Streptomycin Resistance of *Pseudomonas syringae* pv. *papulans* in Apple Orchards and Its Association with a Conjugative Plasmid

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ABSTRACT

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Pseudomonas syringae pv. *papulans* strains resistant to streptomycin were isolated from blister spot lesions in 10 of 22 western New York apple orchards. The growth of a streptomycin-sensitive strain was inhibited by 2.5 μg of streptomycin per milliliter of culture medium. Resistant strains grew on medium containing 100 μg/ml but not on medium containing 250 μg/ml. There was a significant association between the number of streptomycin sprays applied in orchards in 1985 and the detection of streptomycin-resistant strains of *P. s.* pv. *papulans*. Streptomycin-resistant strains were isolated from dormant buds and from fruit lesions the year after initial detection of resistance. A 68-megadalton (MDa) plasmid was present in streptomycin-resistant field strains of *P. s.* pv. *papulans* but was not observed in streptomycin-sensitive field strains. Streptomycin resistance was transferred from streptomycin-resistant donor strains Psp 34 and Psp 36 to streptomycin-sensitive recipient strains after matings on nitrocellulose membranes at frequencies of 4.7×10^{-3} and 2.0×10^{-2} per recipient cell, respectively. Transconjugants contained the plasmids of recipient strains plus the 68-MDa plasmid of the donor strain. The 68-MDa plasmid of Psp 36 was designated pCPP501. Transfer of streptomycin resistance from donor strain Psp 33 occurred at the much lower frequency of 2.0×10^{-6} per recipient cell and resulted in more complex changes in the plasmid content of transconjugants.

Additional key word: blister spot.

Blister spot of apple, caused by *Pseudomonas syringae* pv. *papulans* (Rose) Dye, results in lesions associated with stomata on fruit and severely reduces fresh market value (4). The disease is predominant on, but not restricted to, the Mutsu cultivar (5,23). The bacterium overwinters primarily in buds and leaf scars (7) and infects fruit during a specific stage of fruit development (5). Recently, leaf infections of apple have also been reported (2). Growers have successfully controlled the disease with streptomycin sprays; however, in 1985 incidents of control failure after proper use of streptomycin were brought to our attention. This paper reports the presence of *P. s.* pv. *papulans* strains that are resistant to streptomycin in New York apple orchards, the ability of resistant strains to overwinter in dormant buds, and the association of streptomycin resistance with a conjugative plasmid.

MATERIALS AND METHODS

Orchard survey. Twenty-two Mutsu apple orchards in the western New York area were sampled for the presence of blister spot. This region covers about a 200- × 10-km band along the southern shore of Lake Ontario. Six to 10 fruit with blister spot symptoms were collected from each orchard in October and November 1985 and were transported to the laboratory in plastic bags. Where possible, the rate and number of streptomycin sprays applied in 1985 were recorded for each orchard. Two of the orchards sampled in 1985 were resampled in October 1986.

Assays for streptomycin resistance. Individual fruit samples were surface disinfested by soaking them for 10 min in a 0.5% sodium hypochlorite solution and then rinsing in distilled water. Isolations were made from typical blister spot lesions by cutting one lesion per fruit from five or six fruits per orchard, triturating lesion tissues in 0.1 ml of sterile distilled water (SDW) with a sterile mortar and pestle, and streaking the suspension on King's medium B (KB) (15). Lesions were usually 2–3 mm in diameter and penetrated less than 1 mm into the fruit flesh. Plates were incubated for 24–48 hr at 28 C after which time a typical colony of *P. s.* pv. *papulans* from each plate was subcultured onto KB. Typical colonies of *P. s.* pv. *papulans* produce a pale blue fluorescence under ultraviolet light and are flat and nonmucoid as compared with *P. s.* pv. *syringae* colonies, which produce a green-blue fluorescence and are domed and mucoid.

Purified strains were tested for resistance to streptomycin by evenly spreading 0.1 ml of suspensions containing 10^6 colony-forming units (cfu) per milliliter onto KB. After the surface moisture had dried, 12.7-mm-diameter filter paper disks (Schleicher & Schuell Inc., Keene, NH) were dipped in solutions of streptomycin sulfate containing 0, 10, 100, 250, or 1,000 μg/ml, blotted to remove excess moisture, and then placed equidistant apart on the surface of the plates. Plates were incubated at 28 C and checked for the presence of inhibition zones after 24, 48, and 72 hr.

The effect of various doses of streptomycin on the growth of the streptomycin-sensitive strain Psp 32 and the streptomycin-resistant strains Psp 33, Psp 34, and Psp 35 was determined by a plate count method. An overnight culture of the strains grown in Luria-Bertani (LB) broth (19) at 28 C was diluted appropriately and spread onto solid LB medium amended with 0, 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 μg of streptomycin per milliliter. Plates were incubated at 28 C for 48 hr, and the percent recovery was calculated by dividing the number of colonies that grew on amended media by that on nonamended medium.

Frequency of lesions containing resistant strains. The frequency with which resistant strains were associated with individual lesions in three of the orchards previously assayed was determined by directly isolating from 25 or 50 lesions per orchard onto KB medium containing a gradient of streptomycin using a modification of the method reported by Szybalski (26). Gradient plates were prepared by first pouring KB containing 500 μg of streptomycin per milliliter into plates that were elevated about 5 mm on one edge. The plates consequently contained medium about 5 mm thick at the nonelevated edge and no medium covering about one third of the plate. Plates were then laid flat on the bench and unamended KB was poured into each until the entire plate was filled to a depth of about 5 mm.

Lesions were individually cut from surface-disinfested fruit with a sterile scalpel, held with sterile forceps, and the internal tissues were streaked once across the diameter of the plates in the direction

of the streptomycin gradient. Five to six lesions were streaked per plate. A known sensitive strain was streaked on gradient plates as a control. Plates were incubated at 28 C for 48 hr and then evaluated for growth of resistant strains. Strains that grew greater distances across the gradient than the known sensitive strain were considered resistant.

Overwintering of resistant strains. To test for the ability of streptomycin-resistant strains to overwinter in apple buds, we collected buds from two orchards previously shown to contain streptomycin-resistant strains and one where streptomycin resistance was not previously found. About 500 buds were randomly collected from each of the three orchards on 20 February 1986. Isolations of *P. s.* pv. *papulans* were made from the buds by first surface sterilizing them in 0.5% sodium hypochlorite plus 0.05% tergitol for 10 min and thoroughly rinsing three times in distilled water. Ten samples of 10 buds each were assayed by removing the outer bud scales and triturating the 10 inner buds in 10 ml of SDW with a Waring blender. Serial water dilutions of bud tissue suspensions were plated on a medium for *Pseudomonas* spp. (6) that is semiselective and differential for *P. s.* pv. *papulans*. Plates were incubated at 28 C for 48 hr, at which time typical *P. s.* pv. *papulans* colonies were tested for resistance to streptomycin. Colony morphology of *P. s.* pv. *papulans* and *P. s.* pv. *syringae* on semiselective medium is similar to that observed on KB medium. Twenty seven, 51, and 52 strains were tested from orchards H, S, and R, respectively, by streaking them on KB and KB amended with 100 μg of streptomycin per milliliter. Growth was recorded after 96 hr at 28 C.

Bacterial strains. Field strains of *P. s.* pv. *papulans* that were used for plasmid characterization and conjugation studies were isolated from eight different orchards in the western New York area. All strains were isolated from blister spot lesions on Mutsu apple fruit except for strain Psp 21, which was isolated from a blister spot lesion on Golden Delicious fruit, and strain Psp 35, which was isolated from a Mutsu bud. Strains Psp 5 Rif^r and Psp 32 Rif^r are rifampicin-resistant mutants of Psp 5 and Psp 32, respectively, which were selected by the method of Liang et al (18). Psp 5 Rif^r is resistant to at least 2,000 μg of rifampicin per milliliter and Psp 32 Rif^r is resistant to up to 400 μg of rifampicin per milliliter. *Erwinia stewartii* strain SW2, used as a molecular size standard in plasmid characterization, was obtained from D. L. Coplin (10). Strain SW2 contains 13 plasmids with estimated masses of 210, 69.8, 51.6, 49.2, 43.3, 34.5, 33.0, 29.5, 23.2, 16.9, 8.8, 2.8, and 2.7 megadaltons (MDa).

Plasmid characterization. Plasmids were isolated by a modification of the method of Kado and Liu (14). The cells from 750 μl of an overnight culture grown in LB broth were pelleted in a microfuge tube, resuspended in 70 μl of distilled water, lysed with 300 μl of 3% sodium dodecyl sulfate in 50 mM Tris-HCl (pH 12.6), incubated at 55 C for 25 min, and extracted with 700 μl of a solution containing 25 volumes phenol: 24 volumes chloroform: 1 volume isoamyl alcohol. Plasmids were characterized by electrophoresis in a horizontal 0.7% agarose gel in Tris-acetate buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.2) at 5 V/cm (19).

Conjugation. Streptomycin-resistant strains Psp 33, Psp 34, and Psp 36 were mated on cellulose nitrate membrane filters with streptomycin-sensitive strains Psp 5 Rif^r and Psp 32 Rif^r to test for the conjugative transfer of streptomycin resistance. Cultures were first grown overnight in LB broth at 28 C, diluted threefold with LB broth, and grown for an additional 3 hr. Subsequently, 2.5 ml of a donor and recipient culture were mixed and placed on a cellulose nitrate filter (0.2 μm pore size) by vacuum filtration. After incubating filters on LB medium at 28 C for 16–20 hr, filters were washed in sterile 0.05 M potassium phosphate buffer, pH 6.5. Suspensions were diluted appropriately and spread on LB medium amended with either 40 μg of streptomycin per milliliter, 50 μg of rifampicin per milliliter, or 40 μg of streptomycin per milliliter plus 50 μg of rifampicin per milliliter to determine the number of donor, recipient, and transconjugant cells present, respectively. One to 20 transconjugant colonies was selected per mating for plasmid characterization. Matings were replicated three times, and differences in the frequency of transfer to recipient and from donor strains were analyzed by a two-way analysis of variance.

RESULTS

Orchard survey. Streptomycin-resistant strains of *P. s.* pv. *papulans* were detected in 10 of 22 orchards (Table 1). All strains tested from three of the orchards were resistant, whereas seven orchards had a mixture of sensitive and resistant strains (Table 1). All strains grew up to the margins of filter paper disks that were soaked in 0 and 10 μg of streptomycin per milliliter. Sensitive strains were indicated by clear zones of growth inhibition around disks containing all other concentrations. Growth of resistant strains was not inhibited around disks containing 1,000 μg/ml. All resistant strains reacted identically at the various concentrations of streptomycin using this assay.

There was 100% recovery of the streptomycin-sensitive strain Psp 32 when plated on the medium containing 1 μg/ml of streptomycin but less than 1% recovery on the medium containing 2.5 μg/ml. The recovery of the streptomycin-resistant strains Psp 33, Psp 34, and Psp 35 was not significantly different from the nonamended control medium when the media contained 1–100 μg/ml of streptomycin, but was less than 1% on the medium containing 250 μg/ml.

The number of streptomycin sprays previously applied in the orchards appeared to affect the development of resistance. Orchard sprays generally consisted of 50 or 100 μg/ml of streptomycin (3). Resistance was not detected in the three orchards that were not sprayed in 1985 or in 10 of 12 that received three or fewer sprays. However, resistance was detected in six of seven orchards that were sprayed four or more times (Table 1). The Kruskal-Wallis ranking procedure (16) indicated a significant association ($P = 0.05$) between the number of streptomycin sprays applied in 1985 and the isolation of *P. s.* pv. *papulans* strains resistant to streptomycin.

Frequency of lesions containing resistant strains. Gradient plates were useful for assaying the presence of streptomycin-resistant *P. s.* pv. *papulans* directly from lesions. In almost all cases, no bacteria other than *P. s.* pv. *papulans* were observed on the plates. Orchards H, I, and J had 0 of 25, 0 of 50, and 43 of 50 lesions that yielded resistant strains, respectively. These results compared favorably with the filter paper disk method for determining the presence of resistance in the orchards.

Overwintering of resistant strains. Streptomycin-resistant

TABLE 1. Presence of *Pseudomonas syringae* pv. *papulans* resistant to streptomycin in western New York orchards

Orchard	Strains tested (no.)	Resistant strains (no.)	Streptomycin sprays, 1985 (no.)
A	3	2	4
B	6	0	2
C	3	0	2
D	5	0	3
E	5	4	4
F	4	0	4
G	5	0	0
H	5	0	NA[a]
I	5	0	3
J	5	4	NA
K	4	0	0
L	6	2	NA
M	6	0	3
N	6	0	3
O	5	1	4
P	5	0	0
Q	3	1	3
R	4	4	8
S	3	3	4
T	5	5	4
U	3	0	2
V	5	3	3

[a] NA indicates spray records not available.

strains were detected in dormant apple buds collected from the orchards that were previously determined to contain resistant strains. Fifty-one of 52 strains from orchard R and 32 of 51 from orchard S grew on KB amended with 100 μg/ml of streptomycin. In contrast, none of 27 strains from orchard H (no resistance previously detected) were resistant.

Further evidence of persistence of resistance was obtained by analyzing blister spot lesions from orchards R and S in October 1986 for the presence of resistant *P. s.* pv. *papulans*. Lesions were streaked on gradient plates as previously described. Fifteen of 15 isolations yielded resistant strains from orchard R and 10 of 13 from orchard S.

Plasmid characterization of field strains. The plasmid content of *P. s.* pv. *papulans* strains was highly diverse (Fig. 1). However, the streptomycin-resistant strains Psp 33, Psp 34, Psp 35, Psp 36, Psp 37, Psp 39, and Psp 40 all contained a plasmid of approximately 68 MDa, designated pCPP501 in Psp 36. The streptomycin-sensitive strains Psp 5, Psp 21, Psp 32, and Psp 38 did not contain a plasmid of this size.

Strains Psp 36, Psp 37, Psp 38, and Psp 39 were all isolated from the same orchard yet contained different plasmids (Fig. 1). Several strains from this same orchard had identical plasmid content (data not shown). Strains Psp 35 and Psp 40 were isolated from different orchards and had the same plasmid content (Fig. 1).

Conjugation. Resistance to streptomycin was transferred from resistant- to sensitive-strains of *P. s.* pv. *papulans* by mating on nitrocellulose membranes. The frequency of transfer from strains Psp 33, Psp 34, and Psp 36 was 2.0×10^{-6}, 4.7×10^{-3}, and 2.0×10^{-2} per recipient cell, respectively (Table 2). There was a significant difference ($P = 0.01$) in the frequency of transfer among donor strains but not among recipient strains. The observed frequency of spontaneous mutation for resistance to rifampicin among donor strains was 3.9×10^{-8}, and for resistance to streptomycin among recipient strains was 3.4×10^{-10}.

Transfer of streptomycin resistance in *P. s.* pv. *papulans* was associated with changes in the plasmid content of transconjugants (Fig. 2). Twenty-nine of 30 streptomycin-resistant transconjugants from matings with donor strains Psp 34 and Psp 36 contained the plasmids of the recipient strain plus the 68-MDa plasmid of the donor strain (B, C, F, and G in Fig. 2). One transconjugant contained the 68-MDa plasmid but had undergone other plasmid rearrangements (D in Fig. 2). Transconjugants resulting from matings of donor strains Psp 33 and recipient strain Psp 5 Rif^r contained a 75-MDa plasmid not present in the recipient strain and lost a 21-MDa plasmid (A in Fig. 2). Transconjugants resulting from matings of donor strain Psp 33 and recipient strain Psp 32 Rif^r also contained a 75-MDa plasmid not present in recipient strains but lost a 40-MDa plasmid (E in Fig. 2). The native plasmid lost after mating with Psp 33 differed depending on the recipient strain, but the 21-MDa plasmid of Psp 5 Rif^r and the 40-MDa plasmid of Psp 32 Rif^r were consistently lost in four distinct transconjugants characterized from each recipient.

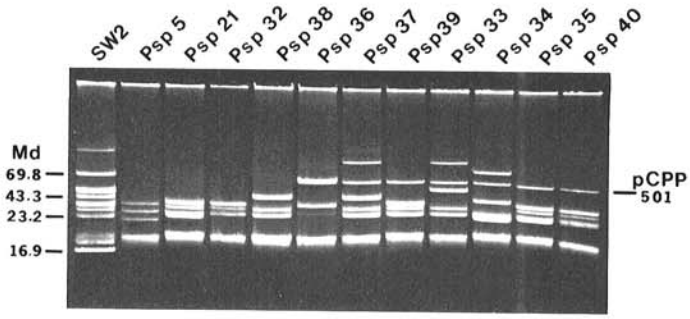

Fig. 1. Plasmid characterization of field strains of *Pseudomonas syringae* pv. *papulans*. Strains Psp 5, Psp 21, Psp 32, and Psp 38 are sensitive to streptomycin. Strains Psp 36, Psp 37, Psp 39, Psp 33, Psp 34, Psp 35, and Psp 40 are resistant to streptomycin.

TABLE 2. Frequency of conjugational transfer of streptomycin resistance in *Pseudomonas syringae* pv. *papulans*

Recipient strains	Donor strains			Recipient means
	Psp 33	Psp 34	Psp 36	
Psp 5 Rif^r	2.4×10^{-6}[a]	5.2×10^{-3}	1.8×10^{-2}	6.1×10^{-4}
Psp 32 Rif^r	1.6×10^{-6}	4.2×10^{-3}	2.2×10^{-2}	5.4×10^{-4}
Donor means	2.0×10^{-6}	4.7×10^{-3}	2.0×10^{-2}	5.7×10^{-4}

Analysis of variance

Source of variation	Degrees of freedom	Mean square[b]	F
Recipient strains	1	0.0158	0.79
Donor strains	2	27.8159	1,383.87**
Interaction	2	0.0265	1.32
Error	12	0.0201	

[a] Frequency of transfer of streptomycin resistance per recipient cell.
[b] Calculated from the log10 of the frequency of transfer. Asterisks (**) denote statistical significance, $P = 0.01$.

DISCUSSION

Resistance of *P. s.* pv. *papulans* to streptomycin presents a serious economic problem to apple growers because no alternative bactericides are available for control of blister spot. Although copper oxychloride sulfate and copper hydroxide are being tested, both are phytotoxic to the fruit if applied during periods when fruit are most susceptible (Burr, *unpublished data*).

Resistance to streptomycin in *P. s.* pv. *papulans* was associated with a 68-MDa conjugative plasmid. Characterization of the plasmids of field strains indicated a plasmid of this size present in all resistant strains but in no sensitive strains. Similarly, strains Psp 34 and Psp 36 transferred resistance to streptomycin-sensitive strains at a high frequency in conjugational matings and cotransferred the 68-MDa plasmid to recipient strains. The 68-MDa plasmid of Psp 36 has been designated pCPP501. It is not known if this plasmid is identical to the 68-MDa plasmid present in the other streptomycin-resistant field strains.

In the case of donor strain Psp 33, the reduced frequency of transfer of streptomycin resistance was associated with more complex changes in the plasmid content of transconjugants. These changes could have arisen by the transfer of the larger 75-MDa plasmid to recipient strains and its incompatibility with a smaller native plasmid, or alternatively, by the transfer of the 68-MDa plasmid, its integration into a smaller native plasmid, and subsequent deletions. Either a lower frequency of transfer of the 75-MDa plasmid or the reduced probability of more complex plasmid rearrangements could explain the much lower freqency of transfer of streptomycin resistance from Psp 33.

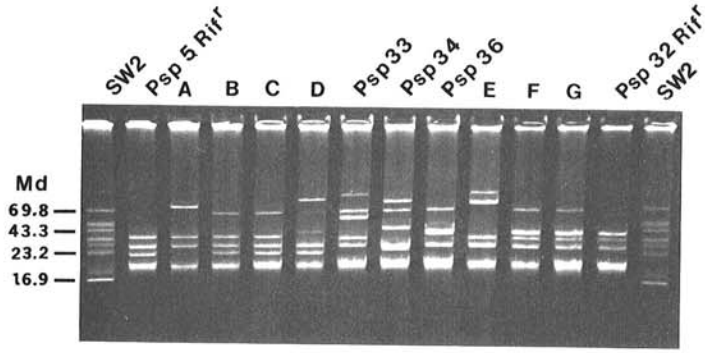

Fig. 2. Plasmid characterization of streptomycin-resistant transconjugants of *Pseudomonas syringae* pv. *papulans* resulting from a mating of streptomycin-resistant donor strains Psp 33, Psp 34, and Psp 36 and streptomycin-sensitive recipient strains Psp 5 Rif^r and Psp 32 Rif^r. Streptomycin resistant transconjugants A–G resulted from matings of: A, Psp 5 Rif^r × Psp 33; B, Psp 5 Rif^r × Psp 34; C and D, Psp 5 Rif^r × Psp 36; E, Psp 32 Rif^r × Psp 33; F, Psp 32 Rif^r × Psp 34; and G, Psp 32 Rif^r × Psp 36.

