Expression of the *strA-strB* Streptomycin Resistance Genes in *Pseudomonas syringae* and *Xanthomonas campestris* and Characterization of IS*6100* in *X. campestris*

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Expression of the *strA-strB* streptomycin resistance (Sm^r) genes was examined in *Pseudomonas syringae* pv. syringae and *Xanthomonas campestris* pv. vesicatoria. The *strA-strB* genes in *P. syringae* and *X. campestris* were encoded on elements closely related to Tn*5393* from *Erwinia amylovora* and designated Tn*5393*a and Tn*5393*b, respectively. The putative recombination site (*res*) and resolvase-repressor (*tnpR*) genes of Tn*5393* from *E. amylovora*, *P. syringae*, and *X. campestris* were identical; however, IS*6100* mapped within *tnpR* in *X. campestris*, and IS*1133* was previously located downstream of *tnpR* in *E. amylovora* (C.-S. Chiou and A. L. Jones, J. Bacteriol. 175:732–740, 1993). Transcriptional fusions (*strA-strB::uidA*) indicated that a strong promoter sequence was located within *res* in Tn*5393*a. Expression from this promoter sequence was reduced when the *tnpR* gene was present in a *cis* position relative to the promoter. In *X. campestris* pv. vesicatoria, analysis of promoter activity with transcriptional fusions indicated that IS*6100* increased the expression of *strA-strB*. Analysis of codon usage patterns and percent G+C in the third codon position indicated that IS*6100* could have originated in a gram-negative bacterium. The data obtained in the present study help explain differences observed in the levels of Sm^r expressed by three genera which share common genes for resistance. Furthermore, the widespread dissemination of Tn*5393* and derivatives in phytopathogenic prokaryotes confirms the importance of these bacteria as reservoirs of antibiotic resistance in the environment.

The aminoglycoside antibiotic streptomycin has been utilized in agriculture since the late 1950s to control populations of phytopathogenic bacteria. Continued usage of streptomycin, however, has been followed by a reduction in efficacy due to resistance in several important phytopathogens (9). Plasmid-encoded streptomycin resistance (Sm^r) determinants have been previously cloned and characterized (7, 32, 33, 42). Hybridization analyses indicated that a homologous Sm^r determinant was present in *Erwinia amylovora*, *Pseudomonas syringae* pv. papulans, *P. syringae* pv. syringae, and *Xanthomonas campestris* pv. vesicatoria strains isolated in North and South America (7, 32, 42). Subsequently, this Sm^r determinant was identified as a homolog of the tandem *strA-strB* aminoglycoside phosphotransferase genes (42) which are also present in clinical bacteria on small broad-host-range plasmids such as pBP1 and RSF1010 (27, 36).

The *strA-strB* homologs in *E. amylovora* are encoded by Tn*5393*, a 6.7-kb transposable element whose complete sequence is known (7). Since previous data indicated that the *strA-strB* genes in *P. syringae* pv. syringae and *X. campestris* pv. vesicatoria were encoded by Tn*5393*-like elements (40–42), we have designated these Tn*5393*a and Tn*5393*b, respectively. Tn*5393* is similar to elements of the Tn*3* subgroup of the Tn*3* family and Tn*2501*; in these transposons, the *tnpA* and *tnpR* genes are transcribed divergently from promoters located on opposite DNA strands within a central intergenic region termed *res* (Fig. 1) (18, 37). During the transposition of Tn*3*, the bifunctional protein (TnpR) resolves cointegrates and re-

presses the transcription of *tnpA* and *tnpR* by binding to *res* at three distinct sites (16, 21).

Although *E. amylovora*, *P. syringae* pv. syringae, and *X. campestris* pv. vesicatoria each contained Tn*5393*, the MICs of streptomycin differed widely among them (7, 32, 42). Strains of *E. amylovora* containing *strA-strB* exhibit MICs of streptomycin of 500 to 1,000 μg/ml (8). A 1.2-kb insertion sequence, IS*1133*, was detected upstream of *strA-strB* in *E. amylovora*, and deletion analysis suggested that a promoter sequence present within IS*1133* was responsible for expression of these genes (7). The MIC of streptomycin in *X. campestris* pv. vesicatoria (250 μg/ml [41]) differed from that observed in *P. syringae* pv. syringae (75 μg/ml [42]) and could be caused by disparate regulatory elements or differential expression of *strA-strB* from the same promoter.

Since little is known about regulatory elements which function in both *P. syringae* and *X. campestris*, the main objective of this study was to analyze the expression of *strA-strB* in *P. syringae* pv. syringae and *X. campestris* pv. vesicatoria. In this study, we identified a putative promoter within Tn*5393*a which was involved in the expression of *strA-strB*. This promoter was negative regulated by the Tn*5393*a repressor encoded by *tnpR*. In *X. campestris* pv. vesicatoria, we found that IS*6100*, an IS*6* family insertion sequence which has been recently detected in a number of diverse gram-negative and gram-positive bacterial genera, is located within *tnpR* upstream of *strA-strB*. Our results show that IS*6100* increased the expression of *strA-strB* in *X. campestris* pv. vesicatoria, which further demonstrates the importance of insertion sequences in modulating gene expression.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used in the present study are listed in Table 1. Plasmids were propagated in *Escherichia coli* DH5α or HB101 before transfer into *P. syringae* pv. syringae FF5

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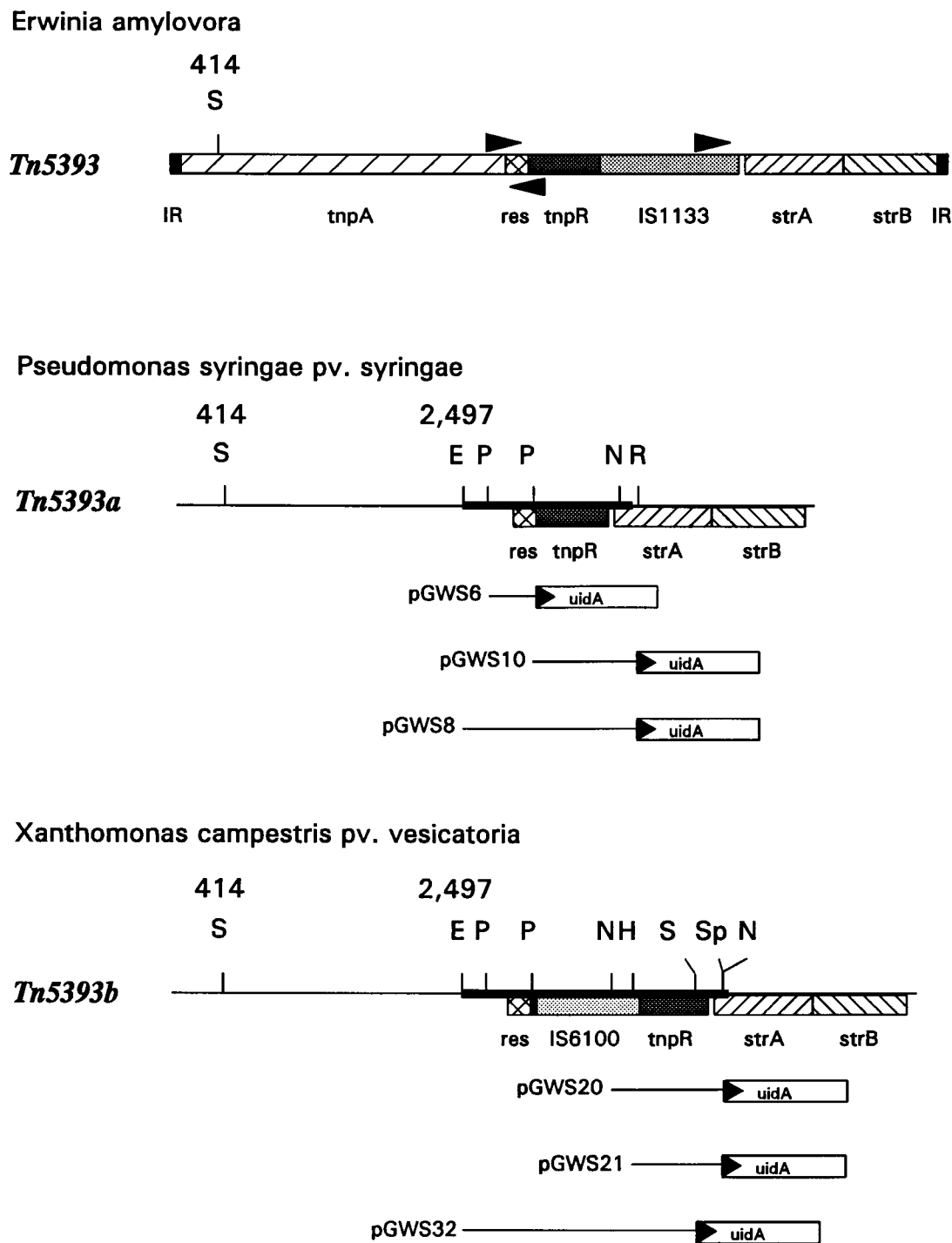
FIG. 1. Physical and functional maps of Tn5393 from E. amylovora, Tn5393a from P. syringae pv. syringae, and Tn5393b from X. campestris pv. vesicatoria. The genes and nucleotide assignments are based on the published sequence of Tn5393 from E. amylovora (7): IR, inverted repeat; tnpA, transposase; res, recombination site; tnpR, resolvase. strA and strB encode aminoglycoside phosphotransferases. Genes which were characterized in this study or in previous studies (7, 42) are boxed. Regions from P. syringae pv. syringae and X. campestris pv. vesicatoria which were sequenced in this study are shown as a heavy line. The arrows show the direction of transcription from putative promoters identified in E. amylovora. The transcriptional fusions constructed in this study and their orientation with respect to a promoterless glucuronidase gene, uidA, are indicated (open rectangles and adjacent arrows). Promoter activities are presented in Table 2. Restriction sites utilized in the construction of strA-strB::uidA fusions are indicated. E, H, N, P, R, S, and Sp represent restriction sites for EcoRV, HindIII, NruI, PstI, RsaI, SstI, and SspI, respectively.

or X. campestris pv. vesicatoria XV16. E. coli strains were cultured in LB medium (31) at 37°C. P. syringae and X. campestris were grown at 28°C in medium B of King et al. (26) and nutrient agar (Difco, Detroit, Mich.), respectively. Following triparental matings, P. syringae recipients were selected on mannitol-glutamate medium (24). The antibiotics ampicillin, spectinomycin, streptomycin, and tet-

racycline were added to media at concentrations of 40, 25, 25, and 12.5 µg/ml, respectively.

Cloning and sequencing of regions upstream of strA-strB from P. syringae and X. campestris. A cosmid library was constructed from the 68-kb Cuʳ Smʳ plasmid pPSR1, an indigenous plasmid in P. syringae pv. syringae A2. pPSR1 was purified

TABLE 1. Bacterial strains and plasmids and their relevant characteristics

Strain or plasmid	Relevant characteristics	Reference
Bacterial strains		
Escherichia coli		
DH5α		35
HB101		35
Pseudomonas syringae pv. syringae		
FF5	Sm^s, no plasmids	42
A2	Cu^r Sm^r *strA-strB* on pPSR1	42
Xanthomonas campestris pv. vesicatoria		
BV5-4a	Sm^r, *strA-strB* on 68-kb plasmid	32
XV16	Sm^r	15
Plasmids		
pBluescript SK$^+$	Ap^r cloning vector	Stratagene
pLAFR3	IncP1, Tc^r, Mob$^+$	39
pRG960sd	IncP, Sm/Sp^r, Mob$^+$, promoterless *uidA*; contains Shine-Dalgarno sequence	46
pRK2013	Mob$^+$, Km^r, Tra RK2	13
pPSR1	68 kb, Cu^r Sm^r, *strA-strB* on Tn*5393*a	42
pPSR1.7	pLAFR3 containing a 22-kb *Sau*3A fragment from pPSR1 ligated into the *Bam*HI site	This study
pPSR2	190 kb, Cu^r Sm^r, Tn*5393*a	42
pPSR5	68 kb, Sm^r, Tn*5393*a	42
pSM1	3.7-kb *Pst*I of pPSR1 in pBluescript SK+	42
pSM7	2.8-kb *Eco*RV of pPSR1.7 in pBluescript SK+	This study
pSM8	4.5-kb *Eco*RI of pPSR1.7 in pBluescript SK+	This study
pGWS2	3.7-kb *Eco*RV of 68-kb Sm^r plasmid from BV5-4a in pBluescript SK+	This study
pGWS3	3.7-kb *Eco*RV of 68-kb Sm^r plasmid from BV5-4a in pBluescript SK+ in opposite orientation	This study
pGWS4	2.2-kb *Hin*dIII of pGWS3 in pBluescript SK+	This study
pGWS5	0.4-kb *Pst*I of pSM8 in pBluescript SK+	This study
pGWS6	0.4-kb *Pst*I of pGWS5 in pRG960sd	This study
pGWS8	1.45-kb *Eco*RV-*Rsa*I of pSM7 in pRG960sd	This study
pGWS10	0.9-kb *Pst*I-*Rsa*I of pSM1 in pRG960sd	This study
pGWS20	0.9-kb *Nru*I of pGWS3 in pRG960sd	This study
pGWS21	0.6-kb *Hin*dIII-*Ssp*I of pGWS4 in pRG960sd	This study
pGWS32	2.0-kb *Eco*RV-*Sst*I of pGWS2 in pRG960sd	This study

on a CsCl-ethidium bromide gradient and partially digested with *Sau*3A to obtain 20- to 30-kb fragments, which were ligated into pLAFR3 as described previously (39). Cosmids were packaged by using an in vitro packaging kit (Boehringer Mannheim, Indianapolis, Ind.) and transfected into *E. coli* HB101 as specified by the manufacturer. Plasmid pRK2013 was used to mobilize cosmids into Sm^s *P. syringae* pv. syringae FF5; selection of recipients was aided by the intrinsic ampicillin resistance of FF5. A cosmid clone, pPSR1.7, which expressed streptomycin resistance in FF5, and pSM1, which contains *strA-strB* from pPSR1 on a 3.7-kb *Pst*I fragment (42), were used to generate overlapping subclones for nucleotide sequence analysis.

The indigenous plasmids from *X. campestris* pv. vesicatoria BV5-4a were purified on a CsCl-ethidium bromide gradient, digested with *Eco*RV, and probed with a 1.5-kb *Sst*I-*Eco*RV fragment from RSF1010 which contains the *strA-strB* genes (36). A 3.7-kb *Eco*RV fragment which hybridized to this probe was ligated into pBluescript SK+ in both orientations to yield pGWS2 and pGWS3, which were further subcloned for DNA sequencing.

Nucleotide sequencing of both DNA strands of all subclones was performed with Sequenase 2.0 (U.S. Biochemical, Cleveland, Ohio) following the instructions of the manufacturer. The location of the *Sst*I site within the transposase (*tnpA*) gene of Tn*5393*a and Tn*5393*b was determined by hybridizing a 0.7-kb *Sac*II fragment from pSM1 to *Sst*I digests of pPSR1 and to the indigenous plasmids from BV5-4a.

Construction and analysis of *strA-strB::uidA* fusions. Clones consisting of various DNA fragments of *strA-strB* in *P. syringae* and *X. campestris* were constructed by ligating fragments into the *Sma*I site of the promoter probe vector pRG960sd. In some cases, fragments were first cloned into pBluescript SK+, and blunt-end restriction sites from the pBluescript polylinker were utilized for cloning into the *Sma*I site of pRG960sd. Clones were introduced into *P. syringae* pv. syringae FF5 and *X. campestris* pv. vesicatoria XV16 via triparental matings (2) and electroporation (15), respectively.

β-Glucuronidase (GUS) activity in bacteria grown in liquid medium was determined by monitoring the ability of bacterial lysates to catalyze the hydrolysis of 4-methylumbelliferyl glucuronide (22). Prior to processing, cells were grown to late-log phase in medium B of King et al. (26) or nutrient broth containing spectinomycin. Aliquots (1.5 ml) were harvested, suspended in GUS extraction buffer (22), and lysed by sonication. GUS activity, reported as specific activity (units per milligram of protein), was determined fluorometrically as previously described (49). The amount of 4-methylumbelliferone was estimated with a Fluoroscan II spectrofluorometer (ICN Biomedicals, Inc., Costa Mesa, Calif.) which was calibrated with 4-methylumbelliferone standards in 0.2 M Na$_2$CO$_3$. The protein content in the bacterial lysates was determined by the procedure of Bradford (4). Lysates of *P. syringae* pv. syringae FF5 and *X. campestris* pv. vesicatoria XV16 containing pRG960sd were utilized as negative controls in each experiment. GUS assays for each plasmid construct were performed three times with two replications per experiment.

Analysis of the transcriptional start site located within *res* in *P. syringae* pv. syringae. Total cellular RNA from 250-ml overnight cultures of *P. syringae* pv. syringae FF5 containing the Sm^r plasmid pPSR5 was isolated as described previously (35). Primer extension reactions were conducted in a reaction mixture containing 100 mM Tris-HCl (pH 8.3), 140 mM KCl, 50 mM MgCl$_2$, 10 mM dithiothreitol, 5 mM (each) dATP, dGTP, and dTTP (Boehringer Mannheim), 10 μCi of [α-^{32}P]dCTP (New England Biolabs, Beverly, Mass.), 32 U of RNasin (Promega Corp., Madison, Wis.), and 9.6 U of avian myeloblastosis virus reverse transcriptase (U.S. Biochemical). A synthetic oligonucleotide primer, 5′-TGTC CCGTTCGACACCTG (Oklahoma State University Recombinant DNA/Protein Resource Facility), which corresponded to the complement of the sequence located 10 bp downstream of the translational start for *res*, was utilized in the reactions. Prior to the addition of the reaction mixture, 20 μg of RNA and 20 ng of the oligonucleotide primer were mixed, and the mixture was boiled for 1 min and chilled quickly on ice. The reaction was incubated at 42°C for 30 min, a chase solution containing 0.3 μM (each) deoxynucleoside triphosphate was added, and the reaction was incubated for an additional 30 min at 30°C. The reaction mixture was then extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with ethanol. The primer extension reaction mixture was loaded onto an 8% polyacrylamide sequencing gel adjacent to sequencing reactions of pGWS1 which were generated with the same oligonucleotide primer.

