Isolation of Streptomycin-Resistant Isolates of *Erwinia amylovora* in New York

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ABSTRACT

Russo, N. L., Burr, T. J., Breth, D. I., and Aldwinckle, H. S. 2008. Isolation of streptomycin-resistant isolates of *Erwinia amylovora* in New York. Plant Dis. 92:714-718.

Streptomycin is currently the only antibiotic registered for the control of fire blight, a devastating disease of apple (*Malus*), pear (*Pyrus*), and other rosaceous plants caused by the bacterium *Erwinia amylovora*. Resistance of *E. amylovora* to streptomycin was first identified in California pear orchards in 1971 and is currently endemic in many parts of the United States. The Northeast remains the only major U.S. apple-growing region without streptomycin-resistant isolates of *E. amylovora*. In 2002, during a routine survey for streptomycin resistance, isolates from two neighboring orchards in Wayne County, NY were found to be highly resistant to streptomycin at a concentration of 100 µg/ml. This constitutes the first authenticated report of streptomycin resistance in New York State. Infected trees were shipped at the same time from a single nursery in Michigan. Resistance was caused by the acquisition of the *strA-strB* gene pair, inserted into the ubiquitous nontransmissible *E. amylovora* plasmid pEA29. Previously, streptomycin-resistant *E. amylovora* populations from Michigan were described with a similar mechanism of resistance, although the *strA-strB* genes are not unique to Michigan. These findings illustrate how unintentional movement of nursery material could undermine efforts to prevent the spread of antibiotic-resistant *E. amylovora*.

Additional keywords: antibiotic resistance

Fire blight, caused by the Enterobacterium *Erwinia amylovora* (Burrill) Winslow et al., is a devastating disease of rosaceous plants. Most commonly occurring on apple (*Malus* spp.) and pear (*Pyrus* spp.), fire blight affects production of these fruit in over 40 countries (4). Fire blight occurs in three distinct phases: blossom blight, shoot blight, and rootstock blight. Of these, blossom blight, the infection of newly opened blossoms, is the most significant, resulting in devastating crop losses (40) while serving as a precursor to the shoot and rootstock phases of the disease, either of which can be fatal to trees (29). Blossom blight is also the sole phase of fire blight with a prediction system and effective chemical control (4).

Blossom blight is controlled primarily through application of the aminoglycoside antibiotic streptomycin, which was introduced as a pesticide for horticultural use in 1955 (23,25). Although streptomycin was highly effective, streptomycin-resistant isolates of *E. amylovora* were identified in California pear orchards in 1971 (24) and soon after in Washington and Oregon, in

1972 (11,20). Streptomycin resistance has been reported throughout the major pomefruit production areas in the western United States as well as in Michigan, Missouri, British Columbia (Canada), Israel, Lebanon, and New Zealand (11,23,34,35,39).

Two mechanisms of streptomycin resistance have been described in *E. amylovora*. The more common form of resistance occurs through a single base-pair mutation of the streptomycin-binding site. Streptomycin binds to protein S12 of the 30S ribosomal subunit, encoded by the *rpsL* gene, thereby preventing protein synthesis (23). Chiou and Jones (10) determined that streptomycin resistance was caused by a single base-pair mutation in codon 43 of the *rpsL* gene (10). Mutational resistance is highly stable and has been shown to persist in the environment in the absence of selection pressure. Moller et al. (25) reported that streptomycin-resistant populations of *E. amylovora* could still be detected in California pear orchards 10 years after the application of streptomycin had ceased.

Streptomycin resistance has also been attributed to the acquisition of resistance plasmids, which confer enzyme-mediated resistance. Chiou and Jones (7) established that streptomycin resistance in Michigan *E. amylovora* populations was based on the acquisition of the plasmid pEA34, which possesses the *strA-strB* gene cluster. Plasmid pEA34, a conjugative plasmid, likely

