Assessment of Phenotypic Variability in *Erwinia stewartii* Based on Metabolic Profiles

W. J. Wilson and H. R. Dillard, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456; and S. V. Beer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

ABSTRACT

Wilson, W. J., Dillard, H. R., and Beer, S. V. 1999. Assessment of phenotypic variability in *Erwinia stewartii* based on metabolic profiles. Plant Dis. 83:114-118.

One hundred twenty-four bacterial isolates originating from sweet corn or corn flea beetles in the northeastern, midwestern, and mid-Atlantic United States were verified as *Erwinia stewartii* (*Pantoea stewartii* subsp. *stewartii*) and characterized phenotypically by their respiratory response to 91 carbon sources. The unweighted pair group method of averages (UPGMA) was used to construct a dendrogram that revealed homogeneous metabolic profiles at 93% similarity. Two-thirds of the isolates formed 18 separate groups, each sharing the same metabolic profile. One-third of the isolates had distinct metabolic profiles. Most groups shared either isolation source, geographical location, and/or year of isolation. Members of some groups persisted through time and had been isolated from diverse geographical locations. Four representative strains of the proposed *Pantoea stewartii* subsp. *indologenes* were also characterized; their metabolic profiles were most similar to those of *Erwinia herbicola* (*Pantoea agglomerans*).

Stewart's bacterial wilt is a serious disease of corn (*Zea mays* L.) that sporadically causes significant losses to the sweet corn industry (15). The disease is caused by the bacterium *Erwinia stewartii*. Typical symptoms are rapid wilting of corn plants and leaves with water-soaked linear, pale green to yellow lesions and streaks. As the disease progresses, the streaks become dry and brown. Corn crops are at serious risk when infection occurs at the three- to five-leaf stage because this will result in severely stunted plants (6,14).

The corn flea beetle, *Chaetocnema pulicaria* is considered the most important overwintering host and vector of *E. stewartii* (17). Corn flea beetle populations are directly proportional to Stewart's wilt incidence; therefore, the abundance of *E. stewartii* primary inoculum is related to corn flea beetle populations (21). Corn flea beetles are susceptible to freezing temperatures, and in harsh winters flea beetle mortality is high, which leads to low incidence of Stewart's wilt the following growing season (21). These relationships were coupled to develop an

often-used predictive system for disease severity (4,5).

Incidences of Stewart's bacterial wilt were high in New York State during the four growing seasons from 1988 to 1991, and strains of *E. stewartii* were isolated from diseased sweet corn and corn flea beetles at those times. To determine the similarity of strains isolated in New York, a large number was collected from infected plants in New York and from the northeastern, midwestern, and mid-Atlantic United States. Additional strains were acquired from culture collections to represent past Stewart's wilt outbreaks and their geographical locations.

Mergaert et al. (11) proposed that *E. stewartii* be moved to the genus *Pantoea* based on DNA-DNA hybridization studies. Similar experiments also indicated that several previously named *E. herbicola* and *E. ananas* strains be moved as subspecies *indologenes* of the proposed *Pantoea stewartii* and the pathogen be designated subspecies *stewartii*.

Biolog Inc. (Hayward, CA) has developed a technique to identify bacteria based on differential utilization of 95 carbon sources, each dried into a separate well of a 96-well microplate (3). Utilization of a particular carbon is visualized by a purple color change from the reduction of the redox dye tetrazolium violet. The results of 91 carbon sources in the Biolog GN gram-negative microplate have been used to compile a database of 124 *E. stewartii* strains.

Our objective was to use the metabolic profiles of these 124 strains to study phenotypic variability within this species. Four

representative strains of the proposed *Pantoea stewartii* subsp. *indologenes* were included for comparison purposes.

MATERIALS AND METHODS

Bacterial strains. Strains of *E. stewartii* and other bacteria included in the study are listed in Table 1. Bacterial strains from 1991 to 1994 were isolated directly from sweet corn plants and corn flea beetles onto Miller-Schroth selective media (12). Older strains were retrieved from culture collection stocks, as indicated in Table 1. All strains were confirmed as *E. stewartii* by colony morphology, gram-reaction, oxidation-fermentation reaction (O/F), lack of motility, reaction to specific *E. stewartii* primers using a polymerