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Identification of *hrcC*, *hrpF*, and *miaA* Genes of *Xanthomonas campestris* pv. *glycines* 8ra: Roles in Pathogenicity and Inducing Hypersensitive Response on Nonhost Plants

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Nonpathogenic mutants of *Xanthomonas campestris* pv. *glycines* were generated with Omegon-Km to isolate genes essential for pathogenicity and inducing hypersensitive response (HR). Three nonpathogenic mutants and two mutants showing slow symptom development were isolated among 1,000 colonies tested. From two nonpathogenic mutants, 8-13 and 26-13, genes homologous to *hrcC* and *hrpF* of *X. campestris* pv. *vesicatoria* were identified. The nonpathogenic mutant 8-13 had a mutation in a gene homologous to *hrpF* of *X. campestris* pv. *vesicatoria* and failed to cause HR on pepper plants but still induced HR on tomato leaves. The nonpathogenic mutant 26-13 had an insertional mutation in a gene homologous to *hrcC* of *X. campestris* pv. *vesicatoria* and lost the ability to induce HR on pepper leaves but still caused HR on tomato plants. Unlike other phytopathogenic bacteria, the parent strain and these two mutants of *X. campestris* pv. *glycines* did not cause HR on tobacco plants. A cosmid clone, pBL1, that complemented the phenotypes of 8-13 was isolated. From the analysis of restriction enzyme mapping and deletion analyses of pBL1, a 9.0-kb *Eco*RI fragment restored the phenotypes of 8-13. pBL1 failed to complement the phenotypes of 26-13, indicating that the *hrcC* gene resides outside of the insert DNA of pBL1. One nonpathogenic mutant, 13-33, had a mutation in a gene homologous to a *miaA* gene encoding tRNA delta (2)-isopentenylpyrophosphate transferase of *Escherichia coli*. This indicated that tRNA modifications in *X. campestris* pv. *glycines* may be required for expression of genes necessary for pathogenicity. The mutant 13-33 multiplied as well as the parent strain did in the culture medium and *in planta*, indicating that loss of pathogenicity is not due to the inability of multiplication *in vivo*.

Keywords : *hrp* genes, hypersensitive response, pathogenicity genes, soybean bacterial pustule disease.

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Bacterial pustule of soybeans caused by *Xanthomonas campestris* pv. *glycines* is one of the most prevalent bacterial disease in Korea. A pustule formation underneath of soybean leaves is a typical symptom, and lesions are usually confined to leaves (Jones and Fett, 1987). Yield losses caused by bacterial pustule are mainly due to premature leaf defoliation and reduced seed sizes (Hwang and Lim, 1992; Jones and Fett, 1987). Under favorable weather conditions, severe damages can be occurred.

Pathogenicity of this bacterium has been studied previously (Hwang et al., 1992). Auxins have been proposed to play a role in pathogenicity of *X. campestris* pv. *glycines*, however it is unlikely that it is a major pathogenicity factor (Fett and Dunn, 1987). Extracellular enzymes such as proteases, cellulase, and polygalacturonase have been known as pathogenicity factors in many plant bacterial pathogens. These enzymes produced by *X. campestris* pv. *glycines* may play some roles in pathogenicity, however apparently they are not critical factors in causing bacterial pustules (Hwang et al., 1992).

hrp (*h*ypersensitive *r*esponse and *p*athogenicity) genes responsible for pathogenicity and the ability to cause hypersensitive response (HR) in nonhost plants were first described for *Pseudomonas syringae* pv. *phaseolicola*, and have been identified from many gram-negative plant pathogenic bacteria (Lindgren et al., 1986). Major phenotypes of *hrp* mutants are the loss of pathogenicity and the inability of causing HR in nonhost plants. The loss of pathogenicity of *hrp* mutants is linked to the inability of multiplying in host tissues (Hwang et al., 1992). However, it is not very clear whether attenuation in their ability of growth in susceptible host tissues is a cause or a result of loss of pathogenicity.

