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Molecular Bases of High-Level Streptomycin Resistance in *Pseudomonas marginalis* and *Pseudomonas syringae* pv. *actinidiae*

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We have collected eight high-level streptomycin-resistant strains of *Pseudomonas marginalis* and *P. syringae* pv. *actinidiae* which were isolated from kiwifruit orchards in Korea and Japan. The molecular mechanisms of resistance were investigated by the PCR, susceptibility tests, and nucleotide sequence analysis. Of the eight high-level streptomycin-resistant strains, four harbored *strA-strB* genes, which encode streptomycin-inactivating enzymes. While the three Korean strains of *P. marginalis* did not have plasmid and carried the resistant genes in the chromosomes, the Japanese strain of *P. syringae* pv. *actinidiae* had a plasmid containing *strA-strB* genes. The myomycin susceptibility test demonstrated that the high-level resistance to streptomycin of the remaining four strains is associated with mutations in the *rpsL* gene. Nucleotide sequence analyses revealed that they contain a single base-pair mutation in codon 43 of their *rpsL* gene.

Key words: streptomycin resistance, *Pseudomonas marginalis, Pseudomonas syringae* pv. *actinidiae,* kiwifruit

The aminoglycoside antibiotic streptomycin was first introduced as an effective anti-tuberculosis drug in 1944. Since the late 1950s this antibiotic has been used in plant agriculture to control populations of phytopathogenic bacteria. However, continued and varied usage of streptomycin in clinical medicine, animal husbandry and plants has been followed by the advent of streptomycin resistance in several important bacteria. From a collection of 367 isolates of *Mycobacteriun tuberculosis* from the United States in 1994, 12.3% of the isolates were resistant to streptomycin (Cooksey *et al.*, 1996) and 42% of enterococcus strains showed streptomycin resistance in Spain (del Campo *et al.*, 2000). Streptomycin-resistant strains also have been found in important plant pathogens including *Erwinia amylovora* (Chiou and Jones, 1991), *Pseudomonas syringae* pv. *papulans* (Burr *et al.*, 1988; Jones *et al.*, 1991), *P. syringae* pv. *syringae* (Sundin and Bender, 1993; Spotts and Cervantes, 1995), and *Xanthomonas campestris* pv. *vesicatoria* (Minsavage *et al.*, 1990). In recent years, streptomycin-resistant strains of *Salmonella enterica* have been reported from cattle, pigs, and humans in Denmark (Madsen *et al.*, 2000).

The mode of action of streptomycin against bacteria is through its binding to the 30S ribosomal subunit, which affects polypeptide synthesis and ultimately leads to inhibition of translation (Gale *et al.*, 1981). Bacterial resistance mechanisms to streptomycin include production of streptomycin-modifying enzymes, alterations of the ribosomal target site, and prevention of streptomycin access to the target site by permeability barriers (Amyes and Gemmell, 1992). Three types of streptomycin-modifying enzymes are frequently found in clinical bacteria; two aminoglycoside phosphotransferases, APH(6) and APH(3″) (Scholz *et al.*, 1989), and nucleotidyltransferase ANT(3″) (Madsen *et al.*, 2000). Mutations associated with streptomycin resistance have been identified in the genes encoding 16S rRNA (*rrs*) and ribosomal protein S12 (*rpsL*). Ribosomal protein S12 stabilizes the highly conserved pseudoknot structure formed by 16S rRNA (Noller, 1984). Alteration of the ribosomal subunit, which is a binding site of many aminoglycoside antibiotics, causes significant resistance to streptomycin (Mingeot-Leclercq *et al.*, 1999). Reduced uptake of antibiotics is likely to be due to membrane impermeabilization, but the underlying molecular mechanisms are largely unknown.

Bacterial canker on kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* was first described in Japan (Takikawa *et al.*, 1989) and subsequently reported in Korea (Koh *et al.*, 1994). Since bacterial canker disease causes serious damage to kiwifruit plants and destroys the orchards, it is important to control the disease at an early stage. To control of this disease, bacteriocides containing streptomycin have been used extensively in both countries

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(Nakajima *et al.*, 1995). In this work, we collected bacterial strains with high resistance to streptomycin from kiwifruit plants showing canker symptoms in Korea and compared the resistance mechanism of Korean strains with that of Japanese strains.

