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NOTE

Occurrence of the *strA-strB* Streptomycin Resistance Genes in *Pseudomonas* Species Isolated from Kiwifruit Plants

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The occurrence of *strA-strB* streptomycin-resistance genes within transposon Tn*5393* was examined *in Pseudomonas syringae* pv. *actinidiae*, *P. syringae* pv. *syringae*, and *P. marginalis*, isolated from kiwifruit plants in Korea and Japan. PCR amplification with primers specific to *strA-strB* revealed that three of the tested *Pseudomonas* species harbored these genes for a streptomycin-resistance determinant. Tn*5393*, containing *strA-strB*, was also identified with PCR primers designed to amplify parts of *tnpA*, *res*, and *tnpR*. No IS elements were detected within *tnpR*, nor were they found in the intergenic region between *tnpR* and *strA*. Nucleotide sequence analysis indicated that the *strA* sequence of *P. syringae* pv. *actinidiae* contained a single nucleotide alteration at position 593 (CAA→CGA), as compared to Tn*5393a* in *P. syringae* pv. *syringae*. This resulted in an amino acid change, from Gln to Arg.

Key words: *Pseudomonas syringae* pv. *syringae, kiwifruit, strA-strB,* Tn*5393*

Streptomycin is the first broad-spectrum antibiotic commonly used for the treatment of tuberculosis and gram-negative infections in humans. It has been also used widely to control bacterial diseases in plants. However, streptomycin resistance has recently become a widespread characteristic of both clinical and plant pathogenic bacteria (Burr *et al.*, 1993; Show *et al.*, 1993). Two genetically distinct types of resistance mechanism have been described in plant pathogenic bacteria. The first appears to be the result of a mutation in the chromosomal gene *rpsL*, which prevents streptomycin from binding to its ribosomal target. The second is mediated by the *strA-strB* genes, which encode the streptomycin-modifying enzymes, aminoglycoside-3-phosphotransferase and aminoglycoside-6-phosphotransferase. Since mutations on chromosomal genes can be transmitted only by cell division, streptomycin resistance due to *rpsL* mutation is unlikely to spread to other bacterial populations. Conversely, the *strA-strB* genes are of particular interest, as these genes are distributed among at least 21 bacterial genera isolated from humans, animals, and plants (Sundin, 2002). In plant pathogenic bacteria, these genes are generally associated with the Tn*5393*-like transposon (Chiou and Jones, 1993; Sundin, 2002).

Bacterial canker disease causes serious damage to kiwifruit plants. Therefore, it is important to control this disease in its early stages. Streptomycin has been used extensively to control this disease in both Korea and Japan. In this work, we assessed the occurrence of the Tn*5393*-like transposon, and analyzed the nucleotide polymorphisms of the *strA-strB* genes within three streptomycin-resistant *Pseudomonas* species, all of which were isolated from kiwifruit plants in both countries.

P. syringae pv. *actinidiae* Pal1 was found to be the causative agent for bacterial canker in kiwifruits isolated from Japan, and exhibited streptomycin resistance (Han *et al.*, 2003a). *P. marginalis* is an important post-harvest pathogen, capable of inducing soft rot in a wide variety of harvested fruit and vegetables (Liao, 1997). However, the involvement, if any, of this bacterium in post-harvest kiwifruit diseases has yet to be determined. *P. syringae* pv. *syringae* is a known causative agent of floral bud necrosis in kiwifruit (Balestra and Varvarro, 1997). The *P. marginalis* BJW1 and *P. syringae* pv. *syringae* KHP7 strains used in this study were isolated from kiwifruit plants in Korea.

In order to determine the genetic background of the streptomycin-resistant *Pseudomonas* strains, we performed PCR amplification with primers specifically designed to detect *strA-strB* genes, *tnpA*, and IS elements (Fig. 1). The presence of *strA-strB* genes was confirmed by primers