Four major *hrp* gene clusters from *P. syringae* pv. *syringae*, *Ralstonia solanacearum*, *Erwinia amylovora*, and *X. campestris* pv. *vesicatoria* have been identified (Hueck, 1998). These groups belong to a so-called type III protein secretion system which is independent of the *sec* system (Hueck, 1998). Most of proteins involved in the type III secretion system are inner membrane proteins and homolo-

gous to components of flagella biosynthesis apparatus (Alfano and Collmer, 1997; Hueck, 1998). In plant pathogenic bacteria, some *hrp* gene clusters encode for elicitors such as harpins and contain regulatory genes like *hrpRS* of *P. syringae* pv. *syringae* (Hueck, 1998). Harpins, *avr* gene products, and possibly other virulence factors are believed to be secreted through *hrp* secretion apparatus (Alfano and Collmer, 1997; Hueck, 1998). Interestingly, some *hrp* genes share significant homologies with genes involved in virulence factor secretion from animal pathogens (Fenselau et al., 1992; Hueck, 1998). These genes were renamed recently as *hrc* (*h*ypersensitive *r*esponse and *c*onserved) genes (Bogdanove et al., 1996).

From the previous work, two *hrp* genes of *X. campestris* pv. *glycines,* homologous to *hrcQ* (*hrpD1*) and *hrcR* (*hrpD2*) of *X. campestris* pv. *vesicatoria,* have been cloned and characterized (Hwang et al., 1992). These two genes were not called as *hrp* genes at the time because the parent strain 8ra and nonpathogenic mutants generated by nitrosoguanidine treatment did not cause HR on tobacco plants, and no DNA sequences of *hrp* genes were available in the databases. However, it has been found that the wild type strain 8ra and nonpathogenic mutants cause HR on pepper and tomato plants (Hwang et al., 1992; Oh et al., 1999). We therefore mutagenized the parent strain 8ra with Omegon-Km and tried to isolate mutants failed to cause HR on pepper and tomato leaves to identify more *hrp* genes responsible for inducing HR. Here we report two genes homologous to *hrpF* and *hrcC* of *X. campestris* pv. *vesicatoria* and describe that these mutants lost pathogenicity and the ability to cause HR on pepper leaves but still induce HR on tomato leaves. We also present that a gene homologous to *miaA* gene encoding tRNA delta (2)-isopentenylpyrophosphate transferase of *E. coli* is important for pathogenicity of *X. campestris* pv. *glycines*.

Materials and Methods

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. All *X. campestris* pv. *glycines* strains are derivatives of a parent strain 8ra which is resistant to rifampicin. An *E. coli* strain S17-1 carrying pJFF350 was obtained from Dr. Joachim Frey (University of Berne, Switzerland).

Media, culture conditions, and chemicals. Strains of *X. campestris* pv. *glycines* were grown on YDC agar medium (Shaad, 1988) or in L broth (Sambrook et al., 1989) at 28°C. All *E. coli* strains were grown in L broth or L agar plates at 37°C. Antibiotic concentrations were used as follows: rifampicin, 50 μg/ml; tetracycline 10 μg/ml; kanamycin, 25 μg/ml for *E. coli* and 50 μg/ml for *X. campestris* pv. *glycines*; ampicillin, 100 μg/ml. All

