Molecular Analysis of High-Level Streptomycin Resistance in *Erwinia amylovora*

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ABSTRACT

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Streptomycin-resistant and -sensitive strains of *Erwinia amylovora* from New Zealand and five states in the United States were compared on streptomycin- and myomycin-amended media. Strains with high resistance to streptomycin (HR strains) were insensitive to myomycin, an antibiotic resembling streptomycin in its mode of action, whereas strains with medium resistance (MR) or sensitivity (S) to streptomycin were sensitive to myomycin. No mutations were found in the 16S rRNA gene of nine HR strains of *E. amylovora* that could account for streptomycin resistance. Nucleotide sequence and allele-specific amplification-polymerase chain reaction analyses were used to examine S, MR, and HR strains for mutations in the *rpsL* gene. All 102 HR 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. Codon 43, which encodes lysine in S and MR strains, was converted to a codon for arginine in 96 HR strains, a codon for asparagine in three HR strains, and a codon for threonine in three HR strains. In gene complementation studies, sensitivity to streptomycin and myomycin was restored to *E. amylovora* and *Escherichia coli* HR strains with a plasmid carrying the wild-type *E. amylovora rpsL* gene. Conversely, resistance to streptomycin and insensitivity to myomycin in *E. amylovora* and *E. coli* S strains was restored only when the plasmid carried a mutant *rpsL* gene with the lysine-to-arginine substitution in codon 43. We conclude that mutations in a single codon of ribosomal protein S12 gene *rpsL* have resulted in high-level streptomycin resistance in *E. amylovora*. Two genetic bases of streptomycin resistance have now been identified in *E. amylovora*. These results indicate that the genetic mechanism determines the level of streptomycin resistance and the expression of myomycin resistance and that the presence or relative importance of the two mechanisms differs among various geographic regions.

In North America, streptomycin has played an important role in control programs for fire blight of apple and pear, caused by *Erwinia amylovora*, for nearly 40 yr. Although streptomycin-resistant *E. amylovora* became a serious problem in the western United States in the early 1970s (7,18,26), streptomycin continues to play a valuable role in control programs for fire blight in the eastern United States, where monitoring of orchards during the mid-1970s failed to detect resistant strains (2,28). The recent emergence of streptomycin-resistant *E. amylovora* in Michigan has led to renewed interest in the resistance problem (4,16). Understanding the nature of streptomycin resistance in *E. amylovora* could lead to the design of better methods of detecting streptomycin-resistant bacteria and facilitate the development of anti-resistance management programs.

Resistance mechanisms used by bacteria to cope with streptomycin include alterations of the ribosomal target site, production of streptomycin-modifying enzymes, and prevention of streptomycin access to the target site (1). Molecular analyses of strains of *Escherichia coli, Mycobacterium tuberculosis,* and *Haemophilus influenzae* with high levels of resistance to streptomycin indicate that resistance can arise from mutations in the *rpsL* gene, which alters ribosomal protein S12, or in the 16S rRNA gene; both mutations reduce the sensitivity of the ribosome to streptomycin (10,17,20,26). Streptomycin resistance in *E. amylovora* from 12 Michigan apple orchards was mediated by aminoglycoside-modifying enzymes encoded by *strA* and *strB*, and both genes were carried by the transferable plasmid pEa34 and transposon

Tn*5393* (5,6,16). In addition, a streptomycin-resistance mechanism unrelated to *strA* and *strB* was detected in certain strains of *E. amylovora* isolated from three Michigan orchards (16). These strains were resistant to higher concentrations of streptomycin than were strains with *strA* and *strB*. Streptomycin resistance in *E. amylovora* in California likely arose from a mutation in the chromosome, because resistance was not plasmid associated or enzyme mediated and the strains exhibited high levels of resistance to streptomycin (26).