Analysis of codon usage and third-codon-position percent G+C in IS*6100*. IS*6100* elements have recently been detected on nylon-degrading plasmids from *Flavobacterium* sp., in *Pseudomonas* sp. adjacent to Tn*1696*, and as part of the composite transposon Tn*610* from *Mycobacterium fortuitum* (19, 23, 29). We

were interested in determining if the sequence characteristics of IS*6100* reflected an origin in a gram-negative or a gram-positive host. This question was addressed by examining codon usage patterns and percent G+C in the third codon position of its putative transposase gene (29). The codon frequencies of 37 coding sequences from *X. campestris* and 16 coding sequences from *Mycobacterium* spp. were tabulated by using the University of Wisconsin Genetics Computer Group (UWGCG) program CodonFrequency (12). Codon frequency tables of *Pseudomonas* spp. (6) and *Streptomyces* spp. (48) were also included in the analysis. The UWGCG program Correspond (17) was used to determine if the codon usage pattern of the putative transposase gene encoded by IS*6100* was similar to that of the other gram-negative or gram-positive organisms examined. The Correspond statistic D^2 equals the sum over all 64 codons of the equation: $D^2 =$ [frequency$_{(codon\ table\ 1)}$ − frequency$_{(codon\ table\ 2)}$]^2. The frequencies compared represent the number of incidents of each individual codon divided by the total number of codons in the table specifying that amino acid or terminator. The D^2 statistic decreases as patterns of codon usage become more similar. The percent G+C in the third codon position was also calculated from each of the codon frequency tables utilized in this study.

Nucleotide accession numbers. The reported nucleotide sequences were deposited with GenBank/EMBL under accession numbers M77502 (*P. syringae* pv. syringae) and U20588 (*X. campestris* pv. vesicatoria).

RESULTS

Cloning and sequencing of regions upstream of *strA-strB* in *P. syringae* and *X. campestris*. Subclones from *P. syringae* pv. syringae (Tn*5393*a) and *X. campestris* pv. vesicatoria (Tn*5393*b) containing *strA-strB* and adjacent upstream sequences were subjected to nucleotide sequence analysis. The 1,285 nucleotides of Tn*5393*a extending from the *Eco*RV site (nucleotide 2497 in Tn*5393* [7]) were identical to a sequence contained in Tn*5393*. This 1,285-bp sequence in Tn*5393*a included all of *res* and *tnpR* and 60 bp of *strA*. However, Tn*5393*a did not contain IS*1133* and the flanking sequence TAG, which was presumably generated during the insertion of IS*1133* in Tn*5393* (Fig. 1). The 1,285-bp sequence in Tn*5393*a was also present in Tn*5393*b, except the latter contained an additional 888-bp sequence within *tnpR* (Fig. 1). A homology search indicated that 880 bp of this sequence was identical to the insertion sequence IS*6100* and the additional 8-bp sequence, 5′-AACATATC-3′, was duplicated in Tn*5393*b presumably during insertion of IS*6100* within *tnpR* (8-bp duplications are characteristic of IS*6* family elements [14]). We also found the *Sst*I site at position 414 in Tn*5393* to be conserved in Tn*5393*a and Tn*5393*b (Fig. 1).

Quantification of *strA-strB* promoter activity in *P. syringae* and *X. campestris*. Expression of *strA-strB*::*uidA* transcriptional fusions in *P. syringae* pv. syringae FF5 indicated that a strong promoter sequence was located within the 0.4-kb *Pst*I fragment which contained *res* in Tn*5393*a (Fig. 1). GUS activity in FF5(pGWS6), a construct containing the 0.4-kb fragment in pRG960sd, was 100-fold higher than that in FF5(pRG960sd) (Table 2). pGWS10, a clone containing only *tnpR* and a portion of *strA* (Fig. 1), had no detectable promoter activity (Table 2), further indicating that the promoter sequence was located within *res* in Tn*5393*a. Repression of GUS activity in the *cis* configuration was observed in FF5 containing pGWS8, a clone which included the *res* promoter sequence and *tnpR*. GUS activity of this construct was approximately fivefold less than that of FF5(pGWS6) (Table 2). *tnpR* was also tested for repression in *trans* by monitoring GUS activity in FF5(pGWS6, pPSR2). pPSR2 is an indigenous plasmid in *P. syringae* pv. syringae which contains Tn*5393*a. GUS activity in FF5(pGWS6, pPSR2) was only about 1.5-fold lower than that in FF5(pGWS6) (Table 2), possibly because of copy number differences between pGWS6 and pPSR2 (discussed below).

In *X. campestris* pv. vesicatoria XV16(pGWS6), GUS activity was 10-fold higher than in XV16(pRG960sd) (Table 2), indicating that the *res* promoter isolated from *P. syringae* was active in XV16. However, in XV16 containing pGWS32, a clone

TABLE 2. GUS activity of *strA-strB*::*uidA* fusions in *P. syringae* pv. syringae FF5 and *X. campestris* pv. vesicatoria XV16

Strain and plasmid	Mean GUS activity ± SEM (U/mg of protein)	Activity[a]
FF5		
pGWS6	798.4 ± 100.5	105.1
pGWS6, pPSR2	520.4 ± 43.3	68.5
pGWS8	168.7 ± 20.1	22.2
pGWS10	3.3 ± 2.5	
pRG960sd	7.6 ± 3.7	
pPSR2, pRG960sd	9.8 ± 1.3	1.3
XV16		
pGWS6	125.5 ± 3.6	9.8
pGWS32	421.3 ± 8.3	32.9
pGWS20	27.2 ± 14.2	2.1
pGWS21	1.0 ± 1.0	
pRG960sd	12.8 ± 0.8	

[a] Expressed relative to that of the strain containing pRG960sd alone.

containing the *res* sequence and IS*6100*, GUS activity was threefold higher than that observed with XV16(pGWS6), indicating the role of IS*6100* in increasing expression of *strA-strB* in *X. campestris* (Table 2). GUS activity similar to that of the control was observed for pGWS20 and pGWS21, clones lacking the *res* and containing sequences from the right outward end of IS*6100* (Table 2).

Analysis of the transcriptional start site located within *res* in *P. syringae* pv. syringae. Since results obtained with *strA-strB*::*uidA* fusions indicated that a strong promoter was located within *res* in *P. syringae*, we hypothesized that the transcriptional start site for *strA-strB* was located within *res*. An 18-bp synthetic oligonucleotide was utilized to synthesize cDNA from total cellular RNA of *P. syringae* pv. syringae FF5 containing pPSR5, a 68-kb Sm^r plasmid which harbors Tn*5393*a. A single primer extension product indicated that transcription was initiated at a guanine residue located within *res*, 106 bp upstream of the translational start for *tnpR* (Fig. 2).

Codon usage of the putative transposase gene in IS*6100*. Codon usage tables compiled from 37 *X. campestris* and 16 *Mycobacterium* coding sequences and the putative transposase gene of IS*6100* were generated with the UWGCG program CodonFrequency (40). Comparative analysis of the codon usage patterns in IS*6100* and codon frequency tables of *X. campestris*, *Mycobacterium* spp., *Pseudomonas* spp., and *Streptomyces* spp., using the program Correspond, indicated that the codon usage of the IS*6100* transposase was most similar to that of *X. campestris* (Table 3). The percent G+C in the third codon position of the IS*6100* transposase was also most similar to that of *X. campestris*, although it was also similar to that of *Mycobacterium* spp. (Table 3).

DISCUSSION

The Sm^r transposon Tn*5393* and variants reside in many bacteria, including three phytopathogenic genera and many gram-negative bacteria isolated from agricultural habitats where streptomycin is used (7, 38, 40, 44). The sequence identity of *tnpR* and *res* from diverse plant pathogens suggests that Tn*5393* was recently disseminated. Although the origin of Tn*5393* is unknown, a sequence downstream of *strB* in the Sm^r plasmid RSF1010 is conserved in the right inverted repeat of Tn*5393* (IR-5393o) (7, 36), suggesting that this transposon was
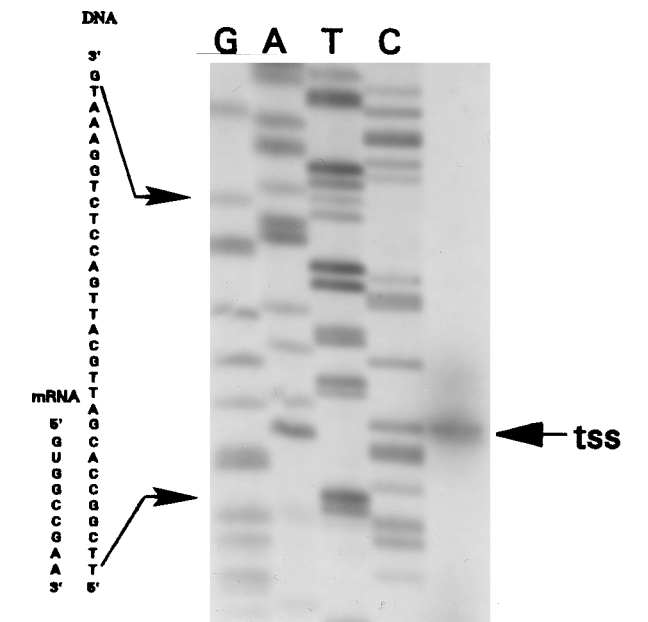
FIG. 2. Transcriptional start site (tss) for *strA-strB* as determined by primer extension. Autoradiograph shows primer extension product and sequencing reactions; the ^{32}P-labeled cDNA product was electrophoresed in parallel with G/A/T/C sequence reactions generated with the same primer. The DNA sequence and predicted mRNA sequence are shown on the left.

present on other plasmids besides those recently characterized in plant pathogenic bacteria.