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or source
E. coli		
HB101	F⁻ hsdS20($r_B^-m_B^-$) *recA13 leuB6 ara-14 proA2 lacY1 galK2 rspL20*(Str^r) *xyl-5 mtl-1 supE55*	Sambrook et al.
DH5	F⁻ 80d *lacZ*M15 *endA1 recA1 hsdR17* (r^{K-} m^{K-}) *supE44 thi-1 gyrA96* (*lacZYA-argF*)U169	Sambrook et al.
X. campestris pv. *glycines*		
8ra	Parent strain, Rif^r	Huguet and Bonas
NP1	Nonpathogenic mutant	Huguet and Bonas
8-13	8ra::Omegon-Km, nonpathogenic mutant	This study
13-33	8ra::Omegon-Km, nonpathogenic mutant	This study
26-13	8ra::Omegon-Km, nonpathogenic mutant	This study
5-15	8ra::Omegon-Km, less virulent mutant	This study
8-31	8ra::Omegon-Km, less virulent mutant	This study
Plasmid		
pLAFR3	Derivative of pRK290, Tet^r	Staskawicz et al.
pTZ18	Amp^r	Mead et al.
pRK2013	Km^r, Tra$^+$, ColE1 replicon	Figurski and Helinski
pJFF350	Km^r, Omegon-Km	Fellay et al.
pBL1	30-kb DNA fragment from 8ra cloned into pLAFR3	This study
pIH5	*Hin*dIII deletion of pBL1	This study
pBK2	Self-ligated *Eco*RI clone from 8-13	Hwang et al.
pBKH3	1.8-kb *Hin*dIII fragment from pBK2 cloned into pTZ18	This study
pBK7	Self-ligated *Eco*RI clone from 13-33	This study
pBK9	Self-ligated *Eco*RI clone from 26-13	This study
pBK12	1.4-kb *Pst*I fragment from pBK7 cloned into pTZ18	This study
pBK13	1.5-kb *Bam*HI fragment from pBK9 cloned into pTZ18	This study
pBM13	9.0-kb *Eco*RI fragment cloned into pLAFR3	This study
pBM17	3.8-kb *Hin*dIII/*Eco*RI fragment cloned into pLAFR3	This study

restriction enzymes and T4 DNA ligase were purchased from New England Biolaboratory (Beverly, MA). A DNA labeling kit (Bioprime® DNA labeling system) and a hybridization and detection kit (Photogene Version™ 2.0 Reagent Assembly) were obtained from GIBCO Bethesda Research Laboratories (Gaithersburg, MD). A Dye Terminal Cycle Sequencing Ready Reaction kit for DNA sequencing was purchased from Perkin Elmer Co. (Foster City, CA).

Plant inoculation. Pepper (*Capsicum annum* L. cv. Dabokkun and Chokwang) and tomato (*Lycopersicon esculentum* Mill. cv. Seokwang) plants were used for HR tests. Cells of *X. campestris* pv. *glycines* were adjusted to $2\text{-}5\times10^8$ cells/ml and injected into fully expanded leaves of whole plants. Tobacco (*Nicotiana tabacum* cv. Samsun) plants were treated similarly for HR tests. The reactions of plants were determined 24 hr after injection. Pathogenicity was determined using soybean (*Glycine max* [L.] cv. Pella) cotyledons, and growth of mutants *in planta* was determined as described previously (Hwang et al., 1992; Hwang and Lim, 1992). Growth study in soybean cotyledons was done twice with three replications.

Mating. All pLAFR3 derivatives were mobilized into *X. campestris* pv. *glycines* strains by triparental mating as described previously (Hwang et al., 1992).

Transposon mutagenesis. *X. campestris* pv. *glycines* 8ra was mutagenized with Omegon-Km as described by Fellay et al. (1989). Transconjugants growing on L agar plates containing kanamycin were isolated and subsequently used for pathogenicity and HR tests.

Recombinant DNA techniques. Restriction enzyme digestions and DNA ligation were done as described by the manufacturer. To isolate chromosomal DNA of bacterial cells, cells were lysed by 1% SDS followed by proteinase K treatment. Polysaccharides were removed from the lysed cell mixtures by hexadecyltrimethyl ammonium bromide (CTAB)/NaCl solution, and solution mixtures containing total genomic DNA were extracted with chloroform/isoamyl alcohol and phenol/chloroform/isoamyl alcohol. Total genomic DNA was precipitated with 0.6 volume of isopropanol and washed with 70% ethanol. Dried DNA was redissolved in 50 μl of water. For colony blot and Southern hybridization analyses, a probe DNA was prepared as described by the manufacturer, and Hybond™-N nylon membranes (Amersham International plc, England) were used. All procedures for hybridizations were done as described by the manufacturer. All other standard molecular biological methods were used as described by Sambrook et al. (1989).

DNA sequencing and data analyses. All DNA sequencing reactions to identify which genes were mutated with Omegon-Km were done using a primer HR (5'-TGCTCAATCAATCACCGG-3') homologous to the end of Omegon-Km as described by the manufacturer. DNA sequence data were analyzed through BLAST Search in National Center for Biotechnology Institute (Gish and States, 1993).

Results

Isolation of HR⁻ and nonpathogenic mutants. After mutagenesis of *X. campestris* pv. *glycines* 8ra with Omegon-Km, one thousand prototrophic colonies were isolated and tested for their pathogenicity on soybean cotyledons and the ability to induce HR on pepper and tomato plants. Three nonpathogenic mutants (8-13, 13-33, and 26-13) and two mutants (5-15 and 8-31) showing slow symptom development were isolated (Fig. 1). Two nonpathogenic mutants, 8-13 and 26-13, completely lost the ability to cause HR on pepper plants, however they retained the ability to induce HR on tomato plants (Fig. 2). One nonpathogenic mutant, 13-33, induced HR on both pepper and tomato plants. As known earlier, the parent strain 8ra, all three nonpathogenic mutants, and nonpathogenic mutant NP1 previously isolated failed to cause HR on tobacco plants (data not shown). Two mutants showing slow symptom development were not characterized further since they were less interested. All three mutants, 8-13, 26-13, and 13-33, retained the same physiological traits as the parent strain 8ra (data not shown).

Identification of *hrp* genes. To identify which genes were knocked out by Omegon-Km in 8-13 and 26-13 mutants, the flanking DNA regions of the site of the transposition

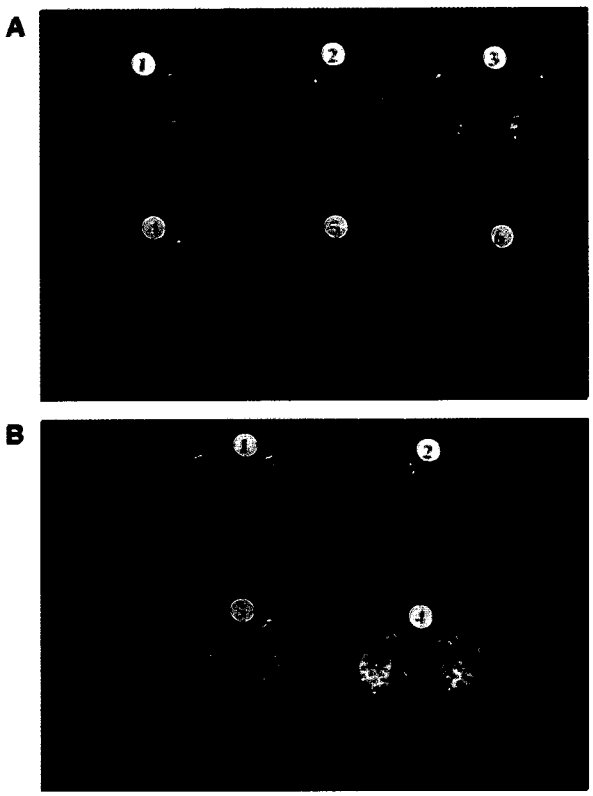

Fig. 1. Pathogenicity assay of *X. campestris* pv. *glycines* strains. Soybean cotyledons of the susceptible cultivar Pella (14 days old) were inoculated with the parent strain 8ra (A-1 and B-1), water (A-2 and B-2), 8-13 (A-3 and B-3), 13-33 (A-4), 15-24 (A-5), 26-13 (A-6), and 8-13(pBL1) (B-4). The cotyledons were photographed 5 days after inoculation.