The objective of this study was to determine the molecular basis for high-level resistance to streptomycin in field strains of *E. amylovora*. Nucleotide sequence analysis of the *rpsL* and 16S rRNA genes from streptomycin-sensitive and -resistant strains of *E. amylovora* suggested that mutations in the *rpsL* gene were responsible for the high level of resistance. A polymerase chain reaction (PCR)-based method was developed for detecting the single-base mutation in strains of *E. amylovora* from different geographic regions. Finally, gene complementation studies were conducted to demonstrate that the mutations were responsible for resistance to streptomycin.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains of *E. amylovora* used in this study were provided under permit by M. N. Schroth, Plant Pathology Program, University of California, Berkeley; K. Mohan, University of Idaho, Parma Research and Extension Center, Parma; J. E. Loper and V. O. Stockwell, USDA-ARS, Horticulture Crops Research Lab, Corvallis, OR; and J. L. Vanneste, Hort Research, Ruakura Research Center, Hamilton, New Zealand, or were isolated from apple orchards in Michigan (4,16). The California strains were isolated from pear orchards in the mid-1970s (26); Idaho strains were isolated by K. Mohan from various hosts between 1991 and 1993; Washington strains were from a state-wide survey of pear orchards in 1988 (15); Oregon strains were from a state-wide survey of pear orchards in 1992 and 1993 by V. O. Stockwell; and New Zealand strains were isolated from the Hawkes Bay region in 1991 (29). *E. coli* strains HB101 and JM109 and plasmid pGEM3Zf(+) were purchased from Promega Corp., Madison, WI. Plasmid pMMB66HE (12) was provided by M. Bagdasarian, Michigan State University, East Lansing.

Cloning and sequencing the *rpsL* gene. The *rpsL* gene in *E. amylovora* was amplified by PCR (23). Two primers, AJ44, 5′-GAAGCAAAAGCTAAAACCAGGAGCT, and AJ45, 5′-GGAATTCGGCCTTACTTAACGGAGA, were designed based on the *rpsL* gene sequences for *E. coli* and *Salmonella typhimurium* (GenBank accession numbers J01688 and M68548, respectively). Reverse primer AJ45 contained an *Eco*RI site to facilitate cloning of the amplification product. The 100-μl PCR mixture contained 10^5 bacterial cells, 0.5 μM each of primers AJ44 and AJ45, 100 μM each of the four deoxynucleotides, 2 units of *Taq* polymerase (Gibco BRL, Grand Island, NY), 1.5 mM $MgCl_2$, and 1× *Taq* reaction buffer (Gibco BRL). Amplifications were performed in a model PTC-150-16 minicycler (MJ Research, Inc., Watertown, MA). The initial reaction cycle was performed at 94 C for 2 min, 55 C for 1 min, 72 C for 1 min; 29 cycles were performed at 92 C for 45 s, 55 C for 45 s, and 72 C for 1 min; and final extension was at 72 C for 4 min. Amplification products were analyzed by agarose gel electrophoresis (24), and the expected 434-bp PCR product was ligated into the *Hin*dII-*Eco*RI sites of pGEM3Zf(+), which was used to transform *E. coli* host cells. Clones containing inserts were sequenced by the dideoxy chain-termination method (25) with primers SP6 and T7 (Promega).

Allele-specific amplification-PCR (ASA-PCR) analysis. Two rounds of PCR were performed for each strain. First, PCR was performed as described above, except reverse primer AJ42 replaced AJ45, to amplify a 514-bp fragment containing the entire *rpsL* gene of *E. amylovora*. Primer AJ42 was located 80-bp upstream from primer AJ45. Second, the PCR-amplified product was diluted 100-fold in water, and 1-μl aliquots were subjected to ASA-PCR in 50 μl of reaction mixture (23). Two sets of ASA-PCR primers, AJ42-AJ52 (5′CGTGGCATGGAAATACTCCG, 5′-CACGACTACCCCTAG) and AJ42-AJ53 (5′CGTGGCATGGAAATACTCCG, 5′-CACGACTACCCCTAA) were used to selectively amplify *rpsL* genes with AGN and AAN nucleotides, respectively, at codon 43. The ASA-PCR mixture contained the same concentration of primers, deoxynucleotides, $MgCl_2$, reaction buffer, and *Taq* polymerase as described for the initial PCR. Ten, 15, 20, and 30 cycles of PCR were performed at 92 C for 45 s, 40 C for 45 s, and 72 C for 1 min, and amplification products were analyzed by electrophoresis in 2% agarose gels (24).