originated in *Pantoea agglomerans* and was acquired by *E. amylovora* through conjugal transfer (7,22). The tandem *strA-strB* genes code for aminoglycoside-3-phosphotransferase and aminoglycoside-6-phosphotransferase, respectively (9). These aminoglycoside-modifying enzymes modify streptomycin, and previously have been described in resistant populations of *Pseudomonas syringae* pv. *papulans*, *P. syringae* pv. *syringae*, and *Xanthomonas campestris* pv. *campestris* (17,23,38), as well as some bacteria of human and animal origin (22). Although the *strA* and *strB* genes function independently, both must be present to confer high levels of antibiotic resistance (9). The *strA-strB* genes have been found on broad host range plasmids such as pRSF1010 (pEa8.7) (30) or located within the transposable element Tn5393 inserted into strain-specific plasmids like pEa34 (8). Tn5393, with *strA-strB*, also has been discovered inserted into the nontransmissible *E. amylovora* plasmid pEA29 and into the chromosome (22). These mobilizable resistance genes have previously been identified in common epiphytic bacteria found in orchards (5,6,13,28,37).

Currently, the Northeast is the only major apple production region in the United States without endemic populations of streptomycin-resistant *E. amylovora*. Resistance surveys conducted by Beer and Norelli (1) and Burr et al. (5,6) failed to identify streptomycin-resistant populations of *E. amylovora* between 1975 and 1992. However, Burr et al. (5,6) did confirm the presence of the streptomycin resistance genes, *strA-strB*, in orchard populations of the blister spot pathogen *P. syringae* pv. *papulans*, often associated with fire blight. Orchard surveys in New York are separated by many years, at which time resistant populations could become established, thereby limiting the effectiveness of eradication measures. The objectives of this study were to survey orchards throughout New York for the presence of streptomycin-resistant isolates of *E. amylovora* and to characterize resistance if found.

MATERIALS AND METHODS

Orchard survey. Active fire blight lesions were collected by regional Cornell Cooperative Extension Associates and by Dr. David A. Rosenberger of Cornell University's Hudson Valley Laboratory, and processed at the New York State Agricultural Experiment Station in Geneva from

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Accepted for publication 13 December 2007.

doi:10.1094/PDIS-92-5-0714

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2002 through 2006. Sampling sites were chosen based on past streptomycin use and reports of inconsistency in control of fire blight using streptomycin. Fire blight lesions were collected in 14 counties from 56 individual farm sites. Samples primarily consisted of infected shoot tissue, although fruit and rootstock tissues were sampled when applicable. An average of three isolations from three different infected tissues were made from each submitted sample.

Isolation of bacteria and resistance screening. Single-colony isolates of *E. amylovora* were isolated from infected tissue and tested for antibiotic resistance according to Burr et al. (5). Samples were surface sterilized in 0.5% sodium hypochlorite solution for 10 min and rinsed with sterile distilled water. Outer bark was removed and internal tissue plated on Crosse and Goodman (CG) medium (12), diagnostic for *Erwinia* spp. Single-colony isolates were identified as *E. amylovora* by colony morphology. Final confirmation was based on the presence of a 1-kb fragment amplified from the ubiquitous *E. amylovora* plasmid pEA29, modified from Bereswill et al. (2). Polymerase chain reactions (PCRs), 50 µl in total volume, were carried out using 2.5 µM primers A and B (2), 12.5 mM MgCl₂, 5 µl of PCR Reaction Buffer (Promega Corp., Madison, WI), 0.1 mM dNTPs, 0.625 units of Taq DNA Polymerase (Promega Corp.), and 10 µl of bacterial sample, and PCR products were analyzed by electrophoresis using 1% agarose gels. Three colonies, when possible, were stored from each sample for resistance screening.

To assess the level of streptomycin resistance, isolates were grown at 28°C in 5 ml of liquid Luria-Bertani (LB) broth and 100-µl aliquots, adjusted to 10⁸ CFU/ml, were spread evenly on LB medium. Bacteria were challenged with filter paper disks (Schleicher & Schuell Inc., Keene, NH) impregnated with a solution of streptomycin sulfate at 0, 10, 50, or 100 µg/ml and incubated at 28°C. Resistance levels were determined by measuring the clear zone of inhibition assessed at 24 and 48 h. Three replicated plates of each single-colony isolate were evaluated for resistance; experiments were repeated to verify results. Resistance level was compared against the streptomycin-susceptible strain Ea273, described by Norelli et al. (27), and the streptomycin-resistant strain CFBP1376str, a streptomycin-resistant variant of strain CFBP1376 originally characterized by Paulin and Samson (31).

Bacterial isolates and plasmid selection. *E. amylovora* isolates CA11 (8) and BCN77 (22), containing the *strA-strB* gene pair, and strain Ea110, containing plasmid pC9, were provided by Dr. George Sundin and Gayle McGhee at Michigan State University (21; Table 1). NY17.1 and NY17.2 are the uncharacterized streptomycin-

resistant isolates of *E. amylovora* that originated from this study.

Pathogenicity assays. Bacteria that displayed resistance to streptomycin were assessed for pathogenicity using both immature green pear fruit (3) and seedling inoculation tests (32). Cross-sections, 5 mm thick, of immature pear fruit were wounded with toothpicks dipped in inoculum and incubated overnight at 28°C for 48 h. Isolates were recorded as pathogenic if the inoculation site became necrotic and ooze exuded from the wound. Actively growing McIntosh seedlings, approximately 10 cm in height, were inoculated by transversely bisecting the two youngest leaves with scissors dipped in a suspension of streptomycin-resistant isolates NY17.1 and NY17.2 (1 × 10⁷ CFU/ml) in 0.5 M potassium phosphate buffer according to Norelli et al. (27). Necrotic lesions accompanied by the production of bacterial ooze confirmed pathogenicity on apple seedlings. Results were compared against the susceptible strain Ea273 and resistant strain CFBP1376str.

Sequencing of the ribosomal protein S12 (*rpsL*) gene. An internal region of the ribosomal *rpsL* gene, containing codon 43, was amplified from streptomycin-resistant New York isolates NY17.1, and NY17.2, along with streptomycin-resistant isolates CA11, Ea88-100, and CFBP1376str and a streptomycin-sensitive strain Ea273. Primers were based on the *rpsL* gene sequence from *E. amylovora* (GenBank accession number L 36465; Table 2). PCR reactions, 50 µl in total volume, were carried out using 0.4 µM primers rpsL212F and rpsL212R, 12.5 mM MgCl₂, 5 µl of PCR Reaction Buffer (Promega Corp.), 0.1 mM dNTP, 0.625 units of Taq DNA Polymerase (Promega Corp.), and 10 µl of bacterial

sample, and PCR products were analyzed by electrophoresis using 1% agarose gels. PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega Corp.). Samples were sequenced using an Applied Biosystems Automated 3730 DNA Analyzer (Foster City, CA) at the Core Laboratories Center (CLC; Ithaca, NY).

***strA-strB* gene identification.** Internal regions of the *strA* and *strB* genes were amplified from New York isolates NY17.1 and NY17.2, along with streptomycin-resistant *E. amylovora* isolates CA11 and CFBP1376str and streptomycin-sensitive strain Ea273. Primers were based on the *E. amylovora strA* and *strB* gene sequences characterized by Chiou and Jones (8; GenBank accession number M 96392; Table 2). PCR reactions, 50 µl in total volume, were carried out using 0.4 µM either strA406-f and strA406-r or strB403-f and strB403-r primers, 12.5 mM MgCl₂, 5 µl PCR Reaction Buffer (Promega Corp.), 0.1 mM dNTP, 0.625 units of Taq DNA Polymerase (Promega Corp.), and 10 µl of bacterial sample, and PCR products were analyzed by electrophoresis using 1% agarose gels. PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega Corp.). Samples were sequenced using an Applied Biosystems Automated 3730 DNA Analyzer at the CLC to verify gene identity.

Plasmid transfer. Plasmid transfer rates were determined according to Chiou and Jones (7). Recipient bacteria were rifampicin-resistant mutants of *E. amylovora* strain Ea273 identified as CU0380. An equal number of cells of recipient strain CU0380 were mixed with each of the donor isolates NY17.1, NY17.2, CA11, and Ea273. Mixtures were incubated overnight

Table 1. Bacterial isolates and plasmids

Strain, plasmid	Relevant characteristics ^a	Source or reference
<i>Erwinia amylovora</i>		
Ea273	Ubiquitous plasmid pEA29, [ATCC 49946]	27
CU0380	R ^f variant of Ea273, mutational resistance	S. Beer ^b
CFBP1376str	Sm ^r variant of CFBP1376, mutational resistance	31
Ea88-100	Sm ^r , mutational resistance	20
CA11	Sm ^r , pEa34 carrying Tn5393 w/ <i>strA-strB</i> genes	8
BCN77	Sm ^r , chromosomal insertion of Tn5393 w/ <i>strA-strB</i> genes	22
NY17.1	Sm ^r , isolated 11/2002	This study
NY17.2	Sm ^r , isolated 11/2002	This study
Ea110 ⁻	R ^f , containing pC9	21
pC9	4.4-kb <i>Pst</i> I fragment from pEA29 cloned into pGEM3zft(+)	21

^a R^f and Sm^r = resistant to rifampicin and streptomycin, respectively.

^b Cornell University, Ithaca, NY.

Table 2. Polymerase chain reaction primers developed in this study

Gene	Primer	Product size (bp)
<i>rpsL</i>	rpsL212-F: 5'-cgtacgcaaaagttgcaaaaa-3' rpsL212-R: 5'-ggatcaggatcacggagtgt-3'	212
<i>strA</i>	strA406-F: 5'-tgactggttgctgtcagag-3' strA406-R: 5'-cggttaagaagtcgggattga-3'	406
<i>strB</i>	strB403-F: 5'-atcgcttgcagctttgtt-3' strB403-R: 5'-cgtgtctctcttccatc-3'	403

at 28°C and dilution plated on LB medium amended with rifampicin, streptomycin, or rifampicin plus streptomycin at 50 µg/ml. Plates were incubated at 28°C and resultant colony growth on medium amended with both antibiotics signified bacterial conjugation. All isolates used in this experiment contained the ubiquitous non-transmissible plasmid pEA29; if the *strA-strB* resistance genes are located on plasmid pEA29 or inserted into the chromosome, no transfer of resistance would be observed.

Plasmid curing. The ubiquitous non-transmissible plasmid pEA29 was cured from *E. amylovora* isolates through incompatibility eviction assay previously described by McGhee and Jones (21). Plasmid curing was performed to determine the effect of pEA29 on streptomycin resistance. Plasmid pC9 (Amp^r; Table 1), containing the pEA29 *ori* region, was electroporated into isolates NY17.1, NY17.2, CA11, and Ea273. Bacteria were grown on LB medium amended with ampicillin at 50 µg/ml to select for pC9. Plasmid pEA29 eviction was verified using the pEA29-specific primers A and B (2) compared with *E. amylovora* chromosomal primers AJ245 and AJ246 (16). PCR reactions, 50 µl in total volume, were carried out using 0.4 µM either A and B, AJ245 and AJ246, with 12.5 mM MgCl₂, 5 µl of PCR Reaction Buffer (Promega Corp.), 0.1 mM dNTP, 0.625 units of Taq DNA Polymerase (Promega Corp.), and 10 µl of bacterial sample. Plasmid-cured isolates were dilution plated on LB medium amended with ampicillin, streptomycin, or ampicillin and streptomycin at 50 µg/ml.

RESULTS

Resistance screening and pathogenicity assay. Between 2002 and 2006, 99 fire blight samples were submitted and processed at the New York State Agricultural Experiment Station in Geneva. In all, 316 isolations were made, and 249 single-colony isolates of *E. amylovora* were screened for resistance to streptomycin. In August 2002, streptomycin-resistant isolates were recovered from symptomatic apple shoots (cv. Idared) collected from a newly planted orchard in Wayne County. Initial sampling occurred on 29 July 2002. Subsequent sampling was conducted on 21 November 2002 and 7 May 2003 to con-

firm the presence of streptomycin-resistant *E. amylovora*. Nine single-colony isolates were confirmed as *E. amylovora* and found to be resistant to streptomycin at 100 µg/ml; no clear zone of inhibition was produced in response to streptomycin at this concentration after 24 or 48 h. A representative streptomycin-resistant isolate was cataloged as *E. amylovora* NY17.1. Streptomycin-resistant *E. amylovora* also was recovered from an orchard located approximately 4.8 km from the original sampling site on 21 November 2002, cataloged as NY17.2. Streptomycin-resistant isolates were pathogenic on both immature pear fruit and apple seedlings (*data not shown*). No resistant isolates were identified in orchards surrounding the two suspect plantings. Investigation revealed that Idared trees planted at both orchards originated from the same nursery shipment. Resistance was confirmed and trees at both orchard sites were removed in the winter of 2003–04. Between 2003 and 2006, no new streptomycin-resistant isolates were identified in New York.