Resistance apparently increased rapidly in orchards since the use of streptomycin for controlling blister spot has only been recommended since 1982 (3). Although a maximum of three sprays was recommended, some growers used additional sprays because the market value of Mutsu is high and some disease started developing. In addition, growers may spray streptomycin during bloom for control of fire blight. Applications during this period would not coincide with the blister spot sprays but would expose *P. s.* pv. *papulans* to streptomycin because the bacteria are epiphytic on leaf and blossom surfaces at that time (6).

The high frequency of transfer of streptomycin resistance from donor strains Psp 34 and Psp 36 could explain the rapid increase of streptomycin-resistant *P. s.* pv. *papulans* in New York orchards. The origin of the 68-MDa plasmid associated with streptomycin resistance is not known. Plasmid characterization of field strains (Fig. 1) indicated a large diversity of plasmids within *P. s.* pv. *papulans*, and it may have been present in the population of *P. s.* pv. *papulans* at a low frequency before the widespread use of streptomycin for blister spot control. It is also possible that the 68-MDa plasmid originated in another species of bacterial epiphyte and was transferred to *P. s.* pv. *papulans*. Plasmid pCPP501 can be transferred at a low frequency (approximately 10^{-7} per recipient cell) from *P. s.* pv. *papulans* to *P. s.* pv. *syringae* strains by conjugation (Norelli, *unpublished data*).

The degree of exposure of *P. s.* pv. *papulans* to streptomycin appeared to affect the development of resistance. In general, resistance was detected in orchards that had been sprayed the greatest number of times. These results may be misleading since only three to six strains of *P. s.* pv. *papulans* were tested from most orchards, and, by testing additional strains, resistance may have been detected. However, resistance was not detected even when 25 or 50 strains of *P. s.* pv. *papulans* were tested from orchards where they were not previously detected using the smaller sample sizes.

Several occurrences of streptomycin-resistant plant pathogenic bacteria have been reported (11,20,22,25,28). However, this is the first report of the field occurrence of a streptomycin-resistant plant pathogenic bacterium associated with a conjugative plasmid. Resistance to streptomycin has been associated with conjugative R plasmids in the Enterobacteriaceae (27). Resistance to copper in *Xanthomonas campestris* pv. *vesicatoria* (24) and *P. syringae* pv. *tomato* (1) and resistance to arsenite in *Corynebacterium flaccumfaciens* subsp. *oortii* (13) have also been associated with conjugative plasmids. There have been several laboratory demonstrations of the conjugative transfer of antibiotic resistance between plant pathogenic bacteria and bacteria associated with plants and animals (8,9,12,17,21). It would appear that the potential spread of pCPP501 or a similar plasmid to other plant pathogenic or human bacteria could pose serious economic and health risks.

