The Analysis of Plasmid-Mediated Streptomycin Resistance in *Erwinia amylovora*

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ABSTRACT

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Streptomycin-resistant mutants of *Erwinia amylovora* were isolated from an apple orchard in Michigan and from crabapple trees adjacent to the same orchard in 1990. Isolates that grew on King's medium B amended with 100 μg/ml of streptomycin sulfate were considered to be resistant strains, whereas isolates that failed to grow on this medium were considered to be sensitive strains. Growth of the resistant strains was not inhibited in a filter-paper disk assay (0.06–5 μg of streptomycin sulfate), but growth of sensitive strains was inhibited at concentrations as low as 0.06 μg of streptomycin sulfate. Only sensitive strains were detected in an additional 19 apple orchards sampled for resistant strains. In colony blot hybridizations, an internal portion of the streptomycin-resistance gene (probe SMP3) from strain Psp36 of *Pseudomonas syringae* pv. *papulans* hybridized with all streptomycin-resistant strains of *E. amylovora*, but not with streptomycin-sensitive strains. Probe SMP3 hybridized to a 2.7-kb restriction fragment from *Ava*I-digested total genomic and plasmid DNA of two resistant strains of *E. amylovora* and to a 1.5-kb fragment in DNA from strain Psp36 of *P. s. papulans*. The probe did not hybridize with digested DNA from sensitive strains. A 33-kb plasmid was present in all streptomycin-resistant field strains but not in streptomycin-sensitive strains. Streptomycin resistance was transferred by matings to four streptomycin-sensitive recipient strains of *E. amylovora* from each of two streptomycin-resistant donor strains. Transconjugants also contained the 33-kb plasmid. DNA from resistant strain Ea88-90 from Washington did not hybridize with the probe, indicating that this strain contains a resistance system unrelated to that in streptomycin-resistant strains from Michigan.

Fire blight, caused by *Erwinia amylovora*, is a devastating disease of apples and pears in North America, New Zealand, much of Europe, and the Mediterranean region (1). Extensive experimentation on the efficacy of streptomycin for the control of fire blight was conducted in North America in the early 1950s. After streptomycin was registered in the United States in the late 1950s, it was used extensively for the control of fire blight on pears in the West and on apples in the East.

Streptomycin-resistant *E. amylovora* was first detected in pear orchards of California in 1971 (14) and soon thereafter in pear orchards of Washington and Oregon (4); in 1988, resistant strains were ubiquitous in pear orchards of Washington (11). In the mid-1970s, attempts to detect resistant strains in apple and pear orchards in western New York state and in apple orchards in Michigan failed (2,22). Except for a report of resistant strains on apple in Missouri (19), detection of streptomycin-resistant *E. amylovora* has been limited to the western United States. However, streptomycin resistance in *Pseudomonas syringae* pv. *papulans* has been a problem in apple orchards of the cultivar Mutsu in the eastern United States (3,8,17).

In California, resistance to streptomycin in *E. amylovora* is caused by a chromosomal mutation (18), but streptomycin resistance in *P. s. papulans* and *Xanthomonas campestris* pv. *vesicatoria* is plasmid-borne (3,15,17). No hybridization was obtained when a streptomycin-resistance probe from *X. c. vesicatoria* was used to probe DNA from a streptomycin-resistant strain of *E. amylovora* from California (15). But the resistance probe did hybridize with DNA from a streptomycin-resistant strain of *P. s. papulans* from New York.

This study reports the detection of streptomycin-resistant *E. amylovora* on apple in Michigan. It shows that resistance is plasmid-borne, and that a DNA probe specific for streptomycin-resistance in *P. s. papulans* hybridizes with DNA from streptomycin-resistant strains of *E. amylovora* but not with DNA from streptomycin-sensitive strains.

MATERIALS AND METHODS

Bacterial strains and plasmids. Streptomycin-resistant strain Ea88-90 of *E. amylovora* was supplied by R. G. Roberts, Tree Fruit Research Laboratory, Wenatchee, WA. All other strains of the bacterium were isolated from apple trees in Michigan during the course of this study. Strains Psp36 and Psp32 of *P. s. papulans* were supplied by T. J. Burr, New York State Agricultural Experiment Station, Geneva. Plasmid pCPP505, containing an internal portion of the streptomycin resistance gene from strain Psp36 of *P. s. papulans* (17), was supplied by J. L. Norelli, New York State Agricultural Experiment Station, Geneva.

