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Synergistic Activation of the Pathogenicity-Related Proline Iminopeptidase Gene in *Xanthomonas campestris* pv. campestris by HrpX and a LuxR Homolog

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Xanthomonas campestris **pv. campestris strain 8004 contains an orphan quorum-sensing (QS) locus, *xccR-pip*$_{Xcc}$, in which the proline iminopeptidase (*pip*$_{Xcc}$) gene (where "*Xcc*" indicates that the *pip* gene is from *X. campestris* pv. campestris) is positively regulated by the LuxR homologue XccR by binding to the *luxXc* box of the *pip*$_{Xcc}$ promoter. The disruption of *pip*$_{Xcc}$ significantly attenuated the virulence of *X. campestris* pv. campestris. An imperfect plant-inducible promoter (PIP) box is located in the upstream region of the *pip*$_{Xcc}$ promoter, which is the putative binding site of the transcriptional activator HrpX. To explore whether the expression of the *pip*$_{Xcc}$ gene is regulated by HrpX, the expression level of a *pip*$_{Xcc}$ promoter-*gusA* fusion gene was assayed in an *hrpX* disruption mutant. The results showed that the lack of HrpX dramatically decreased the β-glucuronidase (GUS) activity. Further analyses using an electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP)-PCR indicated that the imperfect PIP box in *X. campestris* pv. campestris is specifically bound to HrpX. These data demonstrated that the *pip*$_{Xcc}$ gene belongs to the hrp regulon and that the imperfect PIP box of the *pip*$_{Xcc}$ promoter could be a *cis* element for the HrpX protein. We further showed in a pulldown assay that XccR can bind HrpX, suggesting that these two regulatory proteins coactivate the virulence factor by binding to the different *cis* elements of the *pip*$_{Xcc}$ gene and adapt to the host environment during *X. campestris* pv. campestris infection.**

Diseases caused by members of the genus *Xanthomonas* contribute to devastating losses of cultivated crops worldwide (27). Many phytopathogenic bacteria elicit the hypersensitive response (HR) in nonhost plants or pathogenicity in host plants, depending on *hrp* (hypersensitive reaction and pathogenicity) and *hrc* (*hrp*-conserved) genes (2). The *hrp* gene cluster in phytopathogens is regulated by two types of regulators (2, 13). Group I *hrp* genes in *Erwinia amylovora* and *Pseudomonas syringae* are activated by an alternative sigma factor (30), whereas the group II *hrp* genes of *Ralstonia solanacearum* and *Xanthomonas campestris* are activated by an AraC family regulator (HrpX for *Xanthomonas* and HrpB for *Ralstonia*). In many *Xanthomonas* species, HrpX regulates the expression of a genome-wide regulon, including type II and type III secretion systems (14), which also exist in many bacterial pathogens of humans and animals to secrete effector proteins and degradation enzymes (6, 17). The promoters controlled by HrpX often carry a conserved motif called plant-inducible promoter (PIP) box and a −10 box (11, 12). HrpX regulates the PIP box-containing promoters by directly binding to the conserved *cis* element (TTCGC-N15-TTCGC) in xanthomonads (11, 33). A similar sequence (TTCG-N16-TTCG), called *hrp*$_{II}$ box, discovered in *R. solanacearum*, was regulated by HrpB (7). The central cytidine of each half-site is essential for the function of the *cis* element, while the other nucleotides are more flexible (7). Notably, genes with an imperfect PIP box or without a PIP box have also been shown to be expressed in an HrpX-dependent manner (7, 20, 26). Thus, HrpX is believed to be a global regulator, and there are more genes belonging to the HrpX regulon than previously expected.

