Diversity of Plasmids in *Xanthomonas campestris* pv. *vesicatoria*

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ABSTRACT

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Plasmid profiles were determined for 522 strains of *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial spot of tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*). The numbers and sizes of plasmids in strains from culture collections, as well as in strains recently isolated from plants in commercial fields of pepper or tomato, were diverse. Each plasmid was placed into one of 13 classes based on size, as determined by electrophoretic mobility in agarose gels. Plasmid sizes ranged from about 3 to 300 kb. Ten strains had no detectable plasmid. Seventy-one different plasmid profiles were observed, with a maximum of five plasmid size classes in a profile. No plasmid or plasmid profile was characteristic for *X. campestris* pv. *vesicatoria*. Mobility of plasmids was demonstrated in field experiments after inoculation of pepper plants with donor strains containing plasmids in five classes and an antibiotic-resistant recipient strain that contained one unique plasmid. All strains recovered on an antibiotic-amended medium contained the unique plasmid present in the recipient strain. Twenty-nine percent of the 199 cultures recovered on the antibiotic-amended medium in one experiment contained one or more plasmids that were similar in size to those in the donor strains; seven different plasmid profiles were observed. The diverse plasmid content of strains of *X. campestris* pv. *vesicatoria* in nature may be the result of frequent transfer of the plasmids between strains.

Xanthomonas campestris pv. *vesicatoria* causes the bacterial spot disease of tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.). The disease occurs wherever these crops are grown, but is most prevalent when temperatures and rainfall are high (14). When conditions are optimum and the bacterium is present, numerous lesions develop on foliage and fruit, which results in defoliation and culled fruit (14). Control of bacterial spot with copper and/or streptomycin sprays or resistant plants has been erratic because of variation in populations of the bacterium in their resistance to the bactericides and in races that do not interact with resistance genes in the hosts.

Genes for resistance to copper and resistance to streptomycin are plasmid-encoded in *X. campestris* pv. *vesicatoria* (1,6,19). Genes for resistance to copper and avirulence to a cultivar of pepper were found to be linked on a large plasmid, pXvCu1 (28), which also carried genes for prototrophic growth (R. E. Stall, *unpublished data*). Avirulence genes that are associated with resistance of all tomato or all pepper plants to certain strains of *X. campestris* pv. *vesicatoria* are located on other plasmids (4,20).

Extensive surveys of plasmid content in strains of *X. campestris* pv. *vesicatoria* have not been reported, even though the ecologically important traits in bacteria are plasmid-encoded (25). In a preliminary analysis of seven strains, two to five plasmids were detected (11). Lazo and Gabriel (16) also reported plasmids in strains of *X. campestris* pv. *vesicatoria* when five strains were included in a study of plasmid content among strains of pathovars of *X. campestris*.

Important epidemiological information has been obtained from plasmid profiles of large numbers of strains of other pathogens. For example, Noble and Rahman (22) determined plasmid profiles in strains of *Staphylococcus aureus* that were isolated 25 years previously. The epidemiological characteristics of phage type, antibiotic sensitivity, and geographical location were associated with the plasmid data. A large number of strains of *Salmonella* spp. were isolated over a period of 10 years and a large variety of R-plasmids were detected that spread differentially to certain strains (17). In native populations of *Rhizobium* spp., the plant root nodule bacterium, a great diversity in both number and size of plasmids was observed (21). Thirty-three different plasmid profiles were observed in a natural population of about 200 strains of *R. leguminosarum* biovar *viciae* (3). The grouping by plasmid profiles was strongly associated with grouping by serogroup and by intrinsic antibiotic resistance.

This paper reports a comprehensive study of plasmid profiles in strains of *X. campestris* pv. *vesicatoria* obtained from culture collections from distinct geographical locations and from plants in fields of tomato or pepper. The purpose of this analysis was to determine if a single plasmid, or a plasmid profile, was communal in strains of *X. campestris* pv. *vesicatoria*. The extreme variation in the plasmid content in strains led to tests of mobility of plasmids under field conditions.

MATERIALS AND METHODS

Origin of strains. Strains were obtained from a reference collection (R. E. Stall, University of Florida) (UF) of *X. campestris* pv. *vesicatoria* which consisted of 218 strains collected from 1960 to 1988. The strains were mainly from the southeastern United States. Strains in the Taiwan (TW) collection were received from A. T. Tschanz (Asian Vegetable Research and Development Center, Shanhua, Tainan, Taiwan) and S. T. Hsu (National Chung Hsing University, Taichung, Taiwan) and consisted of 28 strains isolated from tomato and pepper over several years. The Buenos Aires (BA) collection of 30 strains was obtained from A.

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M. Alippi (National University of La Plata, La Plata, Argentina), who collected the strains from tomato and pepper during 1984 to 1987.

Collections of additional strains from fields of tomato or pepper were obtained from diseased leaves or fruit taken at random from fields in which bacterial spot was uniformly distributed. Bacteria were isolated by the puncture method from individual lesions (12), cultured on nutrient agar, and streaked for selection of single colonies, which was done two times successively before storage. A population was obtained from tomato plants grown at the INTA Experiment Station, Bella Vista, Argentina (BV), and consisted of 50 strains that originated from 20 samples. The 15 strains of the Ohio population (OH-tomato) were isolated from tomato fields in northwestern Ohio. The Florida tomato population (FL-tomato) included 84 strains from two tomato fields near Bradenton, FL. The 97 strains of the Florida pepper population (FL-pepper) were taken from three pepper fields near Delray Beach, FL. After isolation or recovery from collections, the bacteria were stored at room temperature in sterilized tap water for the duration of these tests. During active use of the cultures, they were stored in petri plates containing a 1:1 combination of lima bean agar (Difco Laboratories, Detroit) and 1.5% water agar.

All strains were tested for pathogenicity on pepper (cv. Early Calwonder) and tomato (cv. Bonny Best) plants. After inoculation, plants were held in growth chambers at 28°C (20). Inoculum consisted of about 10^8 CFU/ml suspended in sterilized tap water. The plants contained six to eight fully expanded leaves, and each suspension was infiltrated into about 1 cm^2 of leaf tissue. Necrosis in leaves 3 to 5 days after inoculation was considered a pathogenic reaction. Each strain was tested two times.

Plasmid extraction and separation. Bacterial strains were grown for 20 to 30 h in tubes containing 2 ml of nutrient broth at 30°C with vigorous shaking. Cells were harvested by centrifugation, cell density adjusted to 0.3 A_{600} in sterilized deionized water, and plasmid DNA extracted by the method of Kado and Liu (15), with modifications of Minsavage et al. (19). Plasmid preparations were sometimes stored for several months at 4°C in disposable microfuge tubes after adding EDTA to a final concentration of 25 mM. Samples were electrophoresed through 0.5% agarose gels (Sea Kem, DNA grade; FMC Corporation, Rockland, ME) in TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0), stained with ethidium bromide (0.5 μg/ml) for 40 min, and photographed using transmitted UV light. Plasmid DNA was subjected to electrophoresis until a bromphenol blue front had moved 5 cm from the well. The electrical current was 5 V cm^{-1} for gels in Mini-Sub Cell and 3 V cm^{-1} for gels in the wide Mini-Sub Cell (Bio-Rad Laboratories, Richmond, CA).

Plasmid sizing. A plasmid preparation from cells of *Erwinia stewartii* strain SW2 (8) was included in each gel for size markers. The 13 plasmids of *E. stewartii*, which ranged in size from 4.1 to 318 kb, were extracted and separated by the same method that was used for *X. campestris* pv. *vesicatoria*. The relative mobility of the 13 plasmids was measured for each gel. Linear regression equations were calculated for each gel, with the $\log_{10}$ of the distance migrated as the independent variable and the $\log_{10}$ of the size (kb) as the dependent variable. Two regressions were calculated for each gel, one regression with the four larger plasmids and a second regression with the nine other plasmids. This was done in order to obtain a straight line regression for the large plasmids. The size of each plasmid of *X. campestris* pv. *vesicatoria* was calculated with the regression equations, based on the value for the distance migrated for each plasmid band in each strain. Three strains of *X. campestris* pv. *vesicatoria* were included in all gels as reference markers, and the values obtained for the plasmids of these strains were used to determine experimental variation. All sizing experiments were done at least two times for each strain.