Materials and Methods

Source and identification of bacterial strains

For the isolation of Korean strains, small pieces of twigs of kiwifruit plants with bacterial canker symptoms were collected from four different cultivation areas from January to April, 1998. Samples were crushed in sterile mortars containing sterile distilled water. The suspensions were diluted and streaked on pepton-sucrose agar (PSA) and incubated for 24~48 h at 27°C. Bacterial colonies were selected and streaked on nutrient agar medium amended with 50 μg/ml of streptomycin to confirm their identity and then stored in glycerol stocks. Each of the isolates was characterized and identified by following the standard methods (Hildebrand *et al.*, 1988). Oxidase negative isolates showing fluorescence on King's B medium were further characterized for gelatine liquefaction, esculin hydrolysis, tyrosinase activity, and utilization of tartrates (GATTa test; Latorre and Jones, 1979). For the pathogenicity tests, the kiwifruit plants were inoculated with cell suspensions of test strains by spraying or puncturing methods (Takikawa *et al.*, 1989). The Japanese strains of *P. syringae* pv. *actinidiae* were provided by Dr. Serizawa (Shizuoka Citrus Experiment Station, Japan) and Dr. Nakajima (Ibaraki University, Japan). Among the strains from Korea and Japan, eight strains grown on media amended with streptomycin at 1,000 μg/ml were selected for further studies. The sources of the bacterial strains used in this study are listed in Table 1.

Preparation of DNA for PCR

One hundred μl of bacterial cultures grown on PS media for 24 h at 30°C were centrifuged to remove the culture medium. The cell pellets were washed twice with sterile water and resuspended in 100 μl of sterile distilled water containing 1% (wt/vol) Chelex 100 resin (Bio-Rad Laboratories, U.S.A.) The cell suspensions were boiled for 10 min and the lysates were then centrifuged briefly. The resulting supernatant was used for PCR assay.

PCR Primers

The 16S rRNA gene of Korean strain BJW1 was amplified for direct sequencing. Template DNA for sequencing was prepared by amplification of the 16S rRNA gene by using bacterial 16S rDNA universal primers (Lane, 1991): 16S-F, 5'-TNANACATGCAAGTCGAICG-3' corresponding to positions 49 to 68 of *Escherichia coli* 16S rRNA (Brosius *et al.*, 1978); 16S-R, 5'-GGYTACCTTGTTACGACTT-3' corresponding to the complement of positions 1510 to 1492. For the detection of the *strA* and the *strB* genes, primers designed previously from the sequence of each gene were used (Palmer *et al.*, 1997): strA-F, 5'-TGAATCGCATTCTGACTGGTT-3'; strA-R, 5'-AAGTTGCTGCCCCATTGA-3', and strB-F, 5'-GGAACTGCGTGGGCTACA-3'; strB-R, 5'-GCTAGATCGCGTTGCTCCTCT-3'. The tandem *strA-strB* structure was verified by primers designed in this work from the 3' region of the *strA* and 5' region of the *strB*: strAB-F, 5'-ATCGGTTGATCAATGTCCGT-3'; strAB-R, 5'-AAACAAAGCTGCAA- AGCGAT-3'. The oligonucleotide primers used to amplify *rpsL* were designed from the nucleotide sequence of *Pseudomonas aeruginosa* PA01 (Stover *et al.*, 2000): rps1, 5'-ACGTGCCTGCGCTGCAA-3' and rps2, 5'-GAACGACCCTGCTTACG-3'. This primer set was used to amplify an 283-bp internal segment of the *rpsL* from *Pseudomonas* strains.

PCR Amplification

The PCR was carried out 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 Shuzo, Japan). The PCR was performed in a GeneAmp PCR system 2400 (Perkin-Elmer, U.S.A.) using the following protocol: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 65°C or 55 for 30 s, and an

Table 1. Bacterial strains used in this study and inhibitory concentration of streptomycin and myomycin.

Bacteria	Stain	Geographic origin	Inhibitory concn (μg/ml)	
			streptomycin	myomycin
Pseudomonas marginalis	BJW1	Bosung, Korea	>2,000	10
	HMY3	Haenam, Korea	>2,000	10
	HOM5	Haenam, Korea	>2,000	50
	NSS2	Namhae, Korea	>4,000	>500
Pseudomonas syringae	PaI1	Kanagawa, Japan	>1,000	50
pv. *actinidiae*	PaI2	Kanagawa, Japan	>1,000	>500
	PaK2	Kanagawa, Japan	>2,000	>500
	PaS33	Shizuoka, Japan	>2,000	>500

extension at 72°C for 30 s, followed by an additional extension at 72°C for 5 min. The annealing temperatures were 65°C for the 16S rDNA amplification and 55°C for detecting the *strA, strB* and *rpsL* genes.

DNA Sequencing

DNA sequencing was performed with an ABI Prism 377XL DNA sequencer (Applied Biosystems, U.S.A.) following the manufacturer's guide.

Determination of inhibitory concentration of antibiotics

All eight strains were assayed for resistance to streptomycin and myomycin. After the strains were grown overnight in PS broth at 30°C with shaking, the cultures were diluted with sterile distilled water. The diluted bacterial suspension was inoculated into 5 μl of PS medium amended with streptomycin at 100, 250, 500, 1,000, 2,000, and 4,000 μg/ml or myomycin at 10, 50, 100, 200, and 500 μg/ml. Bacterial growth was evaluated after 40 h cultivation at 30°C.

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed to determine the plasmid profiles of *Pseudomonas* spp. Plasmids were separated by electrophoresis in 0.5×TBE (Tris-borate-EDTA) buffer at 14°C for 8 h using the CHEF-DR system (Bio-Rad Laboratories, U.S.A.) with a pulse time of 0.1 to 0.6 s and a field strength of 6 V/cm. A 0.65% of pulsed-field grade agarose gel was used for electrophoresis. After PFGE, the gel was stained with ethidium bromide, and DNA bands were visualized with a UV transilluminator.

Southern blot analysis

After electrophoresis, the DNA was transferred to positively charged nylon membrane (Roche Molecular Biochemicals, Germany) by the standard capillary method (Ausubel *et al.*, 1999). Southern hybridization was performed using a non-radioactive labeling and detection system (Roche Molecular Biochemicals, U.S.A.). The 860-bp PCR products amplified with strAB-F and strAB-R primer were labeled with DIG-11-dUTP according to the random primed labeling technique. A DIG-labeled probe was used for hybridization to membrane blotted with DNA at 68°C according to the manufacturer's specification in standard hybridization buffer. The probe were immunodetected with anti-digoxigenin fragments conjugated to alkaline phosphatase, and then visualized with a chemiluminescence substrate.

Nucleotide sequence accession number

The 16S rDNA sequence of the Korean strain of *P. marginalis* BJW1 has been submitted to GenBank with the accession number AY071916. The partial sequences of *rpsL* genes from the strains of *P. marginalis* and *P. syringae* pv. *actinidiae* have been given accession numbers AY247825 to AY247831.

Results and Discussion

Identification of streptomycin resistance strains

We collected *Pseudomonas* spp. from the affected parts of kiwifruit plants in Korea. Several kinds of *Pseudomonas* spp. including *P. syringae* pv. *actinidiae*, a causative agent of bacterial cankers in kiwifruit, were isolated. However, streptomycin-resistant strains were not found in *P. syringae* pv. *actinidiae* isolated from kiwifruit plants showing canker symptoms in Korea (data not shown). The strains showing high-level streptomycin resistance were identified as *P. marginalis* in physiological and biochemical tests. They were positive in the following tests: levan production, presence of catalase and arginine dihydrolase, and potato rot. They utilized mannitol, sorbitol, trehalose and sucrose. No growth was observed on geraniol, benzoate, cellobiose, D-arabinose and D-aspartate. All strains gave negative reactions in nitrate reduction and growth at 41°C. To confirm the results of biochemical identification tests, the 16S rDNA sequence was amplified from one of the *P. marginalis* strains, BJW1, and the PCR product was subject to direct sequencing. The 16S rDNA sequence of Korean strain BJW1 revealed 99.6% similarity with *P. marginalis*; that was the closest relationship on the 16S rRNA sequence database. All streptomycin-resistant strains isolated from Korea were identified as *P. marginalis*.

P. marginalis is an important postharvest pathogen capable of causing soft rot in a wide variety of harvested fruit and vegetables (Liao *et al.*, 1997). Although *P. marginalis* was isolated together with *P. syringae* pv. *actinidiae* from kiwifruit plants showing canker symptoms, the involvement of this bacterium in postharvest disease of kiwifruit has not been clarified yet. The pathogenicity tests showed that all Korean strains of *P. marginalis* tested did not form pathogenic symptoms on kiwifruit plants. All of 120 Korean strains of *P. syringae* pv. *actinidiae* examined were sensitive to streptomycin. In contrast, the occurrence of streptomycin-resistant strains of *P. syringae* pv. *actinidiae* has been reported in Japan since 1984, and all of the streptomycin-resistant strains had one or two plasmids carrying the streptomycin resistance genes (Nakajima *et al.*, 1995). Four Korean strains of *P. marginalis* and four Japanese strains of *P. syringae* pv. *actinidiae* were used for further studies to determine the molecular mechanisms for high-level resistance to streptomycin in field strains isolated from kiwifruit orchards.

Detection of the strA-strB genes

Although low levels of resistance to streptomycin have been observed in many plant pathogenic bacteria, this antibiotic has been used continuously in control programs for plant disease. However, the recent emergence of high-

level streptomycin-resistant bacteria has led to renewed interest in the resistance problem. The levels of streptomycin resistance were readily distinguished by testing strains on streptomycin-amended media. Analysis of streptomycin resistance in plant pathogenic bacteria demonstrated the involvement of two distinct genetic determinants: the tandem *strA-strB* genes (Chiou and Jones, 1993) and a point mutation in the *rpsL* gene (Chiou and Jones, 1995). The linked *strA-strB* streptomycin-resistant genes are notable because these genes are distributed among commensal and pathogenic bacteria isolated from humans, animals, and plants (Sundin and Bender, 1996).

In order to determine the genetic background for the high-level streptomycin resistant isolates, the strains were analyzed by PCR for the presence of *strA-strB* with three sets of primers which amplify a 301-bp region of the *strA* gene, a 303-bp region of the *strB* gene, and a 860-bp of the internal region of the linked *strA-strB*. Of the eight strains tested, four from Korea and four from Japan, four *Pseudomonas* strains contained *strA-strB* genes (Fig. 1). These genes encode the streptomycin-modifying enzymes aminoglycoside-3-phosphotransferase [APH(3")-Ib] and aminoglycoside-6-phosphotransferase [APH(6)-Id] (Scholz *et al.*, 1989).

The acquisition of genes for inactivating streptomycin confers lower levels of resistance in plant-associated bacteria (Chiou and Jones, 1995). The *strA-strB* genes in plant-pathogenic bacteria are usually encoded by Tn5393, a 6.7-kb transposable element of the Tn*3*-family (Chiou and Jones, 1993). However, although many plant-pathogenic bacteria carried *strA-strB* genes on transposon Tn*5393*, the MICs of streptomycin differed widely among

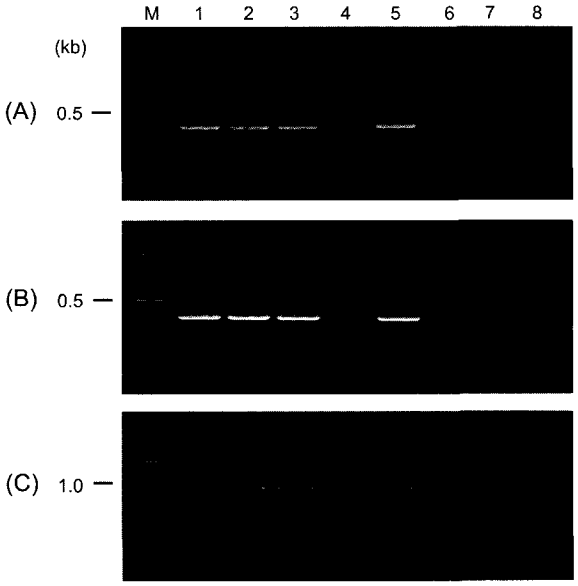

Fig. 1. PCR amplification products using strA (A), strB (B), and strAB (C) primer sets. Lane M, 100 bp ladder (Bioneer); lanes 1 to 4, *Pseudomonas marginalis* strains (BJW1, HMY3, HOM5, NSS2); lanes 5 to 8, *Pseudomonas syringae* pv. *actinidiae* strains (Pal1, Pal2, PaK2, PaS33).

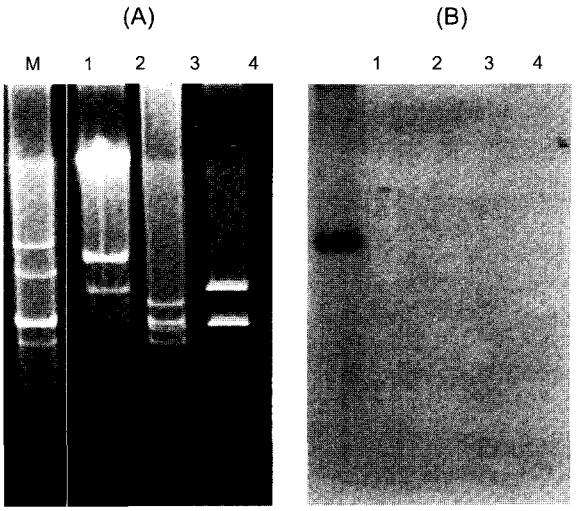

Fig. 2. Plasmid profiles of streptomycin resistance *Pseudomonas syringae* pv. *actinidiae* strains isolated in Japan (A) and hybridization with DIG-labeled *strA-strB* probe (B). Pulsed-field gel electrophoresis of the total DNA proceeded for 8 h at 6V in a 0.65% agarose gel. Lanes 1 to 4, *P. syringae* pv. *actinidiae* strains (Pal1, Pal2, PaK2, PaS33); M, Markers.

them. The insertion sequences IS*1133* or IS*6100* located upstream of *strA-strB* provided strong promoters to express the genes (Sundin and Bender, 1995). In *X. campestris*, expression of streptomycin resistance was increased by the insertion of IS*6100* proximal to *strA-strB*. Similarly, IS*1133* provided a promoter for the high levels of streptomycin resistance in *E. amylovora* (Chiou and Jones, 1993). The high-level resistance of *P. marginalis* and *P. syringae* pv. *actinidiae* to streptomycin indicates a possible involvement of insertion sequences in an expression of streptomycin resistance determinants.

Location of strA-strB genes in the bacteria

The *strA-strB* genes are responsible for the streptomycin resistance in at least 17 genera of environmental and clinical bacteria found worldwide. In bacterial isolates from humans and animals, *strA-strB* are encoded on broad-host-range nonconjugative plasmids (Sundin and Bender, 1996). In bacterial isolates from plants, however, the *strA-strB* genes are usually carried on conjugative plasmids (Sundin and Bender, 1993; Chiou and Jones, 1995; Huang and Burr, 1999). PFGE results showed that Korean strains of *P. marginalis* did not have plasmids (data not shown). In contrast, *P. syringae* pv. *actinidiae* isolated from Japan harbored two to four plasmids with a diverse size range (Fig. 2). Southern blot analyses of total DNA from *P. marginalis* and *P. syringae* pv. *actinidiae* were performed using a DIG-labeled 860-bp DNA fragment as a probe. The results indicated that while the *strA-strB* genes of *P. marginalis* reside on chromosomes, the same streptomycin-resistant determinants of *P. syringae* pv. *actinidiae* are carried in plasmid (Fig. 2 and 3).