X. campestris pv. campestris is the causal agent of black rot on most cultivated crucifer plants (27). Our previous study showed that the *xccR-pip*$_{Xcc}$ locus (where "*Xcc*" indicates that the *pip* gene is from *X. campestris* pv. campestris) is related to pathogenicity, and disruption of either of the two genes results in significantly attenuated virulence of *X. campestris* pv. campestris (34). The proline iminopeptidase (*pip*$_{Xcc}$) gene was regulated by quorum-sensing (QS) LuxR homolog XccR, and the *pip*$_{Xcc}$ promoter-*gusA* fusion gene was significantly induced when the bacteria grew *in planta* (34). QS enables bacterial cell-cell communication via signal molecules, and it monitors the density of bacterial populations (10). In Gram-negative bacteria, the classic QS regulation is mediated by *N*-acylhomoserine lactone (AHL) signal molecules, and LuxI and LuxR are responsible for producing and sensing signals, respectively (10). Only a few isolates of *Xanthomonas* produced detectable AHLs (5). A genome survey showed that *X. campestris* pv. campestris strain 8004 has no cognate LuxI synthase for AHLs (28), and in consequence, no AHL activity was detected. Instead, XccR activates the expression of *pip*$_{Xcc}$, which encodes a hydrolase rather than a LuxI synthase, by binding to the *luxXc* box, highly similar to the *lux* box in the promoter region of the LuxI genes.

In this study, an imperfect PIP box could be found in the *xccR-pip*$_{Xcc}$ intergenic region upstream of the *luxXc* box by sequence analysis. We provide evidence for direct binding of HrpX to the imperfect PIP box *in vivo* and *in vitro*. We further show the *in vitro* binding of HrpX and XccR in pulldown assays, suggesting that the

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TABLE 1 Bacterial strains, plasmids, and primers used in this work

Strain, plasmid, or primer	Relevant characteristics	Source or reference
Strains		
8004	Wild type, Rif^r	32
Δ*hrpX* strain	*hrpX* deletion mutation in strain 8004	This study
Plasmids		
pLAFR6	pLAFR1 with rho-independent terminator and pUC18 polylinker, Tet^r	11
pFR421	pLAFR6(Ppip_{Xcc}-*gusA*)	19
pHM1	Broad-host-range cosmid vector, pSa *ori*, Sp^r	19
pHM1-hrpX-his	pHM1 (*hrpX-his*)	This study
pMAL-p2X	Tac promoter, expression vector, Amp^r	New England BioLabs
pMAL-p2x/hrpX	pMAL-p2X(*hrpX-his*)	This study
pGEM-T	Cloning vector	Promega Co.
pK18*mobsacB*	Suicide plasmid in *X. campestris* pv. campestris, Km^r	29
Primers		
hrpXhisF	5′cccaagcttATGATCCTTTCGACCTACTTCGC3′[a]	
hrpXhisR	5′ccggaattctcagtggtggtggtggtggtgGCGTTGCAGGGTTTCCAT3′	
jxhrpXFwd	5′ttccatatgATGATCCTTTCGACCTACTTCGCA3′	
jxhrpXRev	5′ccgctcgagGCGTTGCAGGGTTTCCATCGG3′	
hrpX del F1	5′gatatcATGATCCTTTCGACCTACTTGA3′	
hrpX del R1	5′gtcgacTGGAAGTGGGTCAGCGCCTT3′	
hrpX del F2	5′gtcgacCGCCCTGGGGGCTGTGCAA3′	
hrpX del R2	5′aagcttTTAGCGTTGCAGGGTTTCCATCG3′	
hrpX outup	5′GCTCACCGCTGCCCTGCATTGCTGC3′	
hrpX outdown	5′TACAATCGTTTGCGCCCACCACAAC3′	
box45F	5′ACGGTGTCGCAATTCGCGCGTTTCGCAATTGCCAACCGGTGTCAT3′	
box45R	5′ATGACACCGGTTGGCAATTGCGAAACGCGCGAATTGCGA3′	
pip-PF	5′GTCGAATTCGAAGGCTCAGTTGGTCGGGTTTG3′	
pip-PR	5′CTCGGTGCACTTCATGACCTGC3′	
pipF1	5′GAAGGCTCAGTTGGTCGGGTT3′	
pipR1	5′GGCAATTGCGAAACGCGCGAA3′	
pipR2	5′GACCTGCGCCCACTTACGG3′	