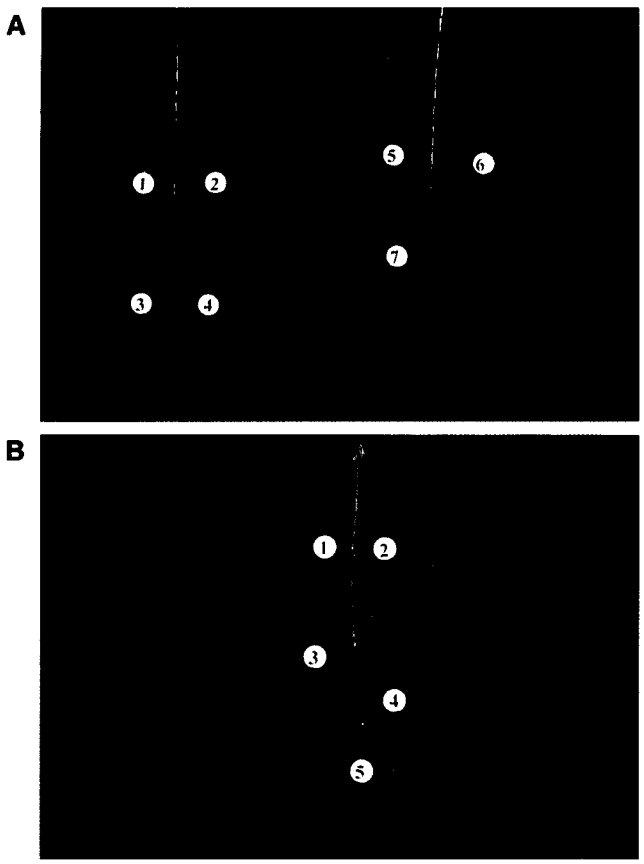

Fig. 2. Response of *X. campestris* pv. *glycines* strains on pepper and tomato plants. Pepper leaves (A) were infiltrated with various bacterial cell suspensions as follows: (1), the parent strain 8ra; (2), the nonpathogenic mutant 8-13; (3) the nonpathogenic mutant 26-13; (4), the nonpathogenic mutant 13-33; (5), a water control; (6), 8-13(pBL1); (7), 8-13 (pIH5). Tomato leaves (B) were injected with bacterial cells as follows: (1), the parent strain 8ra; (2), 8-13; (3), 26-13; (4), 13-33; (5), a water control.

event were cloned and characterized. Total genomic DNA of 8-13 and 26-13 mutants was isolated by the procedure of bacterial genomic DNA preparation as described in Materials and Methods. Since Omegon-Km carries an origin of replication and no *Eco*RI site, 1 μg of total genomic DNA was digested with *Eco*RI, self-ligated, and transformed into *E. coli* DH5. Colonies carrying the flanking DNA fragments were isolated by selection on L agar plates containing kanamycin. Several colonies appeared on the selection medium, and plasmid DNA from each colony was isolated and digested with *Eco*RI to confirm that each plasmid clone carried a single *Eco*RI site. One clone, pBK2, was approximately 15-kilo bases (kb) in size, and had a single *Eco*RI site, three *Hin*dIII sites, eight *Pst*I sites, and three *Sal*I sites. The flanking *Hin*dIII fragment which contains the end of Omegon-Km and part of 8-13 chromosomal DNA in pBK2 was determined by restriction enzyme digestion analyses, and the corresponding 1.8-kb *Hin*dIII fragment was cloned into pTZ18, resulting in pBKH3. The junction of Omegon-Km and 8-13 chromosomal DNA was confirmed by sequencing with the primer HR, and approximately 600 bases were further sequenced from the junction to identify which gene was mutated to show the phenotype. From the BLAST Search analyses with the limited DNA sequencing data, a gene homologous to *hrpF* of *X. campestris* pv. *vesicatoria* and *nolX* that is part of the host specificity genes of *Rhizobium fredii* was found. They showed 64% identity and 76% positives throughout about 80 amino acid residues (data not shown). pBK9 was similarly cloned from the chromosomal DNA of 26-13 digested with *Eco*RI as described above and was approximately 18-kb in size. It had a single *Eco*RI site, two *Hin*dIII sites, and three *Bam*HI sites. The 1.5-kb *Bam*HI fragment containing the end of Omegon-Km and part of chromosomal DNA of 26-13 in pBK9 was cloned into pTZ18, resulting in pBK13. The junction of Omegon-Km and 26-13 chromosomal DNA was confirmed as described above, and approximately 600 bases were further sequenced from the junction. From the BLAST Search analyses with the limited DNA sequencing data, a gene homologous to *hrcC* of *X. campestris* pv. *vesicatoria* and *hrcC* of *Ralstonia solanacearum* was found. They showed 97% identity and 98% positives throughout about 100 amino acid residues (data not shown).