Detection of streptomycin-resistant *Erwinia amylovora*. In June 1990, samples of fire blight were received from an apple grower in southwest Michigan (orchard SW) who suspected a problem with streptomycin-resistant *E. amylovora*. Bacteria were isolated from the samples by placing small bits of tissue from infected spurs and shoots on King's medium B (KB) containing 100 μg/ml of streptomycin and 50 μg/ml of cycloheximide (KBsc). The pathogenicity of eight strains isolated from orchard SW was established by inoculating immature Jonathan apple fruit in the laboratory. Upon reisolation, five strains from orchard SW and two strains from an orchard located at the Botany and Plant Pathology Farm, Michigan State University, East Lansing, were tested for resistance to streptomycin by evenly spreading 10^7 colony-forming units per milliliter onto nutrient-yeast-dextrose agar (4). About 5 h later, six 12.7-mm-diameter filter-paper disks (Schleicher & Schuell Inc., Keene, NH) were placed equidistant on the surface of the plates. Aliquots (50 μl) of solution from a streptomycin sulfate dilution series were applied to the disks.

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Each treatment was replicated four times. Plates were incubated at 22 C, and inhibition zones were measured after 4 days.

Orchard survey. Spurs and terminals with active fire blight lesions were collected in June and early July 1990 from 20 apple orchards throughout the western Michigan fruit belt, including orchard SW. Infected twigs were also collected from seedling crabapple trees in a 2-ha block adjacent to orchard SW. After pulling back the bark, two small pieces of tissue were removed from the water-soaked tissue distal to the necrosis. One piece of tissue was placed on KB with 50 μg/ml of cycloheximide (KBc), and the second was placed on KBsc. Isolations were made from up to 10 samples per location (25 samples, however, were taken from orchard SW). Nonfluorescent white, semimucoid colonies typical of *E. amylovora* that formed on KBc or KBsc in 72 h at 20 C were streaked onto the selective high-sucrose medium of Crosse and Goodman (5). Bacteria characteristic of *E. amylovora* on the high-sucrose medium were saved for study.

Plasmid characterizations. Sensitive and resistant strains of *E. amylovora* from orchard SW and from crabapple trees adjacent to orchard SW were screened for plasmids using a modification of the method of Kado and Liu (9) as described by Burr et al (3). Plasmid DNA was electrophoresed on 0.5% agarose gel, stained, and photographed as described by Sundin et al (21).

Hybridizations. Plasmid pCPP505 was received, maintained, and amplified in *Escherichia coli* strain DH1. Probe SMP3 was prepared as described by Norelli et al (17). Plasmid DNA, extracted by the alkaline lysis method described by Maniatis et al (12) from cultures grown for 16 h at 37 C in Luria-Bertani (LB) medium containing 50 μg/ml of ampicillin and 50 μg/ml of streptomycin, was digested with restriction enzymes *Bam*HI and *Ava*I. A 500-bp restriction fragment (SMP3) was excised from a low melting temperature agarose gel following electrophoresis in Tris-borate EDTA buffer. The fragment was radiolabeled with ^{32}P by the randomized oligonucleotide labeling procedure (Random Primed DNA Labeling Kit, U.S. Biochemical, Cleveland, OH).

Colony hybridizations with DNA probe SMP3 were performed with streptomycin-sensitive and streptomycin-resistant *E. amylovora*. Up to five sensitive strains from each orchard and all resistant strains were transferred to Colony/Plaque Screen hybridization transfer membranes (New England Nuclear Research Products, Boston, MA) that had been placed on the surface of KB agar plates and incubated for 48 h at 20 C. Colonies of *E. coli* with pCPP505 and strain Psp36 of *P. s. papulans* (streptomycin-resistant) and strain Psp32 of *P. s. papulans* (streptomycin-sensitive) were included on each membrane as positive and negative controls, respectively. The bacteria were lysed and the DNA denatured and fixed to the membranes according to the manufacturer's instructions. Hybridizations were performed overnight and the membranes washed according to the manufacturer's recommended procedures. Autoradiographs of membranes were carried out with XAR X-ray film at −70 C.