Determination of minimal inhibitory concentration (MIC). After the strains were grown overnight in Luria-Bertani (LB) broth at 25 C with shaking, the cultures were diluted 100-fold with sterile distilled water. Diluted bacterial suspensions were spotted in 3-μl drops onto duplicate plates of LB medium amended in a geometric series with streptomycin sulfate (Sigma Chemical Co., St. Louis) at 8–4,096 mg/L or myomycin B sulfate (Parke-Davis, Ann Arbor, MI) at 8–512 mg/L. Growth of *E. coli* and *E. amylovora* was evaluated after 20-h incubation at 37 C and 40-h incubation at 30 C, respectively. The MIC was the lowest concentration of antibiotic that inhibited bacterial growth in the spots.

Complementation of *rpsL* genes. Plasmids (pGEM3Zf(+)::*rpsL*) containing DNA cloned from streptomycin-sensitive *E. amylovora* strain EL01 or from streptomycin-resistant strains S5, OR2, and OR4 were digested with *Hin*dIII and *Eco*RI, and each of the 451-bp *Hin*dIII-*Eco*RI fragments was ligated into expression vector pMMB66HE (12). Plasmids were introduced into *E. coli* as described by Nishimura et al (21) and into *E. amylovora* by electroporation (19). Four colonies per transformant were randomly selected, and their MICs to streptomycin and myomycin were determined as described above, except that the medium was supplemented with 0, 0.05, or 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) (12).

Partial sequencing of the 16S rRNA gene. DNA between nucleotides 368 and 984 (*E. coli* numbering) of the 16S rRNA gene for 10 strains of *E. amylovora* was amplified with forward primer AJ41, 5′-GCACAATGGGCGCAAGCCTG, and reverse primer AJ40, 5′-GGTAAGGTTCTTCGCGTTGC, and the reaction

Fig. 1. Nucleotide sequence of the *rpsL* gene from streptomycin-sensitive strain EL01 of *Erwinia amylovora* (GenBank accession number L36465). The deduced amino acid sequence is indicated below the second nucleotide of each codon. The nucleotides and amino acid at codon 43 are shown in bold. The nucleotide and deduced amino acid sequences for 26 additional strains of *E. amylovora* were determined (Table 1). Except for changes at codon 43, the base and amino acid alterations in the strains (strain designation in parentheses) are indicated above the nucleotide and below the amino acid sequences, respectively. The bases corresponding to primers AJ44, AJ52 and AJ53, and AJ45, respectively, are underlined.

TABLE 1. Reaction of field strains of *Erwinia amylovora* from different geographic regions to streptomycin and myomycin and changes detected at codon 43 in the *rpsL* gene by sequence analysis and allele-specific amplification-polymerase chain reaction (ASA-PCR) analysis

Origin	ASA-PCR tested strains (no.)	Strains sequenced (designation)	Reaction to streptomycin[a]	Reaction to myomycin[b]	Codon 43 (amino acid)[c]
Idaho	4	PFB4	S	S	AAA (Lys)
Michigan	2	EL01	S	S	AAA (Lys)
Michigan	3	CA11, JR13b	MR	S	AAA (Lys)
Idaho	1	FB-93-5	HR	IS	AGA (Arg)
California	5	850, 829, 263	HR	IS	AGA (Arg)
Michigan	12	S5, R11, SC1, SC2, JR18b	HR	IS	AGA (Arg)
Oregon	1	OR1	HR	IS	AAT (Asn)
Oregon	2	OR2, OR3	HR	IS	AAC (Asn)
Oregon	3	OR4, OR5	HR	IS	ACA (Thr)
Oregon	23	OR10, OR20	HR	IS	AGA (Arg)
Washington	42	Ea88, JL1177, LA014, LA058	HR	IS	AGA (Arg)
New Zealand	13	NZR4, NZR10, NZS4	HR	IS	AGA (Arg)