***miaA* gene is important for pathogenicity but not required for inducing HR on pepper and tomato plants.** Since the mutant 13-33 lost pathogenicity but retained the ability to cause HR on pepper and tomato plants, it was believed that this mutant belongs to a different group from two mutants that have mutations in *hrp* genes. pBK7 was cloned from the mutant 13-33 by similar methods described above. It was approximately 13.0-kb in size and had a single *Eco*RI site, three *Bam*HI sites, two *Hin*dIII sites, and eight *Pst*I sites. A 1.4-kb *Pst*I fragment carrying the junction of Omegon-Km and 26-13 chromosomal DNA from pBK7 was cloned into pTZ18, resulting in pBK12. Approximately 750 bases of pBK12 from the junction of the insertion site of Omegon-Km were obtained. BLAST Search analyses revealed that a gene knocked out by Omegon-Km was a homolog of *miaA* gene encoding tRNA delta (2)-isopentenylpyrophosphate transferase of *E. coli*. Two genes shared 42% identity and 57% positives throughout more than 100 amino acid residues (data not shown).

To determine if a mutation in *miaA* gene affects the growth rate of *X. campestris* pv. *glycines*, overnight culture of bacterial cells were diluted 100 fold in L broth or M9 minimal medium and continued to grow until OD_{600} values reached 1.2 at 28°C. The growth rate of 13-33 was the same as that of the wild type strain both in L broth and M9 mini-

mal medium (data not shown). We also tested the growth pattern of 13-33 *in planta*. When approximately 5×10^5 cells of the parent strain 8ra and nonpathogenic mutant 13-33 were inoculated into susceptible soybean cotyledons, the number of cells of 13-33 increased about 100-fold during the first 4~5 days (Fig. 3). However, cells of 13-33 grew slower and less than the parent strain did (Fig. 3). This pattern of growth was repeated twice. This indicated that loss of pathogenicity of 13-33 is not due to the inability of multiplication in host plants.

Complementation. *E. coli* colonies carrying cosmid clones from the genomic library were spread on L agar containing tetracycline and blotted into the membranes for colony hybridizations. The 1.8-kb *Hin*dIII fragment of pBK2 was isolated from the agarose gels and labeled as a probe DNA as described in Materials and Methods. One cosmid clone showing a positive signal was isolated and mobilized into the nonpathogenic mutant 8-13. Transconjugants were isolated and tested for pathogenicity and the ability to cause HR on pepper and tomato plants. A cosmid clone pBL1 restored pathogenicity of 8-13 and also the ability of 8-13 to cause HR on pepper leaves (Fig. 1 and 2). However, pBL1 was not able to complement the mutant 26-13 phenotype (Fig. 4). Restriction map analyses showed that pBL1 is identical to a cosmid clone pIH1 identified previously (Hwang et al., 1992). Southern hybridization, deletion, and subcloning analyses indicated that a 3.8-kb *Hin*dIII/*Eco*RI fragment in a 9.0-kb *Eco*RI fragment of pBL1 contains *hrpF* locus (Fig. 4). Thus the 9.0-kb *Eco*RI fragment and the 3.8-kb *Hin*dIII/*Eco*RI fragment were cloned into pLAFR3 resulting in pBM13 and pBM17, respectively. When pBM13 was introduced into the mutant 8-13, pathogenicity was restored and the ability of causing HR on pepper leaves was recovered whereas pBM17 failed to complement the mutant phenotypes (Fig. 4). This indicated that the 3.8-kb *Hin*dIII/*Eco*RI fragment does not contain a complete *hrpF* gene of *X. campestris* pv. *glycines*.