[a] The lowercase letters in primer sequences indicate the restriction enzyme recognition sites and fusion tag sequences.

two proteins are coactivators of pip_{Xcc}. This conclusion is consistent with the results that deletion of either *hrpX* or *xccR* abolished the pip_{Xcc} activity.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacteria and plasmids used in this work are listed in Table 1. *Escherichia coli* strains were grown in Luria-Bertani (24) medium at 37°C. *X. campestris* pv. campestris strains were cultured at 28°C either in NYG medium (5 g/liter tryptone, 3 g/liter yeast extract, 20 g/liter glycerol, pH 7.2) as a nutrient-rich condition or in MMX [4 g/liter K_2HPO_4, 6 g/liter KH_2PO_4, 2 g/liter $(NH_4)_2SO_4$, 1g/liter citric acid-Na_3, 0.2 g/liter $MgSO_4 \cdot 7H_2O$, 5 g/liter glucose, pH 7.0] as a minimal medium. Bacterial cell density was monitored by measuring the optical absorbance at 600 nm. Antibiotics were used at the following final concentrations: 50 μg/ml rifampin, 20 μg/ml kanamycin, 100 μg/ml ampicillin, 80 μg/ml spectinomycin, and 3 μg/ml tetracycline for liquid medium and 10 μg/ml for solid medium.

Plasmid construction. To determine pip_{Xcc} promoter activity, plasmid pFR421 was generated, which contains a 438-bp EcoRI-BspHI fragment PCR amplified with primers pip-PF and pip-PR (Table 1) from the *X. campestris* pv. campestris strain 8004 chromosome (34). The fragment spanned from −438 to −1 relative to the pip_{Xcc} translational start site (TSS).

To construct pHM1-hrpX-his, the coding region of the *hrpX* gene was PCR amplified from the *X. campestris* pv. campestris 8004 genomic DNA with primers hrpXhisF and hrpXhisR (Table 1). PCR products were first cloned into pGEM-T vector and then digested with HindIII and EcoRI restriction enzymes and ligated into pHM1 vector. The 6×His tag was fused to the C terminus of HrpX. The *hrpX* HindIII/EcoRI fragment was blunt ended by DNA polymerase I Klenow fragment (NEB, Hitchin, Hertshire, United Kingdom) and ligated into the flush-ended PstI site of pMAL-p2X to generate phrpX-MBP (maltose binding protein).

To make the plasmid that expressed the chimeric HrpX protein in *E. coli*, the *hrpX* gene was amplified by PCR with primers jxhrpXFwd and jxhrpXRev (Table 1). The fragment was digested with NdeI and XhoI restriction enzyme and cloned into pET30a to generate pET30a-hrpX.

Protein expression, purification, and antibody preparation. Prokaryotic expression plasmids pET30a-hrpX and phrpX-MBP were transformed into *E. coli* BL21 or TB1. Fusion proteins were expressed in *E. coli* cells after induction of an early log culture overnight by isopropyl-β-D-thiogalactopyranoside (IPTG) (0.1 mM) at 16°C. The MBP-tagged protein was purified by affinity chromatography with amylose resin (NEB) and eluted with maltose. The His-tagged protein was purified by Ni-nitrilotriacetic acid (NTA) resin (Novagen) under denaturing conditions. Amicon YM10 (Millipore) was used for protein concentration or for changing the protein suspension buffer. For production of antibody against HrpX, the His-tagged protein expressed from pET30a-hrpX was purified and pooled to immunize and boost rabbits, and serum was taken after the fourth booster injection.

