory proteins (26) are underlined.

***hrpXv* promoter activity and mapping of the transcription start site.** To analyze regulation of expression of the *hrpXv* gene itself, the 2.8-kb *Eco*RI-*Pst*I fragment from pBX1 containing 1.6 kb upstream of the predicted *hrpXv* translation start was fused to the *gusA* gene of the promoter probe plasmid pL6GUSB, resulting in plasmid pPX2 (Fig. 1C). pPX2 was introduced into wild-type strain 85E and the *hrpXv* mutant 85EX. GUS activity of 85E(pPX2) and 85EX(pPX2) was low (0.1 U/10^{10} CFU) in NYG and was induced about 20-fold in XVM2 (2.0 U/10^{10} CFU). Thus, *hrpXv* expression is inducible in XVM2, and the HrpXv protein is not needed for its own expression.

Primer extension analysis (Fig. 4) using oligonucleotide 137 and RNA preparations of strain 85E(pXV74) grown in NYG

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HrpXv MILSTYFAAISALSYAERLPTYTSRMLVGAWPQGLQHLQQRRDGQGAADGADDEVSLFGASGDALLILEYQEEAEDAYRQALKAMRGHQRQLRLLSCRNT 100
HrpB  MLGNIYFALASGLAARERLPEYANAVFAADFDRAYQLVDHHSSQRGKSDDYAGVLAM....ADASLLLECDEEAEEGFRLAQRLIRHSDDQLRVVSCRNT 96

HrpXv AWLMLRQRRLSAALNCFAQLAMDRETPPSLCGESLLGKSLTHFHLGQSTLALQTLERAREVLADLESGASDWLRIATA..LRLDMIAQLRIRRSDRMSDH 198
HrpB  GWQALLRDRYAAAASCFSRMAEDDGATWTQQVEGLIGLALVHHQLGQQDASDDALRAAREA....ADGRSDRGWLATIDLIIYEFAVQAGIRCSNRLLEH 192

HrpXv VFWQATLRQEDATHAFEPSDLRACIADLAESMPVLAQRMRHVRSLL.RIAGGDTFGFDAQIDALPAAATGCPPCARQ...DAQVELALAALAVGRADLAE 294
HrpB  AFWQSAEMGATLLANHGGRNGWTPTVSQGVPMPALIQRRAEYLSLLRRMADGD....RAAIDPLMATLNHSRKLGSRLLMQTKVEVVLAALSGEQYDVAG 288

HrpXv RALAGAGRHH....APRWNLEFEYCQAKISQAMGRTEQALLLYNRYALGAVQCLRSEVQAPRLLESGTP..AHVSDDISARLPAKYRRAYSYMITNAHRS 388
HrpB  RVFDQICNRETTYGARRWNFDFLYCRAKMAAQRGDAAGALKFYTTYMQDALRCLRTETVNVRRASAAVPVASRASDDVSARLSAKYRRAYRYIIENIERS 388

HrpXv DLSINEVAAQIGVTGRALQQAFKSATGLSPTQVLRRYRMQSIRDELL.AEGGCGSVLQAASRWGVGSRSALAKGYRQHFNEAPMETLQR 476
HrpB  DLTTREVAAHINVTERALQLAFKSAVGMSPSSVIRRMRLEGIRSDLLDSERNPSNIIDTASRWGIRSRSALVKGYRKQFNEAPSETIWR 477
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FIG. 3. Amino acid sequence alignment of the HrpXv protein with the HrpB protein of *B. solanacearum*, using the BESTFIT program.

or XVM2 showed a strong signal with RNA of XVM2-induced cells and a much weaker signal with RNA of bacteria grown in NYG that was only visible after longer exposure of the X-ray film. The same, strong signal was obtained with RNA prepared from bacteria grown in susceptible pepper plants (data not shown). The transcription start site is located 59 bp upstream of the predicted ATG start codon (G at position 181 in Fig. 2). We wanted to compare the promoter regions of *hrpX* genes from different *X. campestris* pathovars to identify conserved sequences. Since the published sequences for *hrpXc* and *hrpXo* start only at the conserved *Bam*HI site located 23 bp upstream of the transcription start site of *hrpXv*, we sequenced the region upstream of the *Bam*HI site from the *X. campestris* pv. campestris gene (data not shown). The alignment with the *hrpXv* sequence revealed a high degree of sequence conservation up to position −84, while sequences further upstream were not conserved (Fig. 2). A sequence resembling the −10 box for σ^{70}-dependent transcription in *E. coli* (43) is present in the promoter region, but a corresponding −35 box is missing. No PIP (plant-inducible promoter) box (22) or other similarities with *hrp* promoters of *X. campestris* pv. vesicatoria (7, 22, 54) were identified.