LITERATURE CITED

1. Bender, C. L., and Cooksey, D. A. 1986. Indigenous plasmids in *Pseudomonas syringae* pv. *tomato*: Conjugative transfer and role in copper resistance. J. Bacteriol. 165:534-541.
2. Bonn, W. G., and Bedford, K. E. 1986. Midvein necrosis of Mutsu apple caused by *Pseudomonas syringae* pv. *papulans*. Can. J. Plant Pathol. 8:167-169.
3. Burr, T. J. 1982. Blister spot of apple. NY Food Life Sci. Bull. 95. 2 pp.
4. Burr, T. J., and Hurwitz, B. 1979. The etiology of blister spot of 'Mutsu' apple in New York State. Plant Dis. Rep. 63:157-160.
5. Burr, T. J., and Hurwitz, B. 1981. Seasonal susceptibility of Mutsu apples to *Pseudomonas syringae* pv. *papulans*. Plant Dis. 65:334-336.
6. Burr, T. J., and Katz, B. 1982. Evaluation of a selective medium for detecting *Pseudomonas syringae* pv. *papulans* and *P. syringae* pv. *syringae* in apple orchards. Phytopathology 72:564-567.
7. Burr, T. J., and Katz, B. H. 1984. Overwintering and distribution of *Pseudomonas syringae* pv. *papulans* in apple buds. Plant Dis. 68:383-385.
8. Chatterjee, A. K., and Starr, M. P. 1973. Transfer among *Erwinia* spp. and other enterobacteria of antibiotic resistance carried on R factors. J. Bacteriol. 112:576-584.
9. Cho, J. J., Panopoulos, N. J., and Schroth, M. N. 1975. Genetic transfer of *Pseudomonas aeruginosa* R factors to plant pathogenic *Erwinia* species. J. Bacteriol. 122:253-263.
10. Coplin, D. L., Rowan, R. G., Chisholm, D. A., and Whitmoyer, R. E. 1981. Characterization of plasmids in *Erwinia stewartii*. Appl. Environ. Microbiol. 42:599-604.
11. DeBoer, S. H. 1980. Leaf spot of cherry laurel caused by *Pseudomonas syringae*. Can. J. Plant Pathol. 2:235-238.
12. Gibbins, L. N., Bennett, P. M., Saunders, J. R., Grinsted, J., and Connolly, J. C. 1976. Acceptance and transfer of R-factor RPI by members of the "Herbicola" group of the genus *Erwinia*. J. Bacteriol. 128:309-316.
13. Hendrick, C. A., Haskins, W. P., and Vidaver, A. K. 1984. Conjugative plasmid in *Corynebacterium flaccumfaciens* subsp. *oortii* that confers resistance to arsenite, arsenate, and antimony (III). Appl. Environ. Microbiol. 48:55-60.
14. Kado, C. I., and Liu, S. T. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145:1365-1373.
15. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
16. Kruskal, W. H., and Wallis, W. A. 1952. Use of ranks in one-criterion analysis of variance. J. Am. Statist. Assoc. 47:583-621.
17. Lacy, G. H., and Leary, J. V. 1975. Transfer of antibiotic resistance plasmid RPI into *Pseudomonas glycinea* and *Pseudomonas phaseolicola* in vitro and in planta. J. Gen. Microbiol. 88:49-57.
18. Liang, L. N., Sinclair, J. L., Mallory, L. M., and Alexander, M. 1982. Fate in model ecosystem of microbial species of potential use in genetic engineering. Appl. Environ. Microbiol. 44:708-714.
19. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
20. Ogawa, J. M., Gilpatrick, J. D., and Chiarappa, L. 1977. Review of plant pathogens tolerant to fungicides and bactericides. FAO Plant Prot. Bull. 1977, 25:97-111.
21. Panopoulos, N. J., Cho, J. J., Guimaraes, W. V., and Schroth, M. N. 1975. Conjugative transfer of *Pseudomonas aeruginosa* R factors to plant pathogenic *Pseudomonas* spp. Phytopathology 65:380-388.
22. Schroth, M. N., Thomson, S. V., and Moller, W. J. 1979. Streptomycin resistance in *Erwinia amylovora*. Phytopathology 69:565-568.
23. Smith, M. A. 1944. Blister spot, a bacterial disease of apple. J. Agric. Res. 68:269-298.
24. Stall, R. E., Laschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 76:240-243.
25. Stall, R. E., and Thayer, P. L. 1962. Streptomycin resistance of the bacterial spot pathogen and control with streptomycin. Plant Dis. Rep. 46:389-392.
26. Szybalski, W. 1952. Microbial selection. I. Gradient plate technique for study of bacterial resistance. Science 116:46-48.
27. Watanabe, T., Nishida, H., Ogata, C., Arai, T., and Sato, S. 1964. Episome mediated transfer of drug resistance in Enterobacteriaceae. VII. Two types of naturally occurring R factors. J. Bacteriol. 88:716-726.
28. Young, J. M. 1977. Resistance to streptomycin in *Pseudomonas syringae* from apricot. N.Z. J. Agric. Res. 20:249-251.