Construction of *hrpX* mutant strain. The *hrpX* deletion mutant was generated using a selection/counterselection suicide vector, pK18*mobsacB* (21). To construct the strain with disrupted *hrpX*, two fragments corresponding to the *hrpX* coding regions 1 to 431 and 1008 to 1431 were PCR amplified from the strain 8004 genomic DNA by two pairs of primers,

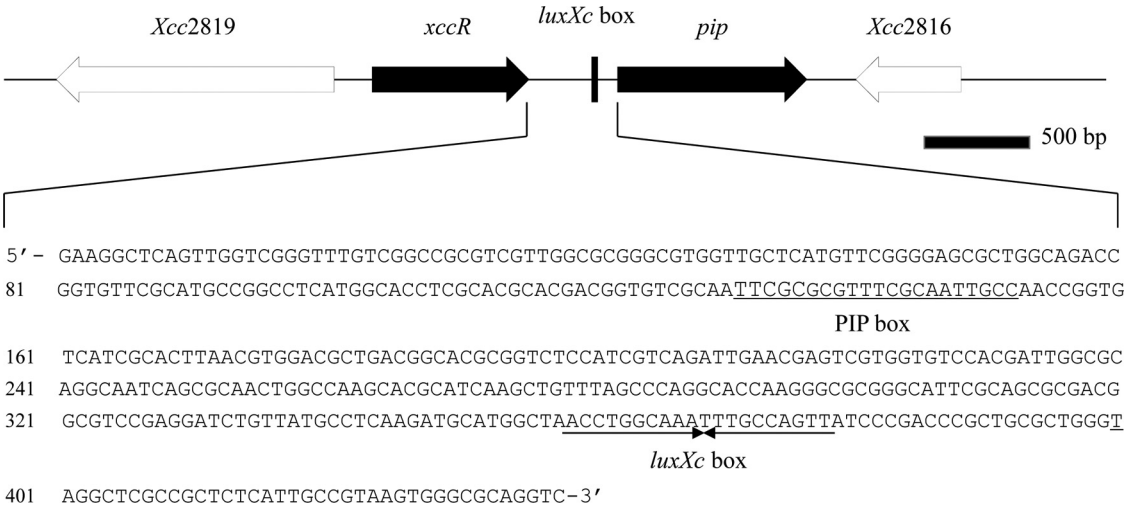

FIG 1 Genomic organization of the *xccR-pip*$_{Xcc}$ locus and structure of the intergenic sequence upstream of the *pip*$_{Xcc}$ gene. The imperfect PIP box and *luxXc* box sequences are indicated.

hrpX del F1/hrpX del R1 and hrpX del F2/hrpX del R2, respectively (Table 1). The resulting EcoRV-SalI and SalI-HindIII fragments were fused into the SmaI/HindIII-cleaved pK18*mobsacB* vector in one ligation reaction. The *hrpX* gene in the strain 8004 genome was truncated by homologous recombination. To detect positive clones with the truncated *hrpX* gene, a PCR primer pair, hrpX outup and hrpX outdown, flanking the *hrpX* coding region (Table 1), was designed.

GUS assay. The β-glucuronidase (GUS) activity of different *X. campestris* pv. campestris strains grown in medium and *in planta* was measured by the fluorometric method using the substrate 4-methylumbelliferyl β-D-glucuronide (MUG) (34). GUS activity was normalized to bacterial cell numbers. One unit of enzyme activity is defined as the amount of enzyme that releases 1 pmol of 4-methylumbelliferone (MU) min^{-1} at pH 7.0 at 37°C. The experiments were repeated at least three times for each of the conditions, each time in triplicate.

ChIP-PCR. Strain 8004 harboring the pHM1-hrpX-his plasmid was grown in 10 ml NYG medium to an optical density at 600 nm of 1.5. The proteins and chromatin DNA were cross-linked by adding formaldehyde to a final concentration of 1% for 10 min. The cross-linking reaction was stopped by the addition of glycine. The assay was performed using a chromatin immunoprecipitation (ChIP) assay kit (Millipore, Billerica, MA), following the manufacturer's instructions. The resulting purified DNA was used for PCR analysis. A small aliquot of untreated sonicated chromatin was reverse cross-linked and used as the total input DNA control. The experiments were repeated at least three times.