Transcriptional fusions generated in the present study indicated that a strong promoter was located in a 0.4-kb *Pst*I fragment containing essentially all of *res* (pGWS6, Table 2). Furthermore, GUS activity was markedly reduced when fusion clones included *tnpR* (pGWS8, Table 2). The function of TnpR as a repressor and its negative effect on the expression of downstream genes was previously shown for Tn*3* and the related transposon γδ (16, 21, 34). In this study, GUS activity was less affected when *tnpR* was present in *trans* on pPSR2 [see FF5(pGWS6, pPSR2), Table 2]. However, GUS activity in FF5(pGWS6, pPSR2) may have been affected by copy number

TABLE 3. Correspond analysis and percent G+C in the third codon position for the transposase gene of IS*6100* and four bacterial genera

Organism or gene	Correspond analysis[a]	% G+C in third codon position[b]
IS*6100* transposase		78.0
Xanthomonas campestris[c]	0.952	80.8
Pseudomonas spp.	1.128	73.9
Mycobacterium spp.[c]	1.577	82.0
Streptomyces spp.	2.796	92.4

[a] Values presented are the D^2 statistic, which was calculated with the UWGCG program Correspond (17). The Correspond analysis compares the pattern of codon usage of the IS*6100* putative transposase gene (29) with codon frequency tables for *X. campestris* (40), *Pseudomonas* spp. (6), *Mycobacterium* spp. (40), and *Streptomyces* spp. (48). The Correspond D^2 statistic increases as patterns of codon usage become more dissimilar.

[b] Calculated for the IS*6100* putative transposase gene (29) and for the genes utilized to generate the codon frequency tables for *X. campestris*, *Pseudomonas* spp., *Mycobacterium* spp., and *Streptomyces* spp. (6, 40, 48).

[c] Codon usage tables for *Xanthomonas* and *Mycobacterium* spp. and the sequences used to compile the tables are available on request.

ACATGTTCCTTTTTCAAAATCTGATAGCGTTCAAGACGCTTTGTTTATGA　　50

AGCTGGTTGAGATACATTTCCAGAGGTCAATGCAATCGTGGCCGAAGCGC　　100
　　　　－35　　　　　　　　　　　　　　－10

CGCCTCAAACCAACGTTTGTGATACATGCTGATCGGATATGCCCGCGTCT　　150

CCAAAGCCGATGGCTCGCAGTCTCTCGACCTGCAGCACGACGCCTTGCGC　　200
　　　　　　　　　　　　　　　　　　　　　　　　　　tnpR →

GCCGCAGGTGTCAACGGGACA　　221
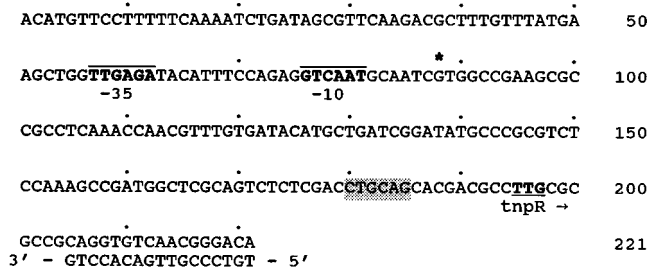3' - GTCCACAGTTGCCCTGT - 5'

FIG. 3. Nucleotide sequence of the entire recombination site (*res*) and a portion of *tnpR* from Tn*5393*a in *P. syringae* pv. syringae A2. The transcriptional start site is shown by the asterisk. A putative σ^{70} promoter is indicated (overlined bold characters). The putative translation initiation codon (TTG) of *tnpR* (position 195), as previously determined (7), is shown (underlined bold characters). The *Pst*I site at the 3' end of *res* (see Fig. 1) is shaded. The sequence of the oligonucleotide primer utilized in the primer extension reaction is indicated (complement of nucleotides 205 to 221).

differences between pGWS6 and pPSR2 and by the presence of *res* on pPSR2.