[a]S = sensitive to streptomycin sulfate, no growth at 8 mg/L; MR = moderately resistant, minimal inhibitory concentration (MIC) of 512–1,024 mg/L; and HR = highly resistant, MIC greater than 4,096 mg/L.
[b]S = sensitive to myomycin sulfate, no growth at 16 mg/L; IS = insensitive, MIC greater than 512 mg/L.
[c]The second nucleotide of codon 43 of the *rpsL* gene in most strains was identified by ASA-PCR. Nucleotide sequence analysis of the *rpsL* gene was carried out for selected strains.

conditions described for cloning the *rpsL* gene. This region of the rRNA gene was examined because mutations causing streptomycin resistance in *E. coli* and *M. tuberculosis* are located around nucleotides 530 and 912 (10,17,20). The amplified fragment was extracted from the reaction mixtures by the Wizard PCR preps DNA purification system (Promega). Automated fluorescent sequencing of the 617-bp fragments was performed with the same primers by the MSU-DOE-PRL Plant Biochemistry Facility, East Lansing, with the ABI (Applied Biosystems Inc., Foster City, CA) catalyst 800 for *Taq* cycle sequencing and the ABI 373A sequencer for the analysis of products.

Nucleotide sequence accession numbers. The nucleotide sequence of the *rpsL* gene (Fig. 1) and the partial 16S rRNA gene of *E. amylovora* strain EL01 were submitted to GenBank and assigned accession numbers L36465 and L36466, respectively.

RESULTS

Levels of streptomycin and myomycin resistance. Testing of strains on streptomycin-amended media resulted in the identification of three readily distinguishable phenotypes (Table 1). Strains were considered sensitive (S) if they failed to grow on media amended with streptomycin at 8 mg/L. Among the 105 streptomycin-resistant strains, three from Michigan grew on media amended with streptomycin at 512–1,024 mg/L but not media amended at 2,048 mg/L. These strains were designated as having medium resistance (MR). The remaining 102 strains grew on media amended with streptomycin at 4,096 mg/L and were designated as having high resistance (HR). All HR strains also grew on media amended with myomycin at 512 mg/L and were designated myomycin-insensitive (IS) strains; streptomycin S and MR strains did not grow on myomycin-amended media and were designated myomycin-sensitive (S) strains.

Nucleotide sequence of *rpsL* genes from *E. amylovora*. The nucleotide sequence of the 434-bp PCR amplified fragment from S, MR, and HR strains of *E. amylovora* revealed a 375-bp *rpsL* gene that encoded a ribosomal protein, S12, of 124 amino acids (Fig. 1). Comparison of the deduced amino acid sequences for the *rpsL* gene of *E. amylovora* and of *E. coli* (22), *S. typhimurium* (13), *M. tuberculosis* (10), and *H. influenzae* (27) showed 98, 98, 69, and 91% identity, respectively. Only a single amino acid change at codon 43 was detected when the *rpsL* gene from HR strain S5 was compared with the genes from S strain EL01 and MR strain CA11. Codon 43 for lysine (AAA) in S and MR strains was replaced by a codon for arginine (AGA) in the HR strain (Fig. 1).

ASA-PCR analysis. Ten to 15 cycles of ASA-PCR were sufficient for amplification of *rpsL* DNA with a complementary nucleotide in the second position of codon 43 that matched the 3′ end of the 15-mer primer but not for the amplification when the second position of codon 43 mismatched the nucleotide at the 3′ end of the primer (Fig. 2). Discrimination between matching and mismatching codons was not possible after about 20 cycles of ASA-PCR.