EMSA. MBP-HrpX fusion protein was purified through an amylose resin chromatography column (NEB) according to the manufacturer's instructions. The 45-bp DNA duplex containing the PIP box sequence was generated by annealing the synthetic oligonucleotides box45F and box45R. The product was then end labeled with [α-^{32}P]dATP. For the electrophoretic mobility shift assay (EMSA), 0.65 μg of the labeled probe and the MBP-HrpX protein were incubated in a binding buffer [10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM dithiothreitol (DTT), 2.5% glycerol, and 50 ng ml^{-1} poly(dI · dC)] for 20 min at room temperature. For competition, a certain amount of the unlabeled probe was coincubated with the labeled probe and the MBP-HrpX protein. Samples were size fractionated by using a 4% nondenaturing polyacrylamide gel in 0.5× TBE buffer (45 mM Tris-borate and 1 mM EDTA) at 4°C. The gel was dried, and the shifted bands were detected by autoradiography.

His pulldown assay. Purified XccR-MBP, HrpX-His, and MBP were subjected to His tag pulldown analysis. HrpX-his was loaded onto a Ni-NTA column using binding buffer (300 mM NaCl, 50 mM Na-phosphate, pH 8.0, 10 mM imidazole). XccR-MBP was then loaded onto the same column. The column was washed with four volumes of wash buffer (300 mM NaCl, 50 mM Na-phosphate, pH 8.0, 20 mM imidazole), and then the protein was eluted in elution buffer (300 mM NaCl, 50 mM Na-phosphate, pH 8.0, 300 mM imidazole). The eluted proteins were analyzed by SDS-PAGE and verified by Western blotting.

RESULTS

Sequence analysis of the *pip*$_{Xcc}$ promoter region. The 438-bp intergenic sequence between *xccR* and *pip*$_{Xcc}$ in *X. campestris* pv. campestris 8004 was analyzed, and an imperfect PIP box (TTCGC-N4-TTCGC-N2-TTGCC) was found at positions −307 to −287 relative to the *pip*$_{Xcc}$ translational start site (TSS) (Fig. 1). While the consensus sequence of a perfect PIP box (TTCGC-N15-TTCGC) has two conserved half-sites (TTCGC), the imperfect PIP box comprises not only the two conserved half-sites but also one nonconserved half-site (TTGCC). The three sites were separated in the imperfect PIP box by 4 bp and 2 bp. Including the spacing base pairs, the imperfect PIP box may be presented as TTCGC-N11-TTGCC for HrpX dimer binding.

HrpX is essential for *pip*$_{Xcc}$ expression. The PIP box is known to be the *cis* element for HrpX binding in the promoter region that regulates gene expression. The existence of the imperfect PIP box in the *pip* promoter may indicate that the expression of *pip* is HrpX dependent. To verify the role of HrpX in *pip* expression, an *hrpX* mutant was generated by double crossover steps using the pK18*mobsacB* vector (21). The GUS reporter plasmid pFR421 that carried a 438-bp *pip* promoter-*gusA* fusion (34) was introduced into the wild-type 8004 and *hrpX* mutant strains. The GUS activity assay showed that disruption of *hrpX* resulted in decreased GUS activities in NYG medium, MMX medium, and *in planta* (Fig. 2). Compared with that in strain 8004/pFR421, the GUS activity in Δ*hrpX*/pFR421 decreased by 6.7-, 12.8-, and 8.2-fold, respectively, under the three growth conditions. These results suggested that HrpX is indispensable for *pip* expression.

HrpX binding to the imperfect PIP box. A previous report showed that even an imperfect PIP box could be recognized by cognate regulatory factors (12). As a result, more genes with imperfect *cis* elements in the promoters could be assigned to the