Primer extension analysis indicated that the transcriptional start site for *strA-strB* in Tn*5393*a was located within *res* at a guanine residue 106 bp upstream from the *tnpR* translational start codon. These data imply that the *strA-strB* genes in *P. syringae* are cotranscribed with *tnpR* as an operon. Putative −35 (TTGAGA) and −10 (GTCAAT) sequences, resembling *Pseudomonas* σ^{70} promoters (11), were identified within *res* seven nucleotides upstream of the transcriptional initiation site (Fig. 3). This sequence differed from a putative σ^{70} promoter sequence previously identified within *res* of Tn*5393* (7).

Analysis of transcriptional fusions in *X. campestris* pv. vesicatoria XV16 indicated that expression from the 0.4-kb *Pst*I fragment in pGWS6 was 10-fold higher than that in the control. Since GUS activity in *P. syringae* pv. syringae FF5 containing pGWS6 was 100-fold higher than in its control, the relative rates of expression from pGWS6 differed by approximately 10-fold in *P. syringae* pv. syringae and *X. campestris* pv. vesicatoria. The reduced rate of expression from pGWS6 in *X. campestris* pv. vesicatoria XV16 could be caused by a differential rate of expression from the same promoter sequence in these organisms. Recently, it was determined that the copper resistance determinant from *X. campestris* pv. vesicatoria was similar to the *cop* operon from *P. syringae* pv. tomato (47). However, these determinants had diverged at the regulatory level, as the cloned *X. campestris* resistance genes were not expressed in *P. syringae* and the cloned *cop* promoter did not function in *X. campestris* (47). Our data regarding the expression of streptomycin resistance in *Pseudomonas* and *Xanthomonas* spp. may also reflect divergence at the regulatory level.

GUS activity in *X. campestris* pv. vesicatoria XV16(pGWS32), a construct containing *res*, IS*6100*, and part of *tnpR*, was 33-fold higher than in XV16(pRG960sd). pGWS32 contains an intact copy of IS*6100*, a member of the IS*6* family (14, 29). Transcriptional activation of downstream genes by the IS*6* family occurs through hybrid promoters consisting of −35 sequences contributed by the right inverted repeat of the IS element and −10 sequences located downstream of the insertion site (1, 5, 25, 28). However, GUS activity was not observed in XV16 containing pGWS20 or pGWS21, both of which contained the right inverted repeat and upstream sequences within IS*6100* (Table 2). Thus, another sequence located further within IS*6100* functioned as a promoter in *X. campestris* pv. vesicatoria. The nature of this sequence is unknown since se-

quences similar to consensus σ^{70} promoters (20) were not observed within IS*6100*.

IS*6100* has been detected in Tn*610* from *M. fortuitum* (29), adjacent to Tn*1696* in *P. aeruginosa* (19), and on the nylon-degrading plasmid pOAD2 in *Flavobacterium* sp. (23). The presence of identical copies of IS*6100* among taxonomically distinct genera indicates that this element has been disseminated both widely and recently among gram-negative and gram-positive bacteria. Although it was once considered unlikely that conjugal transfer of plasmids could occur between gram-negative and gram-positive bacteria, many studies have demonstrated conjugation between these groups in vitro (10, 30). Evidence of recent natural transfer events comes from the close nucleotide sequence identity of antibiotic resistance genes identified in gram-positive and gram-negative genera (5, 45, 50). In all of these examples, a natural transfer event from a gram-positive donor to a gram-negative recipient was hypothesized because the nucleotide sequence and codon usage of the genes were most closely related to those of gram-positive genera. However, several lines of evidence suggest that IS*6100* was transferred from a gram-negative host to *M. fortuitum*. Data presented by Kato et al. suggest that IS*6100* is more closely related to IS*6* family elements identified in gram-negative hosts than to those originating from gram-positive bacteria (23). Our analysis of codon usage patterns and percent G+C in coding sequences, characteristics which reflect genetic origin (3, 17), further supports the origin of IS*6100* in a gram-negative host.

The expression of resistance genes encoded by transposons is an important component of their dissemination into new genera. Tn*5393*a confers Sm^r to *P. syringae* pv. syringae at 75 μg/ml, a level high enough to provide a selective advantage on plant surfaces sprayed with streptomycin (43). In *X. campestris* pv. vesicatoria, the promoter sequence located within *res* functions at a low level; however, expression of streptomycin resistance in this organism was increased to 250 μg/ml by the insertion of IS*6100* proximal to *strA-strB*. Similarly, a promoter sequence present in IS*1133* was presumed to provide a promoter for the high levels (500 to 1,000 μg/ml) of streptomycin resistance observed in *E. amylovora* (7). Thus, Tn*5393* is a versatile transposon which is capable of acquiring IS elements to increase the expression of streptomycin resistance in different bacterial genera. The wide dissemination of Tn*5393* provides further evidence of the emerging importance of phytopathogenic bacteria as reservoirs of antibiotic resistance determinants in the environment.

ACKNOWLEDGMENTS

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