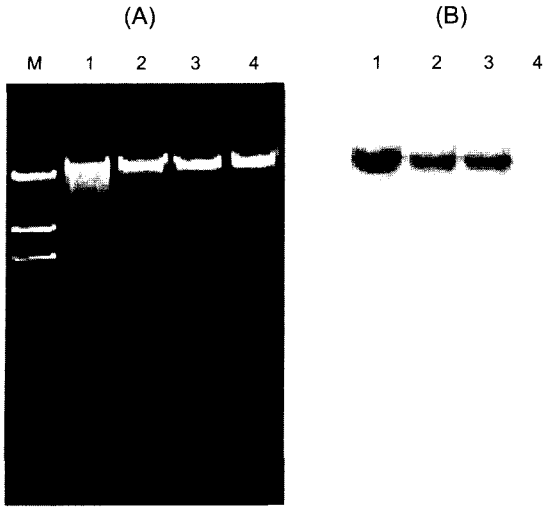

Fig. 3. Agarose gel electrophoresis (A) and Southern blot analysis (B) of *Pseudomonas marginalis* isolated in Korea. The chromosomal DNA was hybridized with DIG-labeled PCR fragments amplified from *strA-strB* genes. Lanes 1 to 4, *P. marginalis* strains (BJW1, HMY3, HOM5, NSS2). Lane M, λ DNA digested with Hind III.

Mutations in the target site of streptomycin

The second mechanism of streptomycin resistance is attributed to a chromosomal mutation that results in the alteration of the target sites for binding streptomycin on bacterial ribosomes. Molecular analyses of the bacterial strains with high-level resistance to streptomycin indicated that the resistance arises from mutations in the *rpsL* gene or in the 16S rRNA gene (Finken *et al.*, 1993; Melancon *et al.*, 1988). In a previous work, *E. coli* strains having a mutation in the *rpsL* gene, but not strains carrying *strA-strB*, showed resistance to myomycin (Davis *et al.*, 1988). The high-level resistance to streptomycin in field strains of *E. amylovora* was also due to mutations in the *rpsL* gene (Chiou and Jones, 1995). All 102 high-level streptomycin-resistant strains of *E. amylovora* contained a single base-pair mutation in codon 43 of their *rpsL* gene that resulted in an amino acid substitution in ribosomal protein S12. Interestingly, all high-level resistance strains also grew on media containing myomycin at 512 μg/μl. Because high-level resistant strains are insensitive to myomycin, this antibiotic has been used for determining the streptomycin-resistant mechanism (Chiou and Jones, 1995). Testing of the strains on myomycin-amended media revealed that four strains, one Korean strain and three Japanese strains, were insensitive to myomycin and did not carry the *strA-strB* resistance gene (Table 1). This result suggests that mutations in a ribosomal protein S12 gene *rpsL* resulted in high-level streptomycin resistance in four *Pseudomonas* strains. Then, the nucleotide sequences of the 283-bp PCR amplified fragments from *Pseudomonas* strains were sequenced and compared. Alignment of the nucleotide sequences from sensitive and resistant strains revealed that the amino acid changes at codon 43 were identified in four resistant strains. They harbored a point mutation at position 128 of the open reading frame which changes codon 43 for lysine (AAA) to arginine (AGA).

There is little doubt that the intensive use of streptomycin in agriculture has contributed to the emerging problem of high-level streptomycin resistance. Although we could not find a streptomycin-resistant strain of *P. syringae* pv. *actinidiae* in Korea, the existence of resistance genes in the strains of *P. marginalis* is meaningful because these commensal bacteria can serve as reservoirs of resistance genes. In this work, we showed that the high-level resistance to streptomycin is due to the gene for inactivating enzyme of streptomycin and mutations of *rpsL* gene in *Pseudomonas* spp. isolated from kiwifruit orchards in Korea and Japan.