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designed from the 5' end of *strA*, and from the 3' end of *strB*: strAB-1, 5'-TTGAATCGAACTAATAT-3'; strAB-2, 5'-CTAGTATGACGTCTGTCG-3'. For the specific detection of the *tnpA* sequence, we used the *tnpA* primers: tnpA-F, 5'-GGCGGGATCTGCTTGTAGAG-3' corresponding to *tnpA* positions 1,193 to 1,212, and tnpA-R, 5'-CTCCGGAGATGTCTGGCTTACT-3', corresponding to *tnpA* positions 1,471 to 1,492. In order to detect IS elements which might be located upstream of *strA-strB*, a PCR primer set was designed from *tnpA* and *strA*: IS-F, 5'-ATGCCCAAGGTCTTCATCAC-3', corresponding to *tnpA* positions 2,773 to 2,792, and IS-R, 5'-TCACCACGTCGAAAAACAAA-3' corresponding to *strA* positions 65 to 84. Three *Pseudomonas* species were cultured for 24 h on peptone-sucrose broth at 30°C, and whole cell DNA was prepared, as previously described (Han *et al.*, 2003b). The PCR amplification of the target sequence was conducted in a total volume of 50 μl of the following reaction mixture: 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 200 μM (each) deoxyribonucleoside triphosphate, 25 pmole of each primer, 2 μl of the template, and 2.5 U of *Taq* DNA polymerase (Takara, Japan). PCR was performed with a GeneAmp PCR system 2400 (Perkin-Elmer, USA.), using the following protocol: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 48°C or 55°C for 30 sec, and an extension at 72°C for 30 sec, followed by an additional extension at 72°C for 5 min. The annealing temperatures were 48°C for the amplification of the *strA-strB* and *tnpA* sequences, and 55°C for the detection of IS elements. The nucleotide sequence analysis was conducted at the Macrogen Co. (Korea) using the double-stranded dideoxy sequencing method. The Basic Local Alignment Search Tool (BLAST) search system was used for sequence comparisons.

The Tn*5393* family, which encodes the *strA-strB* genes, was first discovered in streptomycin-resistant *Erwinia amylovora* (Chiou and Jones, 1993), and was subsequently found in pathogenic bacteria in plants and fish (Sundin and Bender, 1995; LAbée-Lund and Sørum, 2000). Tn*5393* contains *strA-strB* genes downstream from its transposase gene (*tnpA*), resolvase gene (*tnpR*), and central recombination site (*res*) (Fig. 1). A transcriptional fusion study, using *uidA*, indicated that a strong promoter sequence was located within the *res* site, and was expressed along with *tnpR* as one operon in *P. syringae* pv. *syringae*. However, the MICs of streptomycin to *E. amylovora* and *Xanthomonas campestris* pv. *vesicatoria* were higher than those to *P. syringae* pv. *syringae*. IS*1133* and IS*6100*, which were located within and at the end of the *tnpR* sequence, were responsible for the increased expression of *strA-strB* in *Xanthomonas campestris* pv. *vesicatoria* and *E. amylovora*, respectively (Sundin and Bender, 1995) (Fig. 1).

In order to demonstrate that our three *Pseudomonas* species contained *strA-strB* in Tn*5393*, PCR experiments were performed with primers designed to amplify a 1,640-bp region of *strA-strB*, a 300-bp region of *tnpA*, and a 949-bp region which included *res* and *tnpR*. The PCR products amplified with the three *Pseudomonas* species were identical to the size expected for Tn*5393* (Fig. 2A, B). However, no IS elements were present within *tnpR*,

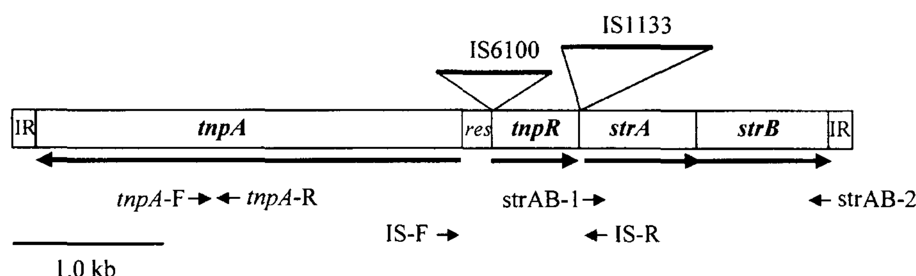

Fig. 1. Map of Tn*5393* including the sites of insertion of IS*1133* and IS*6100* in *Erwinia amylovora* and *Xanthomonas campestris*, respectively. The direction of transcription and the location of oligonucleotide primers used for PCR are indicated with arrows. IR, inverted repeat.

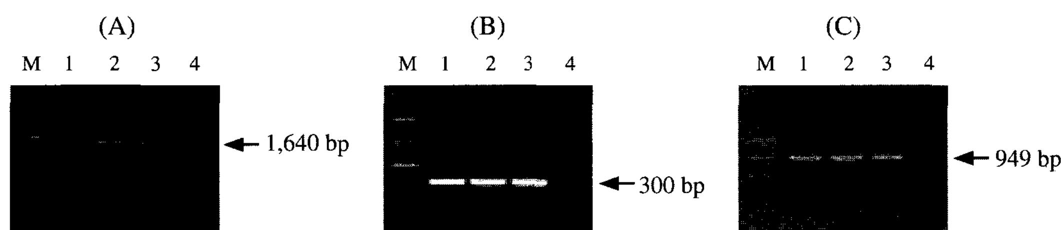

Fig. 2. PCR amplification products using *strAB* (A), *tnpA* (B), and IS primer sets (C). Lane M, 100 bp ladder (Bioneer Co.); lane 1, *Pseudomonas marginalis* BJW1; lane 2, *Pseudomonas syringae* pv. *actinidiae* Pal1; lane 3, *Pseudomonas syringae* pv. *syringae* KHP7; lane 4, negative control.