Heterologous complementation of *hrpX* mutants. For analysis of functionality of the *hrpXv* gene in a different pathovar of *X. campestris*, the 9.4-kb *Eco*RI fragment of pBX1 was cloned in plasmid pUFR043 (pFXI1) with the direction of *hrpXv* transcription in opposite orientation with respect to the *lacZ* promoter. pFXI1 was introduced into *X. campestris* pv. campestris 2D520 (wild type) and JS111 (*hrpXc*) by conjugation, and the transconjugants were tested on tomato. Wild-type strain 2D520 elicits an HR when inoculated into leaves of the nonhost plant tomato, while the *hrpXc* mutant JS111 does not (data not shown). pFXI1 restored the ability of JS111 to elicit the HR on tomato and did not alter the reactions elicited by the parental strain 2D520, indicating that *hrpXv* can functionally complement an *hrpXc* mutant and does not interfere with the wild-type *hrpXc* gene. Similar results have been obtained previously for the *hrpX* genes of *X. campestris* pv. campestris and *X. oryzae* pv. oryzae (31). We also analyzed whether the *hrpXv* mutant could be complemented by the related *hrp* regulatory gene *hrpB* of *B. solanacearum* (Fig. 3). Plasmid pAM5, in which *hrpB* is under control of the *lacZ* promoter, was introduced into *X. campestris* pv. vesicatoria 85EX. Strain 85EX(pAM5) elicited an HR on resistant pepper line ECW-10R and caused watersoaking on the susceptible line ECW (data not shown). However, HR development was only partial

TABLE 2. Expression of *hrp*-Tn*3-gus* fusions in *X. campestris* pv. vesicatoria 85-10 and 85EX after growth in different media

Tn*3-gus* insertion derivative	*hrp* promoter	GUS activity[a] (U/10^{10} CFU)			
		85-10 (wild type)		85EX (*hrpXv*)	
		NYG	XVM2	NYG	XVM2
pXV9::B35	*hrpB*	0.004	5.3	0.005	0.008
pXV9::C17	*hrpC*	0.004	2.1	0.005	0.01
pXV9::D54	*hrpD*	0.004	2.6	0.005	0.02
pXV4::E525	*hrpE*	0.7	16.5	0.6	1.9
pXV2::F312	*hrpF*	0.008	45.5	0.01	0.09

[a] Determined after growth for 14 to 16 h in NYG and XVM2. Values are averages of two to three experiments.

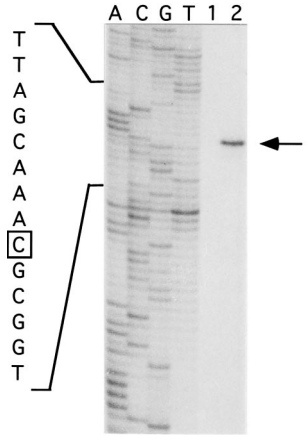

FIG. 4. Mapping of the transcription start site of *hrpXv* by primer extension analysis. RNAs were extracted from strain 85E(pXV74) grown for 16 h in NYG (lane 1) and XVM2 (lane 2), annealed with oligonucleotide 137, and used as templates for reverse transcription. The nucleotide sequence is the reverse complement of the coding strand and was obtained with plasmid pBX1 as a template and oligonucleotide 137. The boxed nucleotide refers to the transcription start site that is indicated by an arrow.