Bacterial conjugation and transposition of transposable elements are important means in the spread of antibiotic resistance determinants between members of the same and sometimes taxonomically unrelated species. Since the *strA-strB* genes can be transferred from phylloplane bacteria such as *P. marginalis* to plant pathogenic bacteria, additional studies demonstrating the transfer mechanism of the antibiotic resistance are needed. To avoid streptomycin resistance in plant pathogenic bacteria, a prudent use guideline and more effective control programs should be developed in the future.

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References

Amyes, S.G.B. and C.G. Gemmell. 1992. Antibiotic resistance in bacteria. *J. Med. Microbiol.* 36, 4-29.

Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1999. Current protocols in molecular biology. John Wiley & Sons, Inc., New York, U.S.A.

Brosius, J., J.L. Palmer, H.P. Kennedy, and H.F. Noller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 75, 4801-4805.

Burr, T.J., J.L. Norelli, B. Katz, W.F. Wilcox, and S.A. Hoying. 1988. Streptomycin resistance of *Pseudomonas syringae* pv. *papulans* in apple orchards and its association with a conjugative plasmid. *Phytopathology* 78, 410-413.

Chiou, C.-S. and A.L. Jones. 1991. The analysis of plasmid-mediated streptomycin resistance in *Erwinia amylovora*. *Phytopathology* 81, 710-714.

Chiou, C.-S. and A.L. Jones. 1993. Nucleotide sequence analysis of a transposon (Tn*5393*) carrying streptomycin resistance genes in *Erwinia amylovora* and other gram-negative bacteria. *J.*

Bacteriol. 175, 732-740.

Chiou, C.-S. and A.L. Jones. 1995. Molecular analysis of high-level streptomycin resistance in *Erwinia amylovora*. *Phytopathology* 85, 324-328.

Cooksey, R.C., G.P. Morlock, A. McQueen, S.E. Glickman, and J.T. Crawford. 1996. Characterization of streptomycin resistance mechanism among *Mycobacterium tuberculosis* isolates from patients in New York city. *Antimicrob. Agents Chemother.* 40, 1186-1188.

Davis, J., M. Cannon, and M.B. Mauer. 1988. Myomycin: Mode of action and mechanism of resistance. *J. Antibiot.* 41, 366-372.

Del Campo, R., C. Tenorio, C. Rubio, J. Castillo, C. Torres, and R. Gomez-Lus. 2000. Aminoglycoside-modifying enzymes in high-level streptomycin and gentamicin resistant *Enterococcus* spp. in Spain. *Int. J. Antimicrob. Agents* 15, 221-226.

Finken, M., P. Kirschmer, A. Meier, A. Wrede, and E.C. Bottger. 1993. Molecular basis of streptomycin resistance in *Mycobacterium tuberculosis*: Alterations of the ribosomal protein S12 gene and point mutations within a functional 16S ribosomal RNA pseudoknot. *Mol. Microbiol.* 9, 1239-1246.

Gale, E.F., E. Cundliffe, P.E. Reynolds, M.H. Richmond, and M.J. Waring. 1981. The molecular basis of antibiotic action. John Wiley & Sons, Inc., New York.

Hildebrand, D.C., M.N. Schroth, and D.C. Sands. 1988. *Pseudomonas*. p. 60-80. *In* N.W. Schaad (ed) Laboratory guide for identification of plant pathogenic bacteria, 2nd ed. APS press, St. Paul.

Huang, T.-C. and T.J. Burr. 1999. Characterization of plasmids that encode streptomycin-resistance in bacterial epiphytes of apple. *J. Appl. Microbiol.* 86, 741-751.

Jones, A.L., J.L. Norelli, and G.R. Ehret. 1991. Detection of streptomycin-resistant *Pseudomonas syringae* pv. *papulans* in Michigan apple orchards. *Plant Dis.* 75, 529-531.

Koh, Y.J., B.J. Cha, H.J. Chung, and D.H. Lee. 1994. Outbreak and spread of bacterial canker in kiwifruit. *Kor. J. Plant Pathol.* 10, 68-72.

Lane, D.J. 1991. 16S/23S rRNA sequencing, p115-175. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Ltd., London, U.K.

Latorre, B.A. and A.L. Jones. 1979. *Pseudomonas morsprunorum*, the cause of bacterial canker of sour cherry in Michigan, and its epiphytic association with *P. syringae*. *Phytopathology* 69, 335-339.