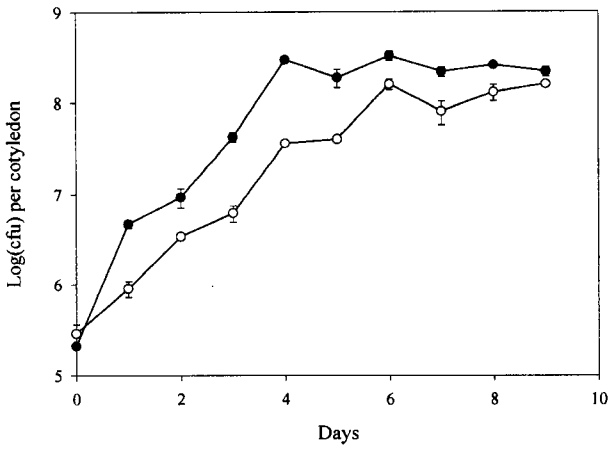

Fig. 3. Growth of *X. campestris* pv. *glycines* parent strain 8ra (closed circles) and nonpathogenic mutant 13-33 (open circles) in soybean cotyledons. Bacterial numbers were counted daily after inoculation, and data are averages of three samples. Similar results were obtained from the repeated experiment, and one of the data is presented here. Vertical bars indicate error ranges.

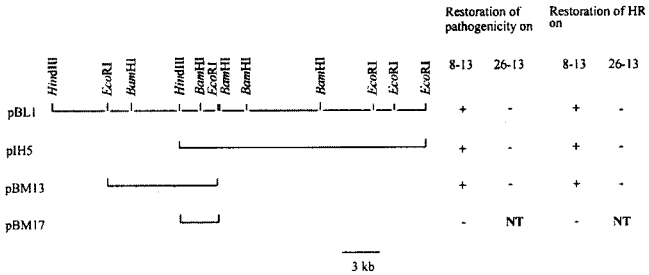

Fig. 4. Restriction map and deletion analysis of pBL1. The transconjugants produced (+) or failed to produce (-) symptoms on soybean cotyledons. The transconjugants induced (+) or did not cause (-) HR on pepper plants.

Discussion

In the previous work, nitrosoguanidine was used to generate nonpathogenic mutants since transposon mutagenesis of *X. campestris* pv. *glycines* 8ra using various transposable elements was not successful (Huguet and Bonas, 1997). However, recently Omegon-Km has been used to mutagenize *X. campestris* pv. *glycines*, and some *hrp* genes of this bacterium have been found (Oh et al., 1999). This transposon has a synthetic 28 bp repeats derived from the ends of *IS*1 and a unique advantage compared to other known transposons (Fellay et al., 1989). It contains an *E. coli*-specific origin of replication within Omegon-Km which allows the rapid and easy cloning of the nucleotide sequences flanking the site of the transposition event (Fellay et al., 1989). Another good trait of this transposon is that transposase necessary for *IS*1 transposition functions resides outside of Omegon-Km, so that further transposition events do not occur and mutants generated by this transposon are very stable (Fellay et al., 1989). Therefore, we exploited Omegon-Km to obtain nonpathogenic mutants of *X. campestris* pv. *glycines* and obtained some nonpathogenic mutants and mutants showing slow symptom development. However, we did not further characterize mutants showing slow symptom development because they are less interested.