***rpsL* gene sequencing.** Comparison of the 212-bp sequences amplified from the *rpsL* gene verified a highly conserved region across all six isolates of *E. amylovora*. Two different single-base-pair muta-

tions were observed in the streptomycin-resistant isolates CFBP1376str and Ea88-100 (Table 3). CFBP1376str was shown to have a deleterious mutation, lysine to threonine, whereas strain Ea88-100 was shown to have a persistent mutation, lysine to arginine. New York isolates NY17.1 and NY17.2 and streptomycin-resistant strain CA11 were identical to the susceptible strain Ea273 at codon 43 of the *rpsL* gene coding for the wild-type amino acid lysine (AAA).

***strA-strB* gene identification.** *strA* and *strB* gene primers amplified 406- and 403-bp regions of the *strA* and *strB* genes from both streptomycin-resistant isolates from New York. Band size was consistent with CA11, which is positive for the *strA-strB* gene cluster (Fig. 1). No amplification occurred in isolates with chromosomal resistance conferred by mutation of the *rpsL* gene or in the wild-type streptomycin-sensitive strain. Sequence results confirmed the identity and conserved nature of the *strA* and *strB* genes previously described from *E. amylovora*.

Plasmid transfer. Plasmid transfer of streptomycin resistance was observed when CA11 was utilized as the donor stain but not with the wild-type strain Ea273. CA11 contains the *strA-strB* gene pair on

Fig. 1. Agarose gel depicting amplification products from a polymerase chain reaction analysis of *Erwinia amylovora* reference isolates and new streptomycin-resistant isolates using primers strA406-F&R and strB403-F&R based on 406- and 403-bp internal fragments of the A *strA* and B *strB* genes, respectively. Lanes 1 and 7, water; lanes 2 and 8, strain Ea273; lanes 3 and 9, CFBP1376str; lanes 4 and 10, CA11; lanes 5 and 11, NY17.1; and lanes 6 and 12, NY17.2.

Table 3. Sequence comparison of *rpsL* gene from streptomycin-sensitive and -resistant isolates of *Erwinia amylovora*

Strain	Sm ^{ra}	Sequence
Ea273	–	CTCGTGTGTACACGACTACCCCTAAAAAACC GAAC TCCGCACTGCGT
CFBP1376str ^b	+	CTCGTGTGTACACGACTACCCCTACAAAACCGAAC TCCGCACTGCGT
CA11	+	CTCGTGTGTACACGACTACCCCTAAAAAACC GAAC TCCGCACTGCGT
Ea88-100 ^c	+	CTCGTGTGTACACGACTACCCCTAGAAAACCGAAC TCCGCACTGCGT
NY17.1	+	CTCGTGTGTACACGACTACCCCTAAAAAACC GAAC TCCGCACTGCGT
NY17.2	+	CTCGTGTGTACACGACTACCCCTAAAAAACC GAAC TCCGCACTGCGT

^a Absence (–) or presence (+) of streptomycin resistance.

^b Deleterious mutation, lysine (AAA) to threonine (ACA), results in unstable streptomycin resistance, which retards colony growth.

^c Persistent mutation, lysine (AAA) to arginine (AGA), results in stable streptomycin resistance.

conjugative plasmid pEa34 (7; Table 4). Growth of CA11 on media containing streptomycin exceeded growth on medium harboring both antibiotics but frequency of plasmid transfer remained high. Growth of the recipient strain CU0380 remained constant in all matings and did not contribute to differences in plasmid transfer. No transfer of resistance was observed between CU0380 and either New York isolate NY17.1 or NY17.2. No spontaneous mutations conferring either streptomycin or rifampicin resistance were observed during the course of the experiment. Experiments were repeated with similar results.