Plasmid transfer in the field. Donor and recipient strains of *X. campestris* pv. *vesicatoria* were inoculated separately into opposite leaves of each of 100 plants of pepper (cv. Early Calwonder) just prior to planting in field plots by infiltration of a 1-cm^2 area with a suspension of about 10^8 CFU/ml. The plants were transplanted into beds covered with plastic film at the Gulf Coast Research and Education Center, Bradenton, FL. The plants were placed in four single rows, 1.8-m apart, and plants were spaced 60-cm apart in each row. Mineral nutrition and pests other than the bacterial spot pathogen were controlled by standard procedures. The test was repeated during three successive years.

A mixture of five strains (Xv 86-12, Xv 86-16, Xv 86-37, Xv 86-39, and Xv CL-90), each containing plasmids of five size classes (A, F, I, K, and M), was selected to be the plasmid donor in these tests. Another strain (Xv P26), which contained a single 15-kb plasmid (class L), was selected as a recipient; this plasmid was found in only 5% of 522 strains of *X. campestris* pv. *vesicatoria*. The recipient strain also was resistant to both rifamycin and chloramphenicol. Resistance to the antibiotics was achieved by successive selection of surviving colonies after transferring 0.5 ml of a suspension of 10^9 CFU/ml onto several plates of nutrient agar containing 75 μg/ml of rifamycin, or 30 μg/ml of chloramphenicol.

After bacterial spot disease progressed on the field plants, 100 leaves (one per plant) were selected at 2-week intervals for 8 weeks. Each leaf was placed into a flask with 10 ml of 0.01% $MgSO_4$ and shaken for 30 min. The resulting bacterial suspensions were streaked onto nutrient agar containing 75 μg/ml of rifamycin and 30 μg/ml of chloramphenicol. Single colonies were transferred to nutrient agar for multiplication. Plasmids were extracted from cells of each culture and visualized as described above.

DNA manipulations. The genomic DNA of the recipient strain (Xv P26) was probed to determine the presence of plasmid DNA integrated into the chromosome. Plasmids of classes F, I, and K, which occurred singly in recipient strains isolated from field plasmid-transfer experiments, were each extracted by alkaline lysis (18) from the recipients. They were then digested with *Pst*I, and the fragments were separated by electrophoresis. As a control, the L-class plasmid in the recipient was also included. An 8-kb fragment from plasmids F and I, a 12-kb fragment from plasmid K, and a 2.5-kb fragment from plasmid L were extracted from the agarose gels by the freeze-squeeze method (29), ligated into pLAFR3 by standard techniques (18), and then transformed into competent cells of *Escherichia coli* DH5α. Clones with inserts similar in size to the original plasmid fragment were then linearized and labeled with a Genius nonradioactive DNA labeling and detection kit (Boehringer Mannheim Biochemicals, Indianapolis, IN). The labeled probes, representing each plasmid, were then hybridized as previously described (19) with *Eco*RI digested genomic DNA of the parent strains, Xv P26 and Xv 86-12.

RESULTS

All strains included in this research were pathogenic on pepper and/or tomato. Some strains produced a hypersensitive reaction on one or the other of the two hosts. Strains that produced a hypersensitive reaction, or a null reaction, on both hosts were discarded.

Plasmid classes. Both large (up to 300 kb) and small (down to 3 kb) plasmids were detected in the strains of *X. campestris* pv. *vesicatoria* (Fig. 1). Linearized chromosomal and plasmid DNA were observed in all gels at the 40- to 45-kb position. The use of the regression equations to calculate plasmid sizes provided reproducible values ($R^2 \geq 0.97$). The standard deviation in size for the plasmid of a mean size of 248 kb was ± 9.4, of a mean size of 43 kb was ± 1.5, and of a mean size of 2.9 kb was ± 0.14.

The plasmids in the strains were grouped into 13 different classes based on size (Table 1 and Fig. 1). As a criterion for the

establishment of classes, plasmids were placed in different classes if they occurred together in any one of the strains. Plasmids were not detected in 10 of the 522 strains. Strains without plasmids were always from culture collections and had been stored for several years. Only three plasmid classes (B, I, and K) were present in all seven collections of strains. Plasmids in class K (25 to 33 kb) were the most common among the strains and were in nearly 60% of the strains. Plasmids in classes B (201 to 259 kb) and I (38 to 46 kb) were next in frequency and were in 48 and 42% of the strains, respectively.

Plasmid profiles. Seventy-one different plasmid profiles, based on the presence or absence of the 12 highest molecular weight plasmid classes, were found in the 522 strains of *X. campestris* pv. *vesicatoria*. Plasmid class M (the smallest plasmid) was not included in profile determinations since this class was not detected reliably in strains having larger plasmids. Profiles consisted of one to five different plasmid classes; 10 profiles had only one plasmid class, 17 had two, 30 had three, 10 had four, and 3 had five. The percentage of strains that contained a particular plasmid class was determined for each of the seven collections (Table 1).

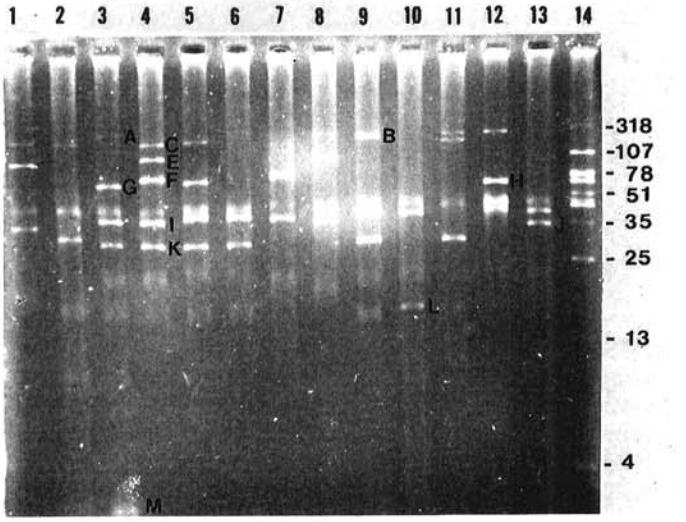

Fig. 1. Agarose gel electrophoresis of plasmid DNA from some strains of *Xanthomonas campestris* pv. *vesicatoria*. Letters indicate size class. Plasmids of strain SW2 of *Erwinia stewartii* are in lane 14 and numbers adjacent to bands indicate size in kilobase pairs. In lanes 1 to 13 are strains 87-18, 86-35, 86-36, 87-6, 87-13c, 87-13g, 84-1, 85-13, E-3, 80-5, 71-31, 79-2, and 65-4, respectively.

TABLE 1. Size classes in which plasmids of *Xanthomonas campestris* pv. *vesicatoria* were grouped

		% of strains with plasmid of designated size						
		Culture collection[a]			Field collection[a]			
Size class	Plasmid size (kb)	UF	TW	BA	FL-pepper	FL-tomato	OH-tomato	BV-tomato
A	> 260	21	0	0	85	6	0	0
B	201–259	45	28	71	3	92	100	49
C	170–200	15	25	0	12	0	0	0
D	150–169	2	0	0	0	0	0	0
E	120–149	1	0	0	72	35	0	0
F	80–119	12	48	0	0	2	0	0
G	60–79	2	27	0	12	1	0	91
H	47–59	1	0	72	0	10	0	25
I	38–46	42	60	3	87	8	66	19
J	34–37	10	2	0	10	8	32	0
K	25–33	70	62	20	90	29	10	8
L	5–24	1	10	0	0	25	10	0
M	< 5	45	2	25	99	35	10	0

[a] The total number of strains in each collection were: UF (University of Florida), 218; TW (Taiwan), 28; BA (Buenos Aires), 30; FL-pepper (Florida), 97; FL-tomato (Florida), 84; OH-tomato (Ohio), 15; and BV-tomato (Bella Vista), 50.