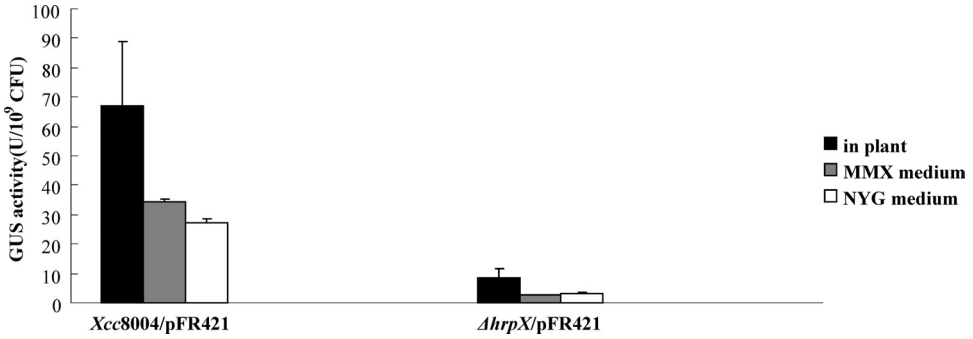

FIG 2 HrpX is essential for $Ppip_{Xcc}$-*gusA* fusion gene expression. The *Xanthomonas campestris* pv. campestris Δ*hrpX*/pFR421[pLAFR6(*P438pip*$_{Xcc}$-*gusA*)] strain exhibited reduced GUS activity compared with that of the 8004/pFR421 strain under three conditions. Relative GUS activity units were defined as nM 4-methylumbelliferone/min/10^9 cells. The graph represents values of experiments from a minimum of three independent samples. *Xcc*8004, *Xanthomonas campestris* pv. campestris strain 8004.

HrpX regulon, such as the pip_{Xcc} gene of strain 8004, in which a potential PIP box-like element is located −307 to −287 upstream from the TSS (Fig. 1). In order to detect the interaction between HrpX and this potential PIP box, ChIP-PCR and EMSA were used to assess the presence of the HrpX protein bound to the *cis* element. ChIP-PCR of strain 8004/pHM1-hrpX-his bacterial extract was performed. The DNA fragments (spanning −438 to −1 and −438 to −287 relative to TSS of pip_{Xcc}) immunoprecipitated with the His monoclonal antibody were amplified by PCR. The amplification of a no-antibody sample was the negative control. The results revealed that the HrpX protein is bound to the potential PIP box (Fig. 3). The result of *in vitro* EMSA was consistent with the ChIP-PCR result. The migrated bands of 45-bp duplex DNA and HrpX-MBP complexes were observed in nondenaturing polyacrylamide gel. Various amounts of unlabeled probe (Fig. 4, right) and various amounts of purified HrpX-MBP (Fig. 4, left) were used as competitors. MBP, as the negative control, did not bind the DNA probe.

HrpX/XccR interplay is responsible for pip_{Xcc} induction. As previously reported, an *xccR* mutant decreased the induction of the pip_{Xcc} promoter in host plants (34). This indicated that the disruption of either *xccR* or *hrpX* can result in a failed induction of pip_{Xcc}. To investigate the relationship between XccR and HrpX in regulating pip_{Xcc} expression, a pulldown assay was carried out to test the binding of the two proteins. The MBP-tagged XccR was expressed in *E. coli* and *X. campestris* pv. campestris harboring the plasmid-expressed HrpX-His. The pulldown result showed that the XccR protein interacted with the HrpX protein (Fig. 5). The MBP protein used as a negative control did not bind any of the tested proteins. This result indicates that the complex formed by XccR and HrpX may contribute to the activation of pip_{Xcc}.

DISCUSSION

In *Xanthomonas* species, the essential role of HrpX in virulence-related gene expression, through binding by a plant-inducible element, the PIP box, has been well defined. According to bioinformatics analysis results, an imperfect PIP box (TTCGC-N4-TTCGC-N2-TTGCC) was found in the pip_{Xcc} promoter region in strain 8004 (Fig. 1). The *X. campestris* pv. campestris genome has 12 candidate promoters with perfect PIP boxes (TTCGC-N15-TTCGC) (13), and an earlier study showed that a substitution in