Plasmid curing. Plasmid pEA29 was evicted from isolates Ea273, NY17.1, and NY17.2 through the introduction of plasmid pC9. Growth on medium amended with ampicillin verified the presence of pC9 in all bacterial strains. Plasmid eviction was verified by the absence of a 1-kb fragment from pEA29 as determined by PCR (*data not shown*). Complete eviction of pEA29 was not achieved for streptomycin-resistant strain CA11, even in the presence of pC9, verified by resistance to ampicillin at 50 µg/ml (Table 5). Based on the nature of streptomycin resistance in CA11 (9,10) results were not influenced by incomplete eviction of pEA29. Results clearly demonstrated that isolates NY17.1 and NY17.2 lost the ability to grow on streptomycin-amended medium after eviction of plasmid pEA29 (Table 5). No spontaneous mutations conferring either streptomycin or ampicillin resistance were observed during the course of the experiment. Experiments were repeated with similar results.

DISCUSSION

In 2002 and 2003, resistant isolates of *E. amylovora* were isolated from two neighboring orchards in Wayne County. New York isolates were found to be highly resistant to streptomycin without losing pathogenicity. These results constitute the first authenticated report of streptomycin-resistant *E. amylovora* in New York. In 2003, both orchards were removed in an effort to prevent the establishment of streptomycin-resistant bacteria. Beyond the two initial plantings sampled in 2002, no streptomycin-resistant *E. amylovora* has been identified in New York since 2003. Early identification and prompt orchard eradication can be attributed in halting the spread of streptomycin resistance and forestalling the loss of streptomycin for future control of fire blight.

Since the discovery of streptomycin-resistant *E. amylovora* in California in 1971, antibiotic usage in apple and pear regions has been reduced dramatically. Reduction in the number of antibiotic applications has been proposed as the main strategy to delay antibiotic resistance development (23,25). Streptomycin usage in New York State, however, historically has

been modest compared with usage in California and Michigan, where resistance was documented in the early 1970s and 1990s, respectively. Moller et al. (25) suggested that, with continued reliance on streptomycin, resistance development is inevitable due to constant mutation and selection in bacterial populations. Although reduction in antibiotic usage is necessary to maintain the effectiveness of streptomycin, there are other means by which resistant bacteria become established in new areas.

Over the past 200 years, fire blight, originally identified in the Hudson Valley, New York, has been unintentionally disseminated throughout the world via the movement of infected plant material (4). Introduction of bacteria on nursery stock poses a great risk for regions without endemic fire blight. Potential impact on apple production is magnified by the probability of importing streptomycin-resistant *E. amylovora*. With regard to the streptomycin-resistant isolates from New York, evidence supports the theory that trees were infected with *E. amylovora* resistant to streptomycin prior to planting. Resistant isolates from Wayne County were identified in orchards that had been planted the previous year and had yet to flower. Fire blight cankers in these plantings were considerable despite the young age of the planting and limited exposure to external sources of *E. amylovora*, which suggested prior infection. Furthermore, only one of the plantings had been treated with streptomycin prior to detection of resistant bacteria. Planting material in both orchards originated from the same nursery located in southwestern Michigan, a region where streptomycin-resistant *E. amylovora* had been described previously (7–10). Trees

were sold following a fire blight epidemic in 2000 that destroyed much of the apple production in southwestern Michigan, resulting in several million dollars' worth of damage and tremendous tree loss (19). The strongest evidence supporting the movement of infected nursery material was the presence of the *strA-strB* gene cluster, found to be the cause of resistance in New York. This type of resistance occurs associated with *E. amylovora* only in Michigan, save for a single California isolate which contained a unique resistance plasmid (30), not observed in the New York isolates.