Since it has been known that *X. campestris* pv. *glycines* does not cause HR on tobacco plants, we believed that this bacterium may have different HR-inducing mechanisms compared to known *hrp* gene systems. The closely related organism *X. campestris* pv. *vesicatoria* causes HR on tobacco plants, and *hrp* genes of this bacterium have been found (Fenselau et al., 1992). However, complete DNA

sequences of *hrp* loci of *X. campestris* pv. *vesicatoria* have not been reported. In this study, we identified two *hrp* genes homologous to *hrpF* and *hrcC* genes of *X. campestris* pv. *vesicatoria* and found that the phenotypes of 8-13 and 26-13 were different from those of typical *hrp* mutants of other plant pathogenic bacteria. Unlike *hrp* mutants of other plant pathogenic bacteria, 8-13 and 26-13 completely lost the ability to induce HR on pepper leaves but still caused HR on tomato leaves. To our knowledge, all known *hrp* mutants of other plant pathogenic bacteria are nonpathogenic and do not have the ability to induce HR on nonhost plants (Hueck, 1998). A *hrcU* mutant of *X. campestris* pv. *glycines* and our previous nonpathogenic mutant NP1 are nonpathogenic but retain the ability to cause HR on pepper and tomato plants (Oh et al., 1999). This phenotype belongs to another group. However, we do not have a plausible explanation as to why various *hrp* mutants of *X. campestris* pv. *glycines* have different phenotypes. This phenomenon suggests that *X. campestris* pv. *glycines* may have another set of *hrp* systems or may have a specificity on nonhost plants. We do not know possible functions of HrpF and HrcC except the fact that HrcC of *X. campestris* pv. *vesicatoria* is located in the inner membrane (Wengelnik et al., 1996).

The fact that pBL1 complemented the mutant 8-13 but not 26-13 led us to believe that a *hrcC* homolog we found resides outside of the insert DNA of pBL1. Considering the size of *hrp* loci and the location of *hrpF* gene in the *X. campestris* pv. *vesicatoria hrp* clusters, pBL1 contains only part of *hrp* genes of *X. campestris* pv. *glycines*. The fact that *hrpF* of *X. campestris* pv. *glycines* is located in the middle of the insert DNA in pBL1 and that pBL1 cannot complement the phenotype of the mutant 26-13 is consistent with our prediction that the *hrp* gene arrangement of *X. campestris* pv. *glycines* is similar to that of *X. campestris* pv. *vesicatoria*. In *X. campestris* pv. *vesicatoria*, *hrpF* is located in one end of the *hrp* loci as a single gene and *hrcC* is located in the other end of it (Wengelnik et al., 1996).

The *miaA* gene is the first gene involved in the synthesis of 2-methyl-N^6-isopentynyladenosine (ms^2i^6A37) in tRNA (Diaz et al., 1987). A mutation in *miaA* gene results in pleiotrophic effects (Diaz et al., 1987, Durand et al., 1997). Among those, the conditional streptomycin-dependent phenotype is associated with a mutation in *miaA* (Taylor et al., 1998). A mutation in *miaA* gene of *Salmonella typhimurium* reduce tetracycline resistance mediated by Tet(O) and Tet(M) (Taylor et al., 1998). Most importantly, tRNA modifications in *Shigella flexneri* influence virulence by regulating posttranscriptional expression of the regulatory genes *virF* (Durand et al., 1997). In fact, the modified nucleoside 2-methylthio-N^6-isopentenyladenosine in tRNA of *S. flexneri* is required for expression of virulence genes (Durand et al., 1997). A *miaA* mutant of *Agrobacterium tumefaciens* resulted in reduced *vir* gene expression (Gray et al., 1992). Similarly, we observed that a *miaA* mutant of *X. campestris* pv. *glycines* lost pathogenicity. This suggests that tRNA modifications in *X. campestris* pv. *glycines* may be required for expression of genes necessary for pathogenicity. Currently, we further characterize which pathogenicity genes need the modified tRNA for their expression.

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