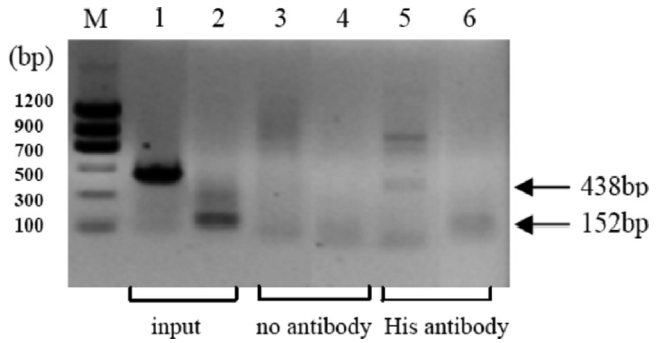

FIG 3 Results of ChIP-PCR assay with or without His tag monoclonal antibody. DNA was amplified by primers designed against the regulatory regions. Lanes 1 and 2 represent the positive controls using chromatin as the template for PCR. The sizes of the resulting fragments are 438 bp (using primers pipF1 and pipR2; Table 1) and 152 bp (using primers pipF1 and pipR1; Table 1), respectively. Negative controls with no antibody are presented in lanes 3 and 4. The results with the addition of anti-His antibody are shown in lanes 5 and 6. The no-template negative control is not shown.

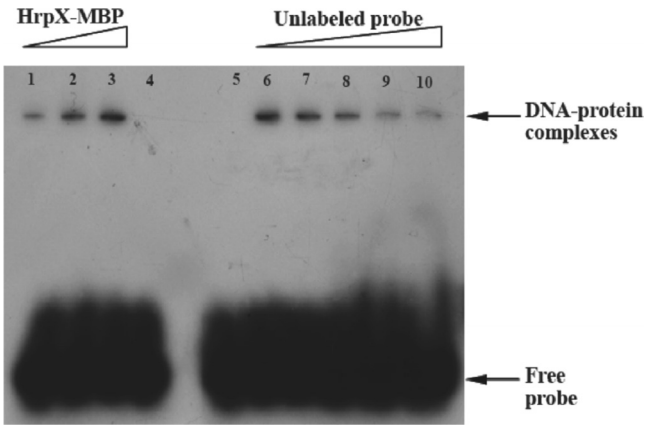

FIG 4 Binding of HrpX to potential PIP box by EMSA. The interaction of the DNA probe with purified HrpX-MBP is shown. Each lane contains 0.65 μg isotope-labeled probe. Lanes 1 to 3 contain 12.28 μg, 24.56 μg, and 49.12 μg HrpX-MBP, respectively; lane 4 contains MBP (44.48 μg) as the negative control; lane 5 contains the free probe as the positive control; and lanes 6 to 10 contain the same concentration of HrpX-MBP (49.12 μg) and various amounts of unlabeled probe (0.65 μg, 3.23 μg, 13 μg, and 19.5 μg, respectively) as competitors.

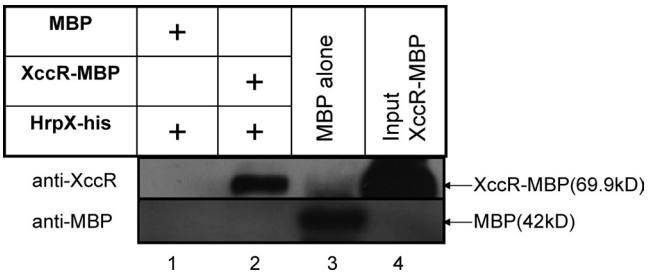

FIG 5 XccR interacts with HrpX *in vitro*. The HrpX-his, MBP, and XccR-MBP strains were used in a His pulldown assay. The precipitated proteins were detected by Western blotting using anti-XccR and anti-MBP antibodies. The recombinant HrpX did not precipitate with MBP (lane 1) but did with XccR-MBP (lane 2). In lane 3, MBP was used as a positive control for immunoblot detection by an anti-MBP antibody. Lane 4 shows an immunoblot of 2 μg purified XccR-MBP.

the central base C in each of the TTCGC elements will drastically decrease the promoter activity (31). In the case of the imperfect PIP box in the pip_{Xcc} promoter, the first two parts were identical to a perfect PIP box half-site consensus sequence, but not the third one. The three parts were separated by 2 and 4 bp. Generally, the separating base stretch between the half-sites ranges from 8 to 16 bp (20), and even a 17-bp spacing retains the promoter activity (12). Thus, the imperfect PIP box in the pip_{Xcc} promoter region of *X. campestris* pv. campestris may be formulated as TTCGC-N11-TTGCC. In this pattern, the central C of the second half-site was replaced by G. We suspect that this replacement may reduce the promoter activity compared with that of the perfect PIP box as previously reported (31). Further experiments need to be conducted to elucidate the imperfect PIP box's contribution to pip_{Xcc} gene expression.