Although evidence strongly suggests that resistant isolates were imported on infected nursery stock, it is difficult to prove conclusively. With the exception of *Rubus* strains, *E. amylovora* isolates are genetically homogeneous, with few differentiating characteristics (26). Using pulsed-field gel electrophoresis analysis, Jock et al. (14) could discern distinct patterns relating to origin in European *Malus* *E. amylovora* isolates, but similar differentiation of North American isolates was not observed (15). The presence of the *strA-strB* gene cluster, although significant, is inconclusive as a marker for bacterial origin. The *strA-strB* genes are common antibiotic resistance genes and have been well characterized in New York populations of epiphytic bacteria that exist in close proximity to *E. amylovora* (13,37).

Movement of plant material is a common practice in the apple industry, where high demand for trees often exceeds local nursery production. Many prominent apple and pear nurseries are located in Washington and Oregon, where streptomycin-resistant *E. amylovora* has been widely

Table 4. Resistance profile of recipient strain CU0380 (rifampicin resistant; Rf^r) after bacterial conjugation with streptomycin-resistant (Sm^r) donor isolates CFBP1376str, CA11, NY17.1, and NY17.2

Donor strain	Recipient	Colony development (CFU/ml) ^a		
		Sm	Rf	Transconjugants Sm with Rf ^b
CFBP1376str	CU0380	5.0 × 10 ⁷	3.0 × 10 ⁷	0
CA11	CU0380	7.0 × 10 ⁷	3.8 × 10 ⁶	1.6 × 10 ⁶
NY17.1	CU0380	1.6 × 10 ⁸	1.0 × 10 ⁷	0
NY17.2	CU0380	2.6 × 10 ⁷	1.3 × 10 ⁷	0

^a Transconjugant growth signifies transfer of streptomycin resistance plasmid.

^b Bacterial growth measured as CFU/ml on media supplemented with either Sm, Rf, or a combination of both at 50 µg/ml.

Table 5. Resistance profile of isolates cured of plasmid pEA29 through incompatibility eviction assay using plasmid pC9

Strain ^b	Colony development (CFU/ml) ^a			
	LB ^c	Amp	Sm	Amp + Sm
Ea273	8.3 × 10 ¹⁰	6.83 × 10 ¹⁰	0	0
CA11 ^d	1.9 × 10 ⁸	1.27 × 10 ⁸	1.17 × 10 ⁸	3.67 × 10 ⁷
NY17.1	8.0 × 10 ⁹	9.83 × 10 ⁸	0	0
NY17.2	7.33 × 10 ⁹	3.03 × 10 ⁹	0	0

^a Ampicillin (Amp) resistance signifies acquisition of the selective plasmid pC9; Sm = streptomycin.

^b Designates isolates cured of plasmid pEA29 by incompatibility eviction assay.

^c Luria-Bertani.

^d CA11 demonstrated incomplete eviction of pEA29; growth of bacteria in culture was inhibited.

documented (11,23,33). Our findings, although inconclusive, do address the potential for infected nursery material to serve as a vehicle to spread resistant bacterial populations.

Alternate materials for control of fire blight have been investigated; however, nothing has proven to be as effective or as durable as streptomycin. Oxytetracycline has been approved for use on pear and is available on a limited basis for apple in California and Michigan, where streptomycin-resistant isolates of *E. amylovora* have been identified. Oxytetracycline, however, is not as effective as streptomycin on streptomycin-sensitive isolates and only outperforms streptomycin in areas where streptomycin resistance occurs (23,36). In Israel, oxolinic acid is used to control fire blight but resistant isolates were identified only 1 year after commercial release (18). New antibiotics, such as gentamicin (Agry-Gent; Gowan Co. LLC, Yuma AZ) and kasugamycin (Kasumin; Arysta Lifescience Corp., Tokyo) are being evaluated for the control of fire blight, with some promising results (36). Unfortunately, although effective, concern over the spread of antibiotic resistance to human pathogens has impeded the registration of new antibiotics for agricultural use (23). Streptomycin is the most effective antibiotic for use on apple and is likely to remain as such; therefore, it is imperative to identify cases of antibiotic resistance early, before bacterial populations become established.

ACKNOWLEDGMENTS

We thank D. Rosenberger, K. Iungerman, and J. Carroll for collection and submission of fire blight samples; and G. Sundin and G. McGhee for additional bacterial isolates and plasmids used in this study.

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