Table 1. Analysis of nucleotide polymorphisms within the *strA* streptomycin resistance genes

Bacterial strain	*strA* sequence polymorphism[a]							GenBank accession no.	Source of isolation
	1	69	80	204	467	470	593		
Erwinia amylovora CA11	T	T	T	G	A	T	A	M95402	plant
Xanthomonas campestris BV5-4a	-	-	-	-	-	-	-	U20588	plant
Pseudomonas marginalis BJW1	-	-	-	-	-	-	-	AY53314	plant (this work)
Pseudomonas syringae pv. *syringae* KHP7	-	-	-	-	-	-	-	AY53313	plant (this work)
Pseudomonas syringae pv. *actinidiae* Pal1	-	-	-	-	-	-	G	AY53312	plant (this work)
Pseudomonas syringae A2	-	-	-	-	-	-	-	M77502	soil
Pseudomonas sp. BixF6	-	-	-	-	-	-	-	AF321547	soil
Aeromonas salmonicida subsp. *Salmonicida* 1682/92	-	-	-	-	-	-	-	AF262622	animal
Corynebacterium striatum M82B	-	-	-	-	-	-	-	AF024666	animal
Pasteurella haemolytica A1	-	C	-	-	T	A	-	M83717	animal
Pasteurella multocida Pm1096	-	C	-	-	-	-	-	U57647	animal
Shigella flexneri 2731	-	-	-	C	-	-	-	AF321551	animal (stool)
Escherichia coli HM69	-	-	G	-	-	-	-	AF321550	clinical
Haemophilus ducreyi CH37	-	C	-	-	-	-	-	L23118	clinical
Pseudomonas aeruginosa MUS	A	-	-	-	-	-	-	AF024602	clinical
Salmonella enterica serovar *typhi* CT18	-	-	-	C	-	-	-	NC_003198	clinical
Yersinia pestis 16/95	-	-	-	-	-	-	-	AJ249779	clinical
Broad range plasmid RSF1010	-	-	-	C	T	A	-	M28829	all

[a]Seven of the 804 nucleotides are polymorphic

nor could they be found in the intergenic region between *tnpR* and *strA*. This is similar to the previously-described case of Tn*5393a* (Sundin and Bender, 1995) (Fig. 2C). These results suggest that the three *Pseudomonas* species collected from kiwifruit orchards in Korea and Japan do, indeed, contain the Tn*5393* family, encoding *strA-strB*. These results were consistent with the previous report that the *strA-strB* genes in plant-pathogenic bacteria were associated with Tn*5393*-like transposons (Sundin and Bender, 1996). In order to confirm our results, the PCR products of Fig. 2 were subjected to nucleotide sequence analysis. The sequences of the 300- and 949-bp fragments of *Pseudomonas* species (Figs. 2B, and 2C) were identical to the corresponding sequences contained in the Tn*5393a* of *P. syringae* pv. *syringae* (Sundin and Bender, 1995). However, while the *strB* sequences were identical to the sequence found in Tn*5393a*, the *strA* sequences exhibited one difference in *P. syringae* pv. *actinidiae* (Table 1). The *strA* sequence of *P. syringae* pv. *actinidiae* Pal1 contained a novel alteration with regard to the Tn*5393a* sequence at position 593 (CAA→CGA), which resulted in a change in the StrA amino acid, from Gln to Arg.

In a previous work, Sundin (2002) compared 19 *strA* sequences, and found that six of the 804 nucleotides were polymorphic. Six polymorphisms could be grouped by the species harboring *strA-strB,* and the location of the genes within a strain. Three of the six nucleotide-polymorphisms resulted in amino acid alterations. In this work, we found an additional *strA* nucleotide polymorphism, which resulted in an amino acid alteration. These results could be useful in epidemiological studies, for example, to track the dissemination of *strA-strB* streptomycin-resistant determinants.

The nucleotide sequences of the streptomycin-resistance genes generated in this study have been deposited in the GenBank database (accession numbers AY53312, AY53313, and AY53314).

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