Plasmids in strains stored in collections. All 13 plasmid classes, arranged in 45 different profiles, were found in the UF collection of 218 strains. No plasmid class was found in all strains (Table 1), but plasmid class K was found the most frequently and in 70% of the strains. Four plasmid profiles comprised 40% of the strains and they contained classes B,K (16%); A,K (9%); B,I,K (8%); and A,F,I,K (7%). Each of 30 other profiles was found in less than 2% of the strains. Nine of the 13 plasmid classes existed in the 28 strains in the TW collection (Table 1). A high percentage of strains with classes I (60%) and K (62%) plasmids occurred in the TW collection. Fifteen different profiles were found and no one profile was dominant. Only five of the 13 plasmid classes were found in 30 strains of the BA collection, but a high percentage (72%) of the strains contained plasmids in classes B (71%) and H (72%) (Table 1). The classes were arranged in only five different profiles.

Plasmids in strains from field populations. Plasmids representing nine of the 13 classes were detected among the 97 strains comprising the FL-pepper population (Table 1). Ten different plasmid profiles were observed, but 70% of the strains had one profile (A,E,I,K). The remainder of the strains were almost evenly distributed among the other nine profiles. In the 84 strains of the FL-tomato population (Table 1), plasmids were distributed into 11 of the 13 classes with 11 different profiles. Strains with two of the profiles (B,K and B,E,K) comprised 65% of the population. Plasmids belonging to six different classes were detected in the 15 strains of the OH-tomato population (Table 1), and they were arranged into only four different profiles. One profile (B,I) was present in 66% of the strains. Five classes of plasmids, arranged in six profiles, were detected in the 50 strains of the BV-tomato population (Table 1). A plasmid representing class G occurred in 91% of the strains, which was unusually high compared to other populations of *X. campestris* pv. *vesicatoria*. Three profiles (G; B,G,H; and B,G,I) were in 85% of the strains.

Plasmid transfer in the field. Bacterial spot developed moderately severely on the plants inoculated with donor and recipient cells in one of the three field tests. Bacteria with the marker antibiotic resistances were isolated from the plants at each of four biweekly assay periods in this test (Table 2). A plasmid of class L was present in all of the strains isolated on the antibiotic medium, which was additional evidence that the recipient bacterium was isolated. The percentage of the recipient cultures with additional plasmids increased over the 8-week assay period (Table 2). Over the whole period, recipient cells with plasmid classes A, F, I, and K were recovered from the field (Fig. 2). Cells with the M-class plasmid were not recovered. A plasmid of class I occurred most frequently in the recipient cells, whereas, the plasmids in classes A, F, and K were detected at about equal frequencies (Table 2). In

TABLE 2. Plasmid profiles of strain Xv P26 recovered on chloramphenicol-rifamycin medium from the field plasmid-transfer experiment

Sample time[a]	Strains collected[b]	Number of strains containing each plasmid profile									% with new plasmid
		A	F	I	K	M	I,K	A,I	F,I,K	L[c]	
1	23	0	0	0	0	0	0	0	0	23	0
2	80	0	0	10	2	0	6	0	0	80	22
3	86	2	1	18	1	0	8	2	1	86	37
4	10	0	1	4	0	0	1	0	0	10	60
Total	199	2	2	32	3	0	15	2	1	199	29

[a] First sampling time occurred 2 weeks after transplanting in the field. Each subsequent sampling time was 2 weeks after the previous time.

[b] The number of strains collected were from 100 leaves sampled. Only one strain was saved from each positive leaf.

[c] Recipient strain, Xv P26, contained a rare L-class plasmid and was also resistant to chloramphenicol and rifamycin. Donor cells contained plasmids of classes A, F, I, K, and M.

17 cultures of the recipient, two additional plasmids occurred, and, in one culture, three additional plasmids were observed. Overall, seven plasmid profiles were detected in the recipient strains (Table 2). In the other two field tests, bacterial spot disease development was very low and the recipient bacterium was not consistently isolated at each assay period. However, when the recipient was recovered in those two years, additional plasmids were also found in some of the recipient cultures.

The possibility existed that plasmids detected in the recipient cells were integrated into the chromosome of the recipient and were released during the development of disease in the field. However, a cloned fragment from each of the F-, I-, and K-class plasmids extracted from the recipient strains did not hybridize with *Eco*RI-digested genomic DNA of cells of Xv P26, the recipient. Hybridizations did occur with the L-class fragment with the L-class plasmid. Hybridizations also occurred when *Eco*RI-digested genomic DNA of strain Xv 86-12, one of the donor strains, was probed with each of the cloned fragments.

DISCUSSION

Crosse (10) and Schroth (26) stressed the importance of knowing the degree of homogeneity of the pathogen population occurring on a given host in a given locality, and the similarity to populations occurring on the same host in other localities, or on other hosts in the same locality. They warned of the possible inaccurate representation of an organism as it occurs in nature if studies are confined to a very few strains. Plasmid content is a source of genetic variation among strains that must be considered. Based on this work, each population of *X. campestris* pv. *vesicatoria* may differ. Differences in plasmid content occurred among strains isolated from different geographic locations and strains from the same location were not homogeneous in plasmid content. No plasmid, or plasmid profile, was present among all the strains of *X. campestris* pv. *vesicatoria* examined.

A few plasmid profiles were found more often, with many other profiles present in lower frequency, in each population of *X. campestris* pv. *vesicatoria*. The most common plasmid profiles differed in each host-locality combination, however. This was consistent with the presence of predominant types of plasmids in strains of *Rhizobium leguminosarum* biovar *trifolii* in each of several sites sampled (13). The apparent differential variation in plasmid profiles for a host-locality combination may contribute to the existence of ecotypes (9). Certain plasmids may have genetic material that is important in a particular host-locality combination.

Categorizing plasmid content based only on plasmid size likely did not accurately assess the diversity of plasmids in *X. campestris* pv. *vesicatoria*. The range of plasmid sizes within several classes was greater than the variance in size, based on experimental error, of a single plasmid within the class (data not shown). Thus, more than one plasmid may have been assigned to the same class. In fact, restriction enzyme digestion patterns of two plasmids of class I were different (5). In addition, two avirulence genes, *avrBsT* and *avrBs3*, are located on plasmids of class I (2,20) and both avirulence genes were not found in the same strain (5). The two plasmids carrying the avirulence genes may be incompatible. On the other hand, the plasmids in classes A, B, and C may be related even though restriction enzyme digestion patterns of plasmids in the three classes are different (6). Copper resistance genes are present on plasmids in all three classes, and plasmids of these three classes are all self-transmissible (6).

Some plasmids are present only in specific chromosomal types among strains of *R. leguminosarum* biovar *viciae*, whereas, others are widespread (30). Cluster analysis of restriction fragment length polymorphism data from plasmid DNA of the strains of *X. campestris* pv. *citri* with different chromosomal types supports the concept of coevolution of plasmid and chromosomal genomes (7,24). Strains with different chromosomal genomes were represented among the strains of *X. campestris* pv. *vesicatoria* in this study. The strains in the BA and BV collections consisted entirely of the B group of strains, whereas, the strains in the other collections were entirely of the A group of strains (27). Plasmids of the classes G and H seemed to be overrepresented in the B group of strains and the A-class plasmid was underrepresented. These plasmids may be associated with a chromosomal type, but that determination must await further study of the plasmid DNA.

The stability of the plasmids in the strains of *X. campestris* pv. *vesicatoria* in culture was not tested. Lazo and Gabriel (16) reported plasmid profiles of xanthomonads to be stable upon serial transfer. However, a specific plasmid in a strain of *X. campestris* pv. *vesicatoria* was lost in successive transfer and culturing of a single strain (20). In this work, plasmids of class E were not found frequently in the culture collections, but were found often in the field collections in Florida. Possibly, E-class plasmids are lost readily during storage of the strains, or it may be a recent introduction into the strains of *X. campestris* pv. *vesicatoria* in Florida. We will continue to monitor that plasmid class in future strains isolated from nature and culture collections.

Many of the plasmids in *X. campestris* pv. *vesicatoria* may be self-transmissible. Detection of the self-transmissible nature of these cryptic plasmids has not been possible in the laboratory, because no efficient method is available for selection of strains with a particular plasmid. However, the mobility of the plasmids between strains during development of disease may be a major factor for the diverse plasmid profiles found among the strains of *X. campestris* pv. *vesicatoria* at single locations. Preferential selection of strains with plasmids by some plant factor may have occurred during disease development to account for the high percentage of recipients with plasmids isolated from the field. Some of the plasmids, particularly the one(s) in class I, may have characteristics that aid in pathogenic fitness to pepper plants. Either that, or the frequency of transfer between the donor and recipient cells must have been extremely high in plant tissue. The latter possibility might occur if conjugations of strains of *X. campestris* pv. *vesicatoria* is induced in pepper plants to a high frequency, similar to the induction of conjugation between strains of *Agrobacterium tumefaciens* in plants (23).

The nearly universal presence of plasmids in *X. campestris* pv. *vesicatoria* strains and their mobility in nature should be considered before using plasmid profiles as markers in epidemiological

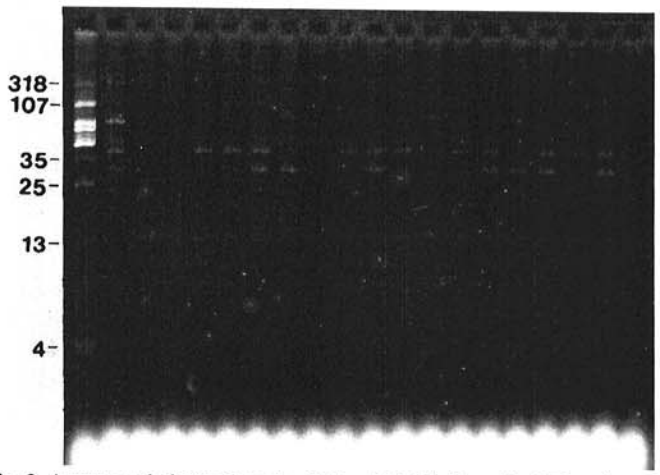

Fig. 2. Agarose gel electrophoresis of plasmid DNA from Xv P26 strains of *Xanthomonas campestris* pv. *vesicatoria* isolated from diseased plants in the field plasmid-transfer experiment. Lane 1, plasmids from *Erwinia stewartii*; lane 2, plasmids from a donor strain Xv 86-12; and lane 3, plasmid in recipient strain, Xv P26. In lanes 4 to 20 are plasmids recovered from double-antibiotic resistant Xv P26 strains isolated from a field experiment. Note that all of the latter strains have a plasmid of 15 kb, which is present in the recipient.

studies. Since so many plasmid profiles exist in nature, experiments involving the tracking of strains with similar plasmid profiles during epidemiological development of disease would be risky. Care should be taken in making conclusions about the spread of strains based on plasmid profiles when a particular strain is used in fields with other strains present. The mobility of plasmids among strains in the field could confuse the results. The mobility of plasmids also would make risky the determination of sources of inoculum by comparisons of plasmid profiles of the bacteria from possible sources of inoculum and the bacteria contributing to an epidemic.

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