Restriction enzyme digests and Southern blots. Plasmid DNA was isolated by alkaline lysis extraction followed by cesium chloride centrifugation (12). Total genomic DNA was prepared by a miniprep procedure (23). The purified plasmid and total genomic DNAs were digested with *Ava*I, and following gel electrophoresis and Southern transfer to GeneScreen hybridization transfer membrane, hybridizations were carried out as described for colony hybridizations.

Bacterial conjugation. Recipient strains were rifampicin-resistant variants of streptomycin-sensitive parental strains EL01, BC06, GR05, and MA05. Donor (HO62-1 and CA11) and recipient strains were grown for 16–24 h at about 22 C on a rotary shaker in 5 ml of LB medium amended with 50 μg/ml of streptomycin (donor strains) or 150 μg/ml of rifampicin (recipient strains). The cultures, each with about 10^{10} cells per milliliter, were mixed in a 1:1 ratio, and 10-μl aliquots were plated on KB medium and incubated 24 h at 22 C. Cell mixtures were suspended in 10 ml sterile distilled water, vortexed, serially diluted, and plated on LB medium amended with 50 μg/ml of streptomycin or 150 μg/ml of rifampicin to determine donor and recipient populations, respectively. Cell mixtures were also plated on LB medium amended with both 50 μg/ml of streptomycin and 150 μg/ml of rifampicin to determine the population of transconjugants. Colonies with good growth on LB medium amended with both antibiotics were considered putative transconjugants. The frequency of spontaneous resistant mutants was determined by plating donor and recipient strains on LB medium amended with rifampicin and streptomycin, respectively.

RESULTS

Detection of resistance. Colonies of *E. amylovora* were recovered on KBsc medium from eight samples of fire blight from orchard SW. When immature fruit were inoculated with these bacteria, all of the strains caused typical symptoms of fire blight, including the production of bacterial ooze.

Level of resistance. Pathogenic strains initially recovered on KBsc medium were highly resistant to streptomycin. Each strain grew to the margin of filter-paper disks containing the highest level (5 μg per disk) of streptomycin sulfate tested. Two strains from the orchard in East Lansing were sensitive to streptomycin, as indicated by the development of clear zones around each disk. Mean size of zones of inhibition for the two sensitive strains were 13.6, 14.2, 15.8, 19.4, 22.2, and 25.0 mm for disks that received 0.06, 0.31, 0.62, 1.25, 2.50, and 5.0 μg of streptomycin sulfate, respectively.

Distribution of resistant strains. *E. amylovora* was recovered on KBc from 137 samples and on KBsc from 20 samples of fire blight collected from 20 apple orchards in western Michigan. All streptomycin-resistant strains (20 out of 25 samples) were recovered from orchard SW. No streptomycin-resistant strains were detected in samples from the remaining 19 orchards. Five streptomycin-resistant strains of the bacterium were also recovered from 15 samples of fire blight collected from crabapple trees adjacent to orchard SW.

Colony hybridization studies. The probe hybridized with plasmid pCPP505 and with DNA from streptomycin-resistant strain Psp36, but not with DNA from streptomycin-sensitive strain Psp32 of *P. s. papulans*. Total DNA from all 25 streptomycin-resistant colonies of *E. amylovora* from orchard SW and the adjacent crabapple trees (but no DNA from 78 colonies of streptomycin-sensitive *E. amylovora*, including five strains from orchard SW) hybridized with the probe in colony blot hybridizations. Probe SMP3 did not hybridize with DNA from streptomycin-resistant *E. amylovora* strain Ea88-90 isolated from pear in Washington.

Plasmid characterizations. Streptomycin-resistant strains from orchard SW and the neighboring crabapple trees contained a plasmid of approximately 33 kb that was not present in streptomycin-sensitive strains isolated from these same locations (Fig. 1). In addition, all strains from these orchards and all other strains of *E. amylovora* examined for plasmid content, including strain Ea88-90 from pear in Washington state, contained a plasmid of approximately 30 kb. Confirmation that this plasmid was the ubiquitous plasmid common to *E. amylovora* (10) was obtained by comparing fragment sizes from *Sal*I, *Pst*I, and *Kpn*I restriction digests of the isolated plasmid with fragment sizes reported in Table 1 of Falkenstein et al (7). Single digests with each of these restriction enzymes of the plasmid isolated from strain Ea88-90 yielded 4, 9, and 5 restriction fragments, respectively. Streptomycin-sensitive strain BC06 contained a large plasmid in addition to the 30-kb plasmid.