Liao, C.H., D.E. McCallus, W.F. Fett, and Y. Kang. 1997. Identification of gene loci controlling pectate lyase production and soft-rot pathogenicity in *Pseudomonas marginalis*. *Can. J. Microbiol.* 43, 425-431.

Madsen, L., F.M. Aarestrup, and J.E. Olsen. 2000. Characterisation of streptomycin resistance determinants in Danish isolates of *Salmonella thyphimurium*. *Ver. Microbiol.* 75, 73-82.

Melancon, P., C. Lemieux, and L. Brakier-Gingras. 1988. A mutation in the 530 loop of *Escherichia coli* 16S ribosomal RNA causes resistance to streptomycin. *Nucleic Acids Res.* 16, 9631-9639.

Mingeot-Leclercq, M., Y. Glupczynski, and P.M. Tulkens. 1999. Aminoglycoside: activity and resistance. *Antimicrob. Agents Chemother.* 43, 727-737.

Minsavage, G.V., B.I. Canteros, and R.E. Stall. 1990. Plasmid-mediated resistance to streptomycin in *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* 80, 719-723.

Nakajima, M., S. Yamashita, Y. Takikawa, S. Tsuyumu, T. Hibi, and M. Goto. 1995. Similarity of streptomycin resistance gene(s) in *Pseudomonas syringae* pv. *actinidiae* with *strA* and *strB* of plasmid RSF1010. *Ann. Phytopathol. Soc. Jpn.* 61, 489-492.

Noller, H.F. 1984. Structure of ribosomal RNA. *Annu. Rev. Biochem.* 53, 119-162.

Palmer, E.L., B.L. Teviotdale, and A.L. Jones. 1997. A relative of the broad-host range plasmid RSF1010 detected in *Erwinia amylovora*. *Appl. Environ. Microbiol.* 63, 4604-4607.

Scholz, P., V. Haring, B. Wittmann-Liebold, K. Ashman, M. Bagdasarian, and E. Scherzinger. 1989. Complete nucleotide sequence and gene organization of the broad-host range plasmid RSF1010. *Gene* 75, 271-288.

Spotts, R.A. and L.A. Cervantes. 1995. Copper, oxytetracycline, and streptomycin resistance of *Pseudomonas syringae* pv. *syringae* strains from pear orchards in Oregon and Washington. *Plant Dis.* 79, 1132-1135.

Stover, C.K., X.Q. Pham, A.L. Erwin, S.D. Mizoguchi, P. Warrener, M.J. Hickey, F.S. Brinkman, W.O. Hufnagle, D.J. Kowalik, M. Lagrou, R.L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L.L. Brody, S.N. Coulter, K.R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G.K. Wong, Z. Wu, I.T. Paulsen, J. Reizer, M.H. Saier, R.E. Hancock, S. Lory, and M.V. Olson. 2000. Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. Nature 406(6977), 947-948.

Sundin, G.W. and C.L. Bender. 1993. Ecological and genetic analysis of copper and streptomycin resistance in *Pseudomonas syringae* pv. *syringae*. *Appl. Environ. Microbiol.* 59, 1018-1024.

Sundin, G.W. and C.L. Bender. 1995. Expression of the *strA-strB* streptomycin resistance genes in *Pseudomonas syringae* and *Xanthomonas campestris* and characterization of IS*6100* in *X. campestris*. *Appl. Environ. Microbiol.* 61, 2891-2897.

Sundin, G.W. and C.L. Bender. 1996. Dissemination of the *strA-strB* streptomycin-resistance genes among commensal and pathogenic bacteria from humans, animals and plants. *Mol. Ecol.* 5, 133-43.

Takikawa, Y., S. Serizawa, T. Ichikawa, S. Tsuyumu, and M. Goto. 1989. *Pseudomonas syringae* pv. *actinidiae* pv. nov.: The causal bacterium of canker of kiwifruit in Japan. *Ann. Phytopathol. Soc. Jpn.* 55, 437-444.