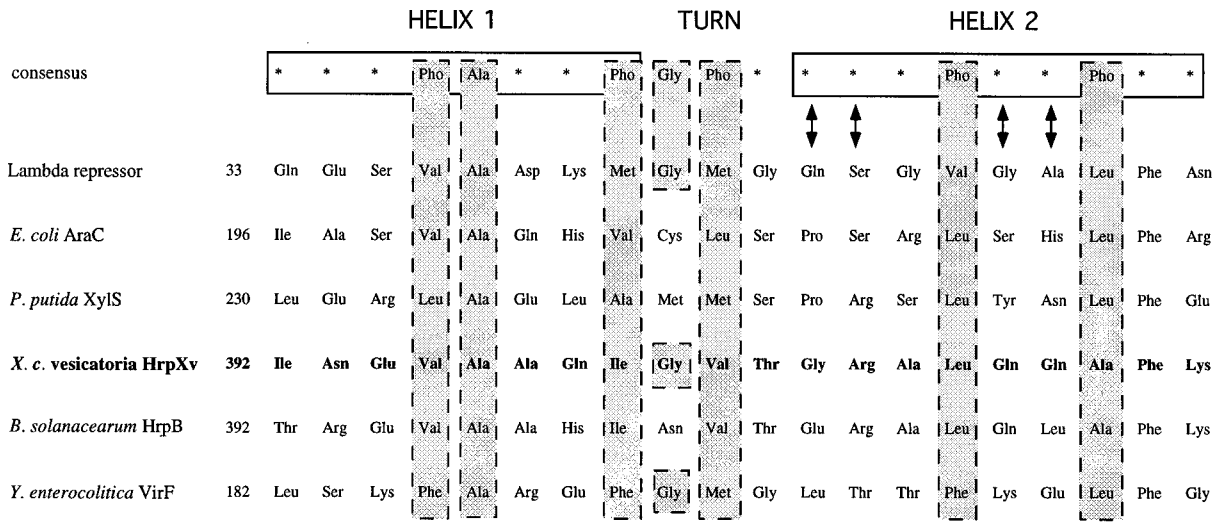

FIG. 5. Alignment of putative helix-turn-helix motifs of proteins of the AraC family with the corresponding region of the lambda repressor protein and the helix-turn-helix consensus motif (12, 41). The numbers on the left refer to the position of the first given amino acid in the sequence of the respective protein. Pho indicates hydrophobic amino acids. The arrows mark the positions in the DNA binding helix that most likely face the major groove of the DNA. Sequences were obtained from the indicated references: lambda repressor (41), AraC (52), XylS (47), HrpB (27), and VirF (15).

(about 30% of the infiltrated area), and watersoaking was delayed by 1 to 2 days. Plasmid pAM5 did not seem to interfere with *hrp* function when introduced into *hrp* wild-type strain 85E; plant phenotypic responses were as with the wild type (data not shown).

DISCUSSION

We isolated and characterized the *hrpX* gene of *X. campestris* pv. vesicatoria. *hrpX* genes have previously been isolated from *X. campestris* pv. campestris and *X. oryzae* pv. oryzae (31, 32). The three putative HrpX proteins show a high degree of sequence conservation (more than 90%), with HrpXv and HrpXo being most closely related. A previous study reported that an *hrpXc* mutant could be complemented for in planta growth by coinoculation with wild-type bacteria. *hrpXc* was therefore suggested to encode an exocellular protein (32). This effect was not observed in a similar experiment with the *hrpXv* mutant, which is perhaps not surprising given the regulatory function of *hrpX*. However, the difference in behavior could be due to the fact that *X. campestris* pv. campestris causes systemic infections, which is not the case for *X. campestris* pv. vesicatoria.

Expression of *hrpXv* is low in NYG and induced in XVM2 but is not autoregulated. The region encompassing 580 bp upstream of the putative translation start site is sufficient for *hrpXv* function and thus contains the promoter. Sequence conservation up to position −84 between the *hrpX* genes of *X. campestris* pv. vesicatoria and *X. campestris* pv. campestris suggests that this region might be sufficient for promoter activity. Interestingly, this sequence is unusually high in A and T residues (64%), while the overall AT content of the 25-kb *X. campestris* pv. vesicatoria *hrp* region is only 37% (41% in the promoter regions). Little work is published on *Xanthomonas* promoters. No similarity between the *hrpXv* upstream region and any *hrp* promoter in the large *hrp* cluster was observed.