Southern analysis. A single 2.7-kb fragment in *Ava*I digests of both plasmid and total genomic DNAs of two streptomycin-resistant strains of *E. amylovora* hybridized with probe SMP3 (Fig. 2, lanes 5, 6, 9, and 10). None of the *Ava*I fragments of DNAs from two streptomycin-sensitive strains of *E. amylovora* hybridized with the probe (Fig. 2, lanes 7, 8, 11, and 12). Hybridization with probe SMP3 also occurred with a 1.5-kb fragment in *Ava*I digests of plasmid and of total genomic DNAs from streptomycin-resistant *P. s. papulans* strain Psp36 (Fig. 2, lanes 1 and 2) and with a 3.7-kb fragment in digested DNAs from

E. coli with plasmid pCPP505 (Fig. 2, lanes 3 and 4).

Bacterial conjugation. Transfer of the plasmid carrying the gene for resistance to streptomycin in donor strains HO62-1 and CA11 was achieved in matings with four recipient strains (Table 1). The frequency of transfer of resistance varied from 2.7×10^{-5} to 4.5×10^{-2}. Spontaneous mutations to streptomycin resistance were less than 2.0×10^{-10}, and spontaneous mutations to rifampicin resistance was about 5.0×10^{-8} per donor cell.

Transfer of streptomycin resistance in *E. amylovora* was associated with transfer of the 33-kb plasmid. All transconjugants resulting from the mating of donor strains CA11 and recipient strains BC06 rif^r and EL01 rif^r contained a 33-kb plasmid not present in the recipient strains (Fig. 3). DNA of putative transconjugants, randomly chosen from antibiotic selection plates, always contained an *Ava*I restriction fragment identical to one in restriction digests of DNA from donor strains but absent in restriction digests of DNA from recipient strains (Fig. 4A). Plasmid DNA from transconjugant BC06 rif^r × HO62-1 contained a 23-kb restriction fragment associated with donor HO62-1 and CA11 but not with recipient BC06 rif^r (Fig. 4A, lanes 4–6). A 2.7-kb *Ava*I restriction fragment in streptomycin-resistant donor and transconjugant strains, but not in streptomycin-sensitive recipient strains, hybridized with probe SMP3 (Fig. 4B, lanes 2–4, 6, and 7).

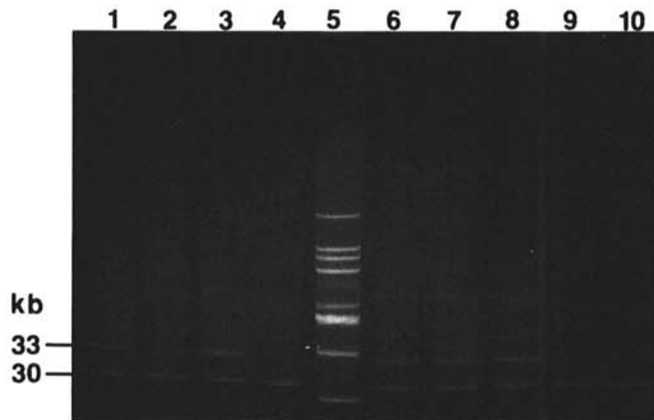

Fig. 1. Plasmids in field strains of *Erwinia amylovora* isolated from apple orchard SW in southwest Michigan and from adjacent crabapple trees. Streptomycin-resistant strains CA13, CA11, HT16, HT01-1, and HT06 are in lanes 1, 3, 6, 7, and 8, respectively, and streptomycin-sensitive strains CA10, CA06, HT03, and HT01 are in lanes 2, 4, 9, and 10, respectively. Lane 5 contains *Erwinia stewartii* strain SW2.

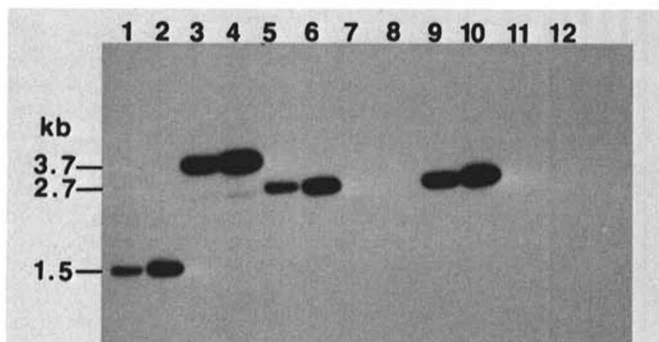

Fig. 2. Autoradiograph of a Southern blot of plasmid DNA, first, and total genomic DNA (including plasmid DNA), second, of *Pseudomonas syringae* pv. *papulans* strain Psp36 (lanes 1 and 2), and DH1 with plasmid pCPP505 (lanes 3 and 4) and streptomycin-resistant (CA11, lanes 5 and 6; HO62-1, lanes 9 and 10) and streptomycin-susceptible (BC06, lanes 7 and 8; EL01, lanes 11 and 12) strains of *Erwinia amylovora* digested with *Ava*I and hybridized to probe SMP3. The sizes (in kb) of hybridizing fragments calculated from *Eco*RI-*Hind*III-digested bacteria phage lambda DNA are given at the far left.

DISCUSSION

The detection of a putative streptomycin-resistance gene in *E. amylovora* that grew on media amended with streptomycin is strong evidence that streptomycin-resistant strains of the bacterium are present in Michigan. In addition, our conjugation and hybridization studies provide evidence that resistance in *E. amylovora* from Michigan is plasmid-borne. In our study, probe SMP3 did not hybridize with DNA from streptomycin-resistant strain Ea88-90 of *E. amylovora* from Washington. The failure of probe SMP3 to hybridize with a streptomycin-resistant strain from the western United States is evidence that the resistance system in the two regions is unrelated.

DNA sequences from streptomycin-resistant *E. amylovora* from Michigan hybridized with DNA sequences from the internal portion of a streptomycin-resistant gene cloned from strain Psp36 of *P. s. papulans*. In previous studies on streptomycin resistance in field strains of *E. amylovora* from California, resistance was found to be chromosomal (18). In addition, a 4.9-kb DNA subclone from streptomycin-resistant *X. c. vesicatoria* did not hybridize with DNA from streptomycin-resistant strain UCBPP 829 of *E. amylovora* from California, but it did hybridize with DNA from strain Psp36 of *P. s. papulans* from New York (15). This is evidence that the DNA sequences in the two pathogens are closely related. Base sequencing and other forms of genetic analyses are needed to establish whether the genes in the two pathogens are identical. Because the DNA sequences associated with resistance in *P. s. papulans* and *X. c. vesicatoria* are related (15), the DNA associated with resistance in *E. amylovora* must also be closely related to the DNA associated with streptomycin-resistance in *X. c. vesicatoria*.

The detection of streptomycin-resistant *E. amylovora* in orchard SW and in crabapple trees next to orchard SW, but not in 19 other orchards, is evidence that resistant strains of the bacterium are currently localized in Michigan's large apple-growing region. The high frequency of transfer of streptomycin resistance from donor strains HO62-1 and CA11 is evidence that transfer of resistance to other strains of the bacterium could result in a rapid increase in streptomycin-resistant *E. amylovora* in Michigan orchards. The spread and buildup of streptomycin-resistant *E.*

TABLE 1. Frequency of conjugational transfer of resistance from streptomycin-resistant to streptomycin-sensitive *Erwinia amylovora*

Recipient strains	Donor strains		Recipient means
	HO62-1	CA11	
EL01	2.8×10^{-5}	2.7×10^{-5}	2.8×10^{-5}
BC06	6.2×10^{-4}	3.8×10^{-4}	5.0×10^{-4}
GR05	3.2×10^{-2}	1.0×10^{-2}	2.1×10^{-2}
MA05	4.5×10^{-2}	2.5×10^{-2}	3.5×10^{-2}

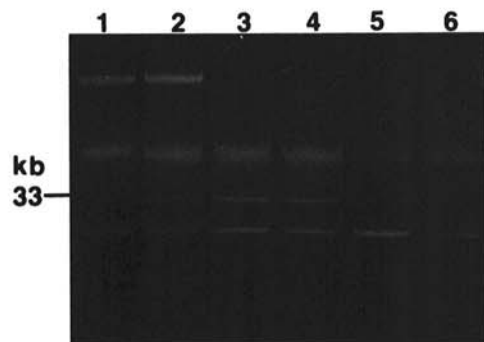

Fig. 3. Agarose gel electrophoresis of cleared lysates of donor, recipient, and transconjugant strains of *Erwinia amylovora*. Lane 1, recipient BC06 rif^r; lane 2, transconjugant BC06 rif^r × CA11; lane 3, donor CA11; lane 4, transconjugant EL01 rif^r × CA11; lane 5, recipient EL01 rif^r; lane 6, strain Ea88-90 with plasmid identical in restriction pattern to pEA29 (7).

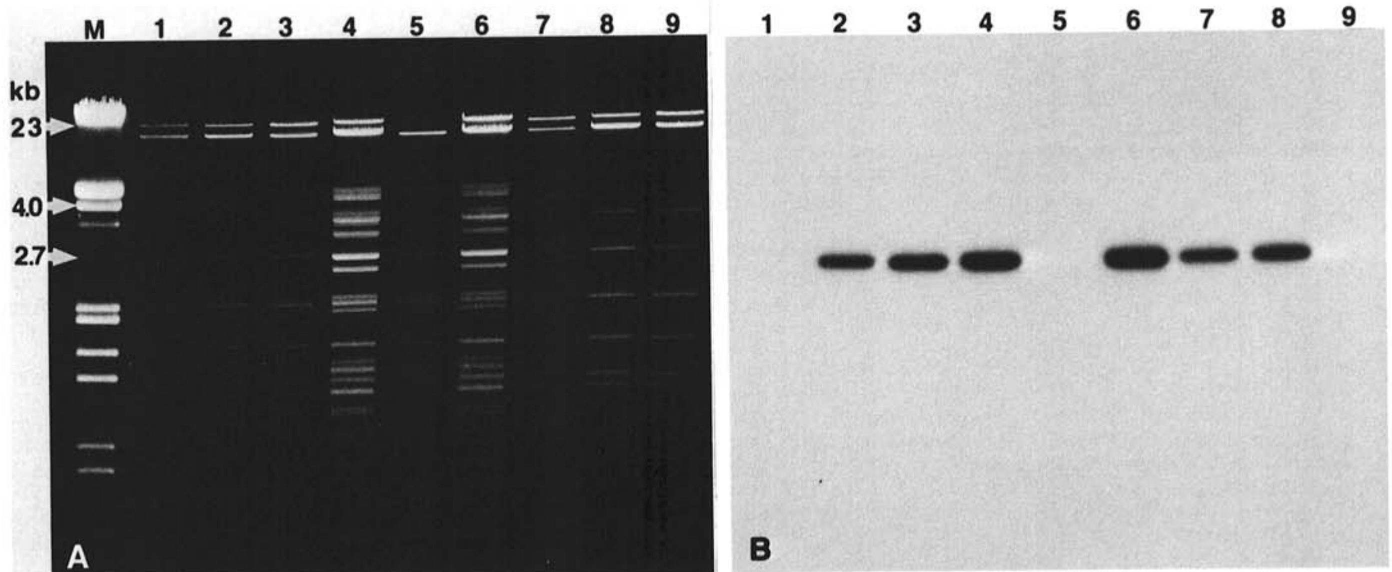

Fig. 4. Ethidium bromide-stained gel (A) and autoradiograph of a Southern blot (B) of plasmid DNAs of donor, recipient, and transconjugant strains of *Erwinia amylovora* digested with *Ava*I and hybridized to probe SMP3. Lane 1, recipient EL01 rif^r; lane 2, transconjugant EL01 rif^r × CA11; lane 3, donor CA11; lane 4, transconjugant BC06 rif^r × CA11: lane 5, recipient BC06 rif^r; lane 6, transconjugant BC06 rif^r × HO62-1; lane 7 donor HO62-1; lane 8, transconjugant EL01 rif^r × HO62-1; and lane 9, recipient EL01 rif^r. The sizes (in kb) of hybridizing fragments calculated from *Eco*RI-*Hind*III-digested bacteria phage lambda DNA are given at the far left.

amylovora presents a serious economic threat to apple and pear growers because of the lack of alternative bactericides for the control of fire blight.

To limit further spread of resistant strains and reduce the likelihood of selecting resistant strains elsewhere in Michigan, it is important that apple and pear growers limit applications of streptomycin to a period from bloom to about the first cover stage of bud development (a maximum of four applications per season). The frequency of spray applications could be reduced even more by using a forecast system to aid in the timing of applications of streptomycin for the control of fire blight (20). Although the occurrence of streptomycin-resistant *E. amylovora* in California and Washington could not be correlated with the use of streptomycin (11,18), it was suggested that selection pressure was not sufficient in the East, due to the limited use of streptomycin, to cause resistant strains to build up to detectable levels (16). Orchard SW has had a history of fire blight problems for over 20 yr, and the management program for fire blight in this orchard has been one of the most intensive in Michigan (A. L. Jones, *personal observation*). Grower records indicate that in 1988, 1989, and 1990 streptomycin was applied in 6, 7, and 8 applications (5.4 kg/ha of actual streptomycin over 3 yr), respectively. The continued and frequent use of streptomycin in orchard SW could have resulted in the buildup of streptomycin-resistant *E. amylovora* to detectable levels. Although Terramycin-streptomycin combinations have been shown to delay resistance in laboratory trials (6), this strategy cannot be utilized until Terramycin is registered on apples.

Because we detected streptomycin-resistant *E. amylovora* in crabapple trees, there is evidence that the resistant strains have started to move out of orchard SW. Now these infected crabapple trees are a potential source of resistant strains for reinfecting orchard SW and for infecting other orchards currently free of resistant strains. Removal of these crabapple trees would reduce the potential for further movement of resistant strains in southwest Michigan. It is also likely that the resistant strains in the two orchards have a common source. Strain HO62-1 from orchard SW and strain CA11 from crabapples carried the resistance gene on the same size plasmid. Also, no restriction-length polymorphism was observed among *Ava*I DNA fragments from the two strains, but *Ava*I digests of DNA from some sensitive strains produced different restriction patterns.

Unlike *P. s. papulans*, with its high diversity in plasmid content (3,8), the plasmid content of *E. amylovora* is predictable and not diverse. All strains have a plasmid of approximately 30 kb, and some strains have an additional plasmid of approximately 65 kb (13). The failure to detect a plasmid of approximately 33 kb in earlier studies on plasmids in *E. amylovora* suggests that it may be a recent introduction into the bacterium. It is possible that a transposable element carrying the resistance gene has been inserted into the approximately 30-kb plasmid, or that *E. amylovora* has acquired a new plasmid. Characterization of the plasmid of approximately 33 kb would help to determine if one of these possibilities is correct.

The streptomycin-resistance gene identified in *E. amylovora* is widely geographically distributed in *P. s. papulans* and in *X. c. vesicatoria* (3,8,15). It is also widely distributed among a diverse group of gram-negative bacteria isolated from apple orchards in New York (17) and in Michigan, including orchard SW (P. Sobiczewski, C.-S. Chiou, and A. L. Jones, *unpublished data*). The presence of a homologous streptomycin-resistance gene in both saprophytic and pathogenic bacteria that inhabit apple suggests that acquisition of streptomycin resistance by *E. amylovora* was by conjugation. We are currently investigating the possibility that the gene can be transferred from saprophytic gram-negative bacteria to *E. amylovora.*

LITERATURE CITED

1. Beer, S. V. 1990. Fire blight. Pages 61-63 in: Compendium of Apple and Pear Diseases. A. L. Jones and H. S. Aldwinckle, eds. American Phytopathological Society, St. Paul, MN.
2. Beer, S. V., and Norelli, J. L. 1976. Streptomycin-resistant *Erwinia amylovora* not found in western New York pear and apple orchards. Plant Dis. Rep. 60:624-626.
3. Burr, T. J., Norelli, J. L., Katz, B., Wilcox, W. F., and Hoying, S. A. 1988. Streptomycin resistance of *Pseudomonas syringae* pv. *papulans* in apple orchards and its association with a conjugative plasmid. Phytopathology 78:410-413.
4. Coyier, D. L., and Covey, R. P. 1975. Tolerance of *Erwinia amylovora* to streptomycin sulfate in Oregon and Washington. Plant Dis. Rep. 59:849-852.
5. Crosse, J. E., and Goodman, R. N. 1973. A selective medium for and a definitive colony characteristic of *Erwinia amylovora*. Phytopathology 63:1425-1426.
6. English, A. R., and Van Halsema, G. 1954. A note on the delay

in emergence of resistant *Xanthomonas* and *Erwinia* strains by the use of streptomycin plus terramycin combinations. Plant Dis. Rep. 38:429-431.

7. Falkenstein, H., Bellemann, P., Walter, S., Zeller, W., and Geider, K. 1988. Identification of *Erwinia amylovora*, the fireblight pathogen, by colony hybridization with DNA from plasmid pEA29. Appl. Environ. Microbiol. 54:2798-2802.
8. Jones, A. L., Norelli, J. L., and Ehret, G. R. 1991. Detection of streptomycin-resistant *Pseudomonas syringae* pv. *papulans* in Michigan apple orchards. Plant Dis. 75:529-531.
9. Kado, C. I., and Liu, S. T. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145:1365-1373.
10. Laurent, J., Barny, M.-A., Kotaujansky, A., Dufriche, P., and Vanneste, J. L. 1989. Characterization of a ubiquitous plasmid in *Erwinia amylovora*. Mol. Plant-Microbe Interact. 2:160-164.
11. Loper, J. E., Henkels, M. D., Roberts, R. G., Grove, G. G., Willett, M. J., and Smith, T. J. 1991. Evaluation of streptomycin, oxytetracycline, and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington State. Plant Dis. 75:287-290.
12. Maniatis, R., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
13. Merckaert, C., Thiry, M. E., Thiry, G. J., and Ledous, L. 1982. Characterization of the plasmids present in *Erwinia amylovora*. Arch. Int. Physiol. Biochem. 90:B54-B55.
14. Miller, T. D., and Schroth, M. N. 1972. Monitoring the epiphytic population of *Erwinia amylovora* on pear with a selective medium. Phytopathology 62:1175-1182.
15. Minsavage, G. V., Canteros, B. I., and Stall, R. E. 1990. Plasmid-mediated resistance to streptomycin in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 80:719-723.
16. Moller, W. J., Schroth, M. N., and Thomson, S. V. 1981. The scenario of fire blight and streptomycin resistance. Plant Dis. 65:563-568.
17. Norelli, J. L., Burr, T. J., Lo Cicero, A. M, Gilbert, M. T., and Katz, B. H. 1991. Homologous streptomycin resistance gene present among diverse gram-negative bacteria in New York apple orchards. Appl. Environ. Microbiol. 57:486-491.
18. Schroth, M. N., Thomson, S. V., and Moller, W. J. 1979. Streptomycin resistance in *Erwinia amylovora*. Phytopathology 69:565-568.
19. Shaffer, W. H., and Goodman, R. N. 1985. Appearance of streptomycin-resistant *Erwinia amylovora* in Missouri apple orchards. (Abstr.) Phytopathology 75:1281.
20. Steiner, P. W. 1990. Predicting apple blossom infections by *Erwinia amylovora* using the Maryblyt model. Acta Hortic. 273:139-148.
21. Sundin, G. W., Jones, A. L., and Fulbright, D. W. 1989. Copper resistance in *Pseudomonas syringae* pv. *syringae* from cherry orchards and its associated transfer in vitro and in planta with a plasmid. Phytopathology 79:861-865.
22. Sutton, T. B., and Jones, A. L. 1975. Monitoring *Erwinia amylovora* populations on apple in relation to disease incidence. Phytopathology 65:1009-1012.
23. Wilson, K. 1988. Preparation of genomic DNA from bacteria. Pages 2.4.1–2.4.5 in: Current Protocols in Molecular Biology. Vol. 1. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, eds. John Wiley & Sons, New York.