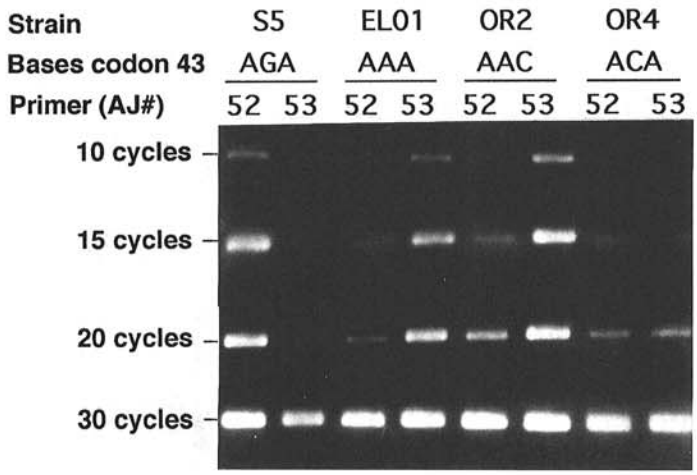

Fig. 2. The effect of the number of cycles on allele-specific amplification-polymerase change reaction (ASA-PCR) of *rpsL* DNA of *Erwinia amylovora*. ASA-PCR primers AJ52 5′-CACGACTACCCCTAG-3′ and AJ53 5′-CACGACTACCCCTAA-3′ were identical except for the base at the 3′ end. Primer AJ52 only detected the complementary C nucleotide, and primer AJ53 only detected the complementary T nucleotide in the second position of codon 43. The lysine residue (AAA) at codon 43 in streptomycin-sensitive strain EL01 was replaced in strains highly resistant to streptomycin by an arginine residue (AGA) in strain S5, an asparagine residue (AAC) in strain OR2, and a threonine residue (ACA) in strain OR4. ASA-PCR was performed simultaneously with both primers on DNA from each strain, and amplification products were analyzed by agarose gel electrophoresis after 10, 15, 20, and 30 cycles.

When all 111 strains were analyzed by ASA-PCR with primers AJ42-AJ53, the *rpsL* gene from six S, three R, and three HR strains (OR1, OR2, and OR3) was amplified after 10–15 cycles (Table 1). Using primers AJ42-AJ52, the *rpsL* gene from 96 HR strains but none of the remaining 15 strains was amplified after 10–15 cycles. Neither primer set amplified DNA from strains OR4, OR5, and OR22 after 10–15 cycles. No amplified DNA was detected when sample DNA was omitted from the PCR reaction mixture.

When the nucleotide sequence of the *rpsL* gene from HR strains OR1–OR5 was determined, codon 43 for lysine (AAA) in S strains was replaced by a codon for asparagine (AAT) in strain OR1,

an alternative codon for asparagine (AAC) in strains OR2 and OR3, and a codon for threonine (ACA) in strains OR4 and OR5 (Fig. 1; Table 1). Outside of codon 43, codon 109 in strain OR1, codons 15, 18, and 62 in strain OR3, and codon 25 in strain OR5 differed from the corresponding codons in S strain EL01 (Fig. 1). Each mutation, except the one in codon 25 in strain OR5, resulted in an altered amino acid in ribosomal protein S12. In addition, sequence data for strains LA014, NZR10, and R11 showed a different nucleotide in the third position of three, one, and one codons, respectively, and for strain LA058 in the first position of codon 61 (Fig. 1).

Complementation of *rpsL* genes in *E. coli* and *E. amylovora*. Streptomycin-resistant *E. coli* HB101 and *E. amylovora* S5 transformed with pMMB66HE continued to exhibit a high level of resistance to streptomycin and insensitivity to myomycin on antibiotic-amended media under inducing conditions (Table 2). When the *rpsL* gene from streptomycin-sensitive *E. amylovora* EL01 was expressed in *E. coli* HB101 and *E. amylovora* S5, sensitivity to streptomycin and myomycin was restored. Expression of the *rpsL* gene from strain EL01 in streptomycin-resistant *E. amylovora* OR2 and OR4 also restored susceptibility to streptomycin and myomycin.

Sensitivity to streptomycin and myomycin in streptomycin-sensitive *E. coli* JM109 and *E. amylovora* EL01 was not altered by the presence of expression vector pMMB66HE (Table 2). When the *rpsL* gene from streptomycin-resistant *E. amylovora* S5 was expressed in *E. coli* JM109 and *E. amylovora* EL01, resistance to streptomycin and insensitivity to myomycin was restored, at least partly. However, sensitivity to streptomycin and myomycin was not altered by the expression of the *rpsL* gene from streptomycin-resistant *E. amylovora* OR2 and OR4.