The sequence downstream of the *hrpXv* stop codon does not contain a rho-independent termination signal. Since the omega insertion has a polar effect on transcription, successful complementation of 85EX and 75X with plasmid pSX3 indicates that no other *hrp* gene is located downstream of *hrpXv* in the same transcription unit. Several open reading frames exist in this region but are not very likely to be expressed, as analyzed by the computer program Codonpreference.

We could demonstrate in this report that the putative product of the *hrpXv* gene is the transcriptional activator of the *X. campestris* pv. vesicatoria *hrp* cluster. *hrpXv* is necessary for activation of transcription of the five *hrp* loci *hrpB* to *hrpF*. Interestingly, induction of *hrpA*, which is located at the left border of the *hrp* cluster, is independent of *hrpXv* (54). Additional copies of the *hrpXv* gene that itself is expressed at a low level in NYG in a chromosomal *hrp*$^+$ background did not allow *hrp* expression under noninducing conditions but, in contrast, led to a reduction of *hrpF* expression in XVM2 by a factor of 10. This finding could indicate that a cofactor is needed for activation of *hrpF*.

HrpXv, like HrpXc and HrpXo (40), is closely related to the predicted HrpB protein of *B. solanacearum*, which is the activator of the *B. solanacearum hrp* cluster (27). The observed heterologous complementation, albeit partial, of the *X. campestris* pv. vesicatoria *hrpX* mutation with the *hrpB* gene is the first example for heterologous complementation of an *X. campestris* pv. vesicatoria *hrp* mutant by its counterpart from *B. solanacearum*. This result was somewhat surprising because only the C-terminal 115 amino acids of the proteins HrpXv and HrpB show a higher degree of sequence conservation (62% identity), while the N-terminal regions are only 32% identical (Fig. 3). Weak homology on the DNA level is probably the reason for our unsuccessful attempts to isolate the *hrpXv* gene with *hrpB* as a probe.

HrpXv belongs to the AraC-and XylS-type family of regulatory proteins, which show weak but significant amino acid similarity in their C-terminal regions (26). For AraC, it has been demonstrated that this region contains a DNA-binding domain (13), and it has been suggested that amino acids 197 to 216 might be involved in DNA binding (12, 25). This sequence shows homology to a domain in the lambda repressor protein that is structured as an alpha-helix–turn–alpha-helix (41), in which the second helix contacts the DNA and the first helix stabilizes the complex. As shown in Fig. 5, hydrophobic amino

acids that are important for this supersecondary structure are conserved in HrpXv and other members of this protein family, suggesting that in HrpXv this domain might be involved in DNA binding.

Inspection of the upstream sequences of *hrpXv* regulated loci revealed a conserved sequence motif, designated the PIP box (22), in close vicinity to the transcription start site of the four loci *hrpB*, *hrpC*, *hrpD*, and *hrpF* (22, 23). The PIP box consists of a direct repeat with the exact spacing of 15 nucleotides (TTCGC-N_{15}-TTCGC). Interestingly, identical or highly similar motifs are present (22) upstream of the *hrpB*-dependent (27) *hrp* transcription units 2, 3, and 4 (50) and the *popA* gene (4) of *B. solanacearum*. The PIP box was not found in the upstream region of the *hrpA1* and *hrpXv* genes of *X. campestris* pv. vesicatoria, which are regulated independently of *hrpXv*. These observations suggest that the PIP box might be involved in HrpXv- and HrpB-mediated *hrp* gene activation. Since the geometry of DNA sequences recognized by regulatory proteins determines the number and orientation of the binding monomers (49) and members of the AraC family bind or are suggested to bind repeated sequences as a dimer (13, 14, 34, 35, 37, 53), it is tempting to speculate that HrpXv might bind the PIP box as a dimer. This hypothesis has to be tested experimentally; so far, overexpression of HrpXv has not been achieved.

In conclusion, *hrp* gene regulation in *X. campestris* pv. vesicatoria seems to be similar to that in *B. solanacearum* but differs in two aspects. First, *hrpB* has been reported to be partly autoregulated (27), which has not been observed for *hrpXv*, and second, expression of the *hrpA1* gene is independent of *hrpXv* in *X. campestris* pv. vesicatoria, while its homolog, *hrpA*, in *B. solanacearum* is cotranscribed with *hrpB* and therefore dependent on *hrpB* (27). A different *hrp*-regulatory cascade is involved in the well-studied *Pseudomonas syringae* pathovars. At least three *hrp* genes, *hrpL* (55, 56), *hrpR*, and *hrpS* (28, 29), and the *rpoN* gene (20), which encodes the transcription factor σ^{54}, are needed for *hrp* and *avr* gene activation in *P. syringae*. None of these genes shows sequence similarity to *hrpXv*.