To explore whether HrpX contributes to the induction of pip_{Xcc} expression, an *hrpX* mutant was constructed by double crossover. The disruption of HrpX in Δ*hrpX*/pFR421 decreased the GUS activity by 6.7-, 12.8-, and 8.2-fold in NYG, MMX medium, and *in planta*, respectively, in comparison with that of the control strain 8004/pFR421, which indicated the importance of HrpX for pip_{Xcc} gene expression (Fig. 2). To verify whether HrpX directly binds the imperfect PIP box, we performed ChIP-PCR and EMSA. As expected, both experiments confirmed the direct binding of HrpX to the imperfect PIP box, and this result may expand the gene members of the HrpX regulon in *X. campestris* pv. campestris. For phytopathogenic bacteria, the HrpG/HrpX regulon responds to the surrounding environment and aids in the nutrient uptake process (16, 33). An acidic pH, low osmotic pressure, and nutritional limitations are thought to partly contribute to the induction of the *hrp* and *hrc* genes (30). Several synthetic plant environment mimicking media (such as MMX medium) were used for *hrp* gene induction and other virulence gene expression experiments (8, 15, 18). The GUS activity of Δ*hrpX*/pFR421 was lower in the MMX medium than *in planta*, which indicated that the synthetic medium cannot absolutely mimic the natural conditions. Interestingly, in the nutrient-rich medium NYG (a non-*hrp*-inducing medium), the GUS activity of Δ*hrpX*/pFR421 decreased drastically compared with that of strain 8004/pFR421 (Fig. 2). This implied that the basic level of HrpX also affected pip_{Xcc} expression in rich medium. Generally, HrpX-regulated genes consist of type III effector protein genes with PIP boxes (12) and genes without PIP boxes (26). As a member of the HrpX regulon, the disruption of pip_{Xcc} impaired the virulence of *X. campestris* pv. campestris in a host plant cabbage. It is worth exploring whether PIP_{Xcc} is a secretory virulence protein or a modification enzyme to arrest the other virulence factors.

Our previous observations showed that the pip_{Xcc} gene could be induced in host plants and that the increased GUS activity of strain 8004/pFR421 depends on the activation of XccR by binding to the *luxXc* box *in planta* (34). Disruption of *xccR* on the 8004 chromosome (strain 8515/pFR421) resulted in failure of the *in planta* induction of *gusA* (34). In this study, the pip_{Xcc} promoter activity also decreased drastically in the *hrpX* mutant. The results of the pulldown assay indicated that XccR can bind HrpX directly. It is likely that HrpX and XccR form a complex as coactivators to regulate pip_{Xcc} expression and that HrpX/XccR interplay is responsible for pip_{Xcc} induction.

Quorum-sensing systems exist widely in bacteria, and QS-dependent functions include virulence, sporulation, plasmid transfer, biosynthesis of antibiotics, and plant nodulation (3, 4, 25). In the *xccR*-pip_{Xcc} locus, XccR is an unpaired LuxR homolog of QS because the cognate LuxI synthase gene is lacking. The LuxR orphans, such as ExpR of *Sinorhizobium*, BisR of *Rhizobium*, QscR of *Pseuodomonas*, and SdiA in *Salmonella*, *Escherichia*, and *Klebsiella*, respond to AHL signals (1, 9, 22, 23). However, XccR could not respond to AHLs. Instead, XccR activates the expression of pip_{Xcc}, which encodes a hydrolase, by binding the *luxXc* box (34). In this study, XccR recruited HrpX to coregulate pip_{Xcc} expression, which indicated that at least two transcriptional factors mediate the function of the *xccR*-pip_{Xcc} locus.

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