Sequence analysis of the 16S rRNA gene. No nucleotide mutations were detected in either the 530-loop or the 912 region of the 16S rRNA gene from HR strains OR1, OR2, OR4, OR10, S5, LA014, LA058, Ea88, and NZR4 when the nucleotide sequences were compared with the nucleotide sequence for S strain EL01 (data not shown).

DISCUSSION

The results of this study indicate that mutation has been more important in the development of streptomycin resistance in *E. amylovora* than gene acquisition in the western United States and New Zealand, but gene acquisition has been more important than mutation in Michigan. The strains we examined from the western United States and New Zealand represented a cross-section of streptomycin-resistant strains from the respective regions. All 90 resistant strains from these regions were of the HR phenotype and all contained a mutation in codon 43 of the *rpsL* gene (Table 1). These results are consistent with the finding that with minor exceptions (7), strains from the West and New Zealand were highly resistant to streptomycin (7,15,26,29). The HR strains we examined from Michigan were from two adjacent orchards in Kent County and one orchard in Van Buren County. The strains were a subset of strains isolated from 14 orchards in which streptomycin resistance has been detected since 1990 (16). Strains in 11 Michigan orchards (both mechanisms were present in one Van Buren orchard) have acquired genes *strA* and *strB* carried in transposon Tn*5393* and, in most strains, the self-transmissible plasmid pEa34 (4,5,16). In 1994, strains with *strA* and *strB* were found in eight additional Van Buren County orchards, indicating the population of *E. amylovora* with this mechanism of resistance is increasing in importance (P. S. McManus and A. L. Jones, *unpublished data*).

Two phenotypes, HR and MR, were distinguished when 105 streptomycin-resistant strains of *E. amylovora* from New Zealand, California, Idaho, Michigan, Oregon, and Washington were screened for variations in the level of streptomycin resistance. The two phenotypes corresponded well to groups, based on variations in MIC values for streptomycin, described by McManus and Jones (16) for strains of *E. amylovora* from Michigan. It is now possible to attribute resistance in HR strains to mutations in the *rpsL* gene and resistance in MR strains to the presence of acquired genes for inactivation of streptomycin (5,6). Because mutations in the *rpsL* gene confer high levels of resistance and the acquisition of genes for inactivating streptomycin confers lower levels of resistance, the mechanism for resistance to streptomycin in strains of *E. amylovora* can be predicted based on their level of resistance to streptomycin.

Strains with mutations in the *rpsL* gene and strains with enzyme-mediated resistance also were differentiated readily on medium containing myomycin, an antibiotic produced by *Nocardia* sp. that resembles streptomycin in biological activity. Previously, Davis et al (8) reported that *rpsL* mutants of *E. coli*, but not strains carrying *strA* and *strB*, were insensitive to myomycin. Because HR strains are insensitive to myomycin, the potential value of myomycin for countering streptomycin-resistant bacteria in the field will be limited to those situations involving strains

TABLE 2. Results of complementation tests in which the *rpsL* gene from a streptomycin-sensitive and three streptomycin-resistant strains of *Erwinia amylovora* was ligated into vector pMMB66HE and transformed into streptomycin-sensitive *Escherichia coli* JM109 and *E. amylovora* EL01 and into streptomycin-resistant *E. coli* HB101 and *E. amylovora* S5, OR2, and OR4