The results described here mark the beginning of an insight into the regulatory network needed for *hrp* gene function. Two of the questions to be addressed are which gene is responsible for activation of the *hrpXv* gene and whether the same or yet another gene regulates expression of *hrpA*.

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REFERENCES

1. **Adler, B., C. Sasakawa, T. Tobe, S. Makino, K. Komatsu, and M. Yoshikawa.** 1989. A dual transcriptional activation system for the 230 kb plasmid genes coding for virulence-associated antigens of *Shigella flexneri*. Mol. Microbiol. **3:**627–635.
2. **Aiba, H., S. Adhya, and B. de Crombugghe.** 1981. Evidence of two functional *gal* promoters in intact *Escherichia coli* cells. J. Biol. Chem. **256:**11905–11910.
3. **Altschul, S. F., W. Gish, W. Miller, E. Meyers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
4. **Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pernollet, and C. A. Boucher.** 1994. PopA1, a protein which induces a hypersensitivity-like response on specific *Petunia* genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum*. EMBO J. **13:**543–553.
5. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1992. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
6. **Bonas, U.** 1994. *hrp* genes of phytopathogenic bacteria. Curr. Top. Microbiol. Immunol. **192:**79–98.
7. **Bonas, U.** Unpublished data.
8. **Bonas, U., R. Schulte, S. Fenselau, G. V. Minsavage, and B. J. Staskawicz.** 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.
9. **Bonas, U., R. E. Stall, and B. Staskawicz.** 1989. Genetic and structural characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Gen. Genet. **218:**127–136.
10. **Brown, I., J. Mansfield, and U. Bonas.** 1995. *hrp* genes in *Xanthomonas campestris* pv. *vesicatoria* determine ability to suppress papillae deposition in pepper mesophyll cells. Mol. Plant-Microbe Interact. **8:**825–836.
11. **Brown, I., J. Mansfield, J. Conrads-Strauch, and U. Bonas.** 1993. Ultrastructure of interactions between *Xanthomonas campestris* pv. *vesicatoria* and pepper, including immunocytochemical localization of extracellular polysaccharides and the AvrBs3 protein. Mol. Plant-Microbe Interact. **6:**376–386.
12. **Brunelle, A., and R. Schleif.** 1989. Determining residue-base interactions between AraC protein and *ara*I DNA. J. Mol. Biol. **209:**607–622.
13. **Bustos, S. A., and R. F. Schleif.** 1993. Functional domains of the AraC protein. Proc. Natl. Acad. Sci. USA **90:**5638–5642.
14. **Carra, J. H., and R. F. Schleif.** 1993. Variation of half-site organization and DNA looping by AraC protein. EMBO J. **12:**35–44.
15. **Cornelis, G., C. Sluiters, C. L. de Rouvroit, and T. Michiels.** 1989. Homology between *virF*, the transcriptional activator of the *Yersinia* virulence regulon, and AraC, the *Escherichia coli* arabinose operon regulator. J. Bacteriol. **171:**254–262.
16. **Daniels, M. J., C. E. Barber, P. C. Turner, M. K. Sawczyc, R. J. W. Byrde, and A. H. Fielding.** 1984. Cloning of genes involved in pathogenicity of *Xanthomonas campestris* pv. *campestris* using the broad host range cosmid pLAFR1. EMBO J. **3:**3323–3328.
17. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
18. **Ditta, G., S. Stanfield, D. Corbin, and D. Helinski.** 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.
19. **Englesberg, E., J. Irr, J. Power, and N. Lee.** 1965. Positive control of enzyme synthesis by gene C in the L-arabinose system. J. Bacteriol. **90:**946–957.
20. **Fellay, R., L. G. Rahme, M. N. Mindrinos, R. D. Frederick, A. Pisi, and N. J. Panopoulos.** 1991. Genes and signals controlling the *Pseudomonas syringae* pv. *phaseolicola*-plant interaction, p. 45–52. *In* H. Hennecke and D. P. S. Veram (ed.), Advances in molecular genetics of plant-microbe interactions. Kluwer Academic Publishers, Dordrecht, The Netherlands.
21. **Fenselau, S., I. Balbo, and U. Bonas.** 1992. Determinants of pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are related to proteins involved in secretion in bacterial pathogens of animals. Mol. Plant-Microbe Interact. **5:**390–396.
22. **Fenselau, S., and U. Bonas.** 1995. Sequence and expression analysis of the *hrpB* pathogenicity operon of *Xanthomonas campestris* pv. *vesicatoria* which encodes eight proteins with similarity to components of the Hrp, Ysc, Spa, and Fli secretion systems. Mol. Plant-Microbe Interact. **8:**845–854.
23. **Fenselau, S., E. Huguet, K. Wengelnik, and U. Bonas.** Unpublished data.
24. **Figurski, D., and D. R. Helinski.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided *in trans*. Proc. Natl. Acad. Sci. USA **76:**1648–1652.
25. **Francklyn, C. S., and N. Lee.** 1988. AraC proteins with altered DNA sequence specificity which activate a mutant promoter in *Escherichia coli*. J. Biol. Chem. **263:**4400–4407.
26. **Gallegos, M. T., C. Michan, and J. L. Ramos.** 1993. The XylS/AraC family of regulators. Nucleic Acids Res. **21:**807–810.
27. **Genin, S., C. L. Gough, C. Zischek, and C. A. Boucher.** 1992. Evidence that the *hrpB* gene encodes a positive regulator of pathogenicity genes from *Pseudomonas solanacearum*. Mol. Microbiol. **6:**3065–3076.
28. **Grimm, C., W. Aufsatz, and N. J. Panopoulos.** 1995. The *hrpRS* locus of *Pseudomonas syringae* pv. *phaseolicola* constitutes a complex regulatory unit. Mol. Microbiol. **15:**155–165.
29. **Grimm, C., and N. J. Panopoulos.** 1989. The predicted protein product of a pathogenicity locus from *Pseudomonas syringae* pv. phaseolicola is homologous to a highly conserved domain of several procaryotic regulatory proteins. J. Bacteriol. **171:**5031–5038.
30. **Inouye, S., A. Nakazawa, and T. Nakazawa.** 1981. Molecular cloning of gene *xylS* of the TOL plasmid: evidence for positive regulation of the *xylDEGF* operon by *xylS*. J. Bacteriol. **148:**413–418.
31. **Kamdar, H. V., S. Kamoun, and C. I. Kado.** 1993. Restoration of pathogenicity of avirulent *Xanthomonas oryzae* pv. oryzae and *X. campestris* pathovars by reciprocal complementation with the *hrpXo* and *hrpXc* genes and identification of HrpX function by sequence analyses. J. Bacteriol. **175:**2017–2025.
32. **Kamoun, S., and C. I. Kado.** 1990. A plant-inducible gene of *Xanthomonas campestris* pv. campestris encodes an exocellular component required for growth in the host and hypersensitivity on nonhosts. J. Bacteriol. **172:**5165–5172.

33. **Kamoun, S., E. Tola, H. Kamdar, and C. I. Kado.** 1992. Rapid generation of directed and unmarked deletions in *Xanthomonas*. Mol. Microbiol. **6:**809–816.
34. **Kessler, B., V. de Lorenzo, and K. N. Timmis.** 1993. Identification of a cis-acting sequence within the *Pm* promoter of the TOL plasmid which confers XylS-mediated responsiveness to substituted benzoates. J. Mol. Biol. **230:**699–703.
35. **Kessler, B., K. N. Timmis, and V. de Lorenzo.** 1994. The organization of the *Pm* promoter of the TOL plasmid reflects the structure of its cognate activator protein XylS. Mol. Gen. Genet. **244:**596–605.
36. **Knoop, V., B. Staskawicz, and U. Bonas.** 1991. Expression of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. vesicatoria is not under the control of *hrp* genes and is independent of plant factors. J. Bacteriol. **173:** 7142–7150.
37. **Lambert de Rouvroit, C., C. Sluiters, and G. R. Cornelis.** 1992. Role of the transcriptional activator VirF and temperature in the expression of the pYV plasmid genes of *Yersinia enterocolitica*. Mol. Microbiol. **6:**379–388.
38. **Lindgren, P. B., R. C. Peet, and N. J. Panopoulos.** 1986. Gene cluster of *Pseudomonas syringae* pv. "phaseolicola" controls pathogenicity of bean plants and hypersensitivity of nonhost plants. J. Bacteriol. **168:**512–522. (Erratum, **169:**928, 1987).
39. **Minsavage, G. V., D. Dahlbeck, M. C. Whalen, B. Kearney, U. Bonas, B. J. Staskawicz, and R. E. Stall.** 1990. Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*-pepper interactions. Mol. Plant-Microbe Interact. **3:**41–47.
40. **Oku, T., A. M. Alvarez, and C. I. Kado.** 1995. Conservation of the hypersensitivity-pathogenicity regulatory gene *hrpX* of *Xanthomonas campestris* and *X. oryzae*. DNA Sequence **5:**245–249.
41. **Pabo, C. O., and R. T. Sauer.** 1984. Protein-DNA recognition. Annu. Rev. Biochem. **53:**293–321.
42. **Prentki, P., and H. M. Krisch.** 1984. *In vitro* insertion mutagenesis with a selectable DNA fragment. Gene **29:**303–313.
43. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13:** 319–353.
44. **Sakai, T., C. Sasakawa, and M. Yoshikawa.** 1988. Expression of four virulence antigens of *Shigella flexneri* is positively regulated at the transcriptional level by the 30 kiloDalton VirF protein. Mol. Microbiol. **2:**589–597.
45. **Schulte, R., and U. Bonas.** 1992. Expression of the *Xanthomonas campestris* pv. vesicatoria *hrp* gene cluster, which determines pathogenicity and hypersensitivity on pepper and tomato, is plant inducible. J. Bacteriol. **174:**815–823.
46. **Shaw, J. J., L. G. Settles, and C. I. Kado.** 1988. Transposon Tn*4431* mutagenesis of *Xanthomonas campestris* pv. *campestris*: characterization of a nonpathogenic mutant and cloning of a locus for pathogenicity. Mol. Plant-Microbe Interact. **1:**39–45.
47. **Spooner, R. A., K. Lindsay, and F. C. Franklin.** 1986. Genetic, functional and sequence analysis of the *xylR* and *xylS* regulatory genes of the TOL plasmid pWW0. J. Gen. Microbiol. **132:**1347–1358.
48. **Staskawicz, B. J., D. Dahlbeck, N. Keen, and C. Napoli.** 1987. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. glycinea. J. Bacteriol. **169:**5789–5794.
49. **Travers, A. A.** 1989. DNA conformation and protein binding. Annu. Rev. Biochem. **58:**427–452.
50. **Van Gijsegem, F., C. Gough, C. Zischek, E. Niqueux, M. Arlat, S. Genin, P. Barberis, S. German, P. Castello, and C. Boucher.** 1995. The *hrp* gene locus of *Pseudomonas solanacearum*, which controls the production of a type III secretion system, encodes eight proteins related to components of the bacterial flagellar biogenesis complex. Mol. Microbiol. **15:**1095–1114.
51. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153:**3–11.
52. **Wallace, R. G., N. Lee, and A. V. Fowler.** 1980. The *araC* gene of *Escherichia coli*: transcriptional and translational start-points and complete nucleotide sequence. Gene **12:**179–190.
53. **Wattiau, P., and G. R. Cornelis.** 1994. Identification of DNA sequences recognized by VirF, the transcriptional activator of the *Yersinia yop* regulon. J. Bacteriol. **176:**3878–3884.
54. **Wengelnik, K., C. Marie, M. Russel, and U. Bonas.** 1996. Expression and localization of HrpA1, a protein of *Xanthomonas campestris* pv. vesicatoria essential for pathogenicity and induction of the hypersensitive reaction. J. Bacteriol. **178:**1061–1069.
55. **Xiao, Y., S. Heu, J. Yi, Y. Lu, and S. W. Hutcheson.** 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.
56. **Xiao, Y., and S. W. Hutcheson.** 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.