	MIC (mg/L)[a]				
	IPTG (mM) + streptomycin			IPTG (mM) + myomycin	
Bacterial strains	(0)	(0.05)	(0.5)	(0)	(0.5)
E. coli[b]					
HB101 (pMMB66HE)	>4,096	>4,096	>4,096	>512	>512
HB101 (pMMB66HE::*rpsL*$^{+}_{EL01}$)	1,024	8	8	>512	8
JM109 (pMMB66HE)	8	8	8	8	8
JM109 (pMMB66HE::*rpsL*$_{S5}$)	8	1,024	>4,096	32	128
JM109 (pMMB66HE::*rpsL*$_{OR2}$)	8	8	8	8	8
JM109 (pMMB66HE::*rpsL*$_{OR4}$)	8	8	8	8	8
E. amylovora[c]					
S5 (pMMB66HE)	>4,096	>4,096	>4,096	>512	>512
S5 (pMMB66HE::*rpsL*$^{+}_{EL01}$)	>4,096	8	8	>512	16
OR2 (pMMB66HE::*rpsL*$^{+}_{EL01}$)	>4,096	8	8	>512	16
OR4 (pMMB66HE::*rpsL*$^{+}_{EL01}$)	>4,096	8	8	>512	16
EL01 (pMMB66HE)	8	8	8	16	16
EL01 (pMMB66HE::*rpsL*$_{S5}$)	8	64	1,024	32	>512
EL01 (pMMB66HE::*rpsL*$_{OR2}$)	8	8	8	16	16
EL01 (pMMB66HE::*rpsL*$_{OR4}$)	8	8	8	16	16

[a] Minimal inhibitory concentrations (MICs) for streptomycin (0–4,096 mg/L) and myomycin (0–512 mg/L) in Luria-Bertani medium amended with isopropyl-β-D-thiogalactopyranoside (IPTG).
[b] Growth of bacteria was scored after 20-h incubation at 37 C.
[c] Growth of bacteria was scored after 40-h incubation at 30 C.

with enzyme-mediated resistance. Currently, myomycin is not commercially available, limiting its application in monitoring for streptomycin-resistant strains and controlling *E. amylovora* with enzyme-mediated resistance.

A mutation in codon 43 of the *rpsL* gene was detected in all 102 HR strains of *E. amylovora* but not in six S strains or three MR strains with *strA* and *strB*. Except for six HR strains from Oregon, a replacement of lysine by arginine was detected at codon 43 in all HR strains. In the six HR strains from Oregon, nucleotide sequence analyses revealed that lysine at codon 43 was replaced by asparagine in three strains and by threonine in two strains (strain OR22 was not sequenced). Our results indicate that a change in codon 43 of the *rpsL* gene confers a high level of resistance to streptomycin in *E. amylovora* and that the amino acid at this position plays an important role in the action of streptomycin.

Our examination of many HR strains by nucleotide sequence and ASA-PCR analyses provides circumstantial evidence that mutations in codon 43 of the *rpsL* gene are responsible for the high level of resistance to streptomycin and insensitivity to myomycin in *E. amylovora*. Also, the lack of mutations in the conserved regions around nucleotides 530 and 912 of the 16S rRNA gene where streptomycin-resistance mutations have been identified in other bacterial systems (10,17,20) is consistent with this conclusion. However, our complementation tests provide direct evidence that mutations in codon 43 are responsible for resistance to streptomycin and insensitivity to myomycin in this important plant pathogen.

Sensitivity to streptomycin is frequently dominant over streptomycin resistance in bacteria with ribosome-mediated resistance (14), as was observed here in streptomycin-sensitive bacteria carrying an exogenous *rpsL* gene. In streptomycin-resistant strains of *E. coli* a lysine-to-arginine mutation at codon 43 is nonrestrictive, whereas lysine-to-threonine or -asparagine mutations are restrictive (3,11). Ribosomes from strains with restrictive mutations, compared to wild-type strains, exhibited a slower rate of translation and a significantly lower missense error frequency. Ribosomes from strains with nonrestrictive mutations and those from wild-type strains did not differ in rate of translation or error frequency. Since strains with mutations of the restrictive type exhibit slower growth rates (9), they are likely less fit. Reduced fitness may explain why very few of the streptomycin-resistant field strains of *E. amylovora* we examined contained mutations for asparagine or threonine. In addition, the fitness of arginine mutants probably explains why streptomycin-resistant strains of *E. amylovora* persist in the field even years after streptomycin application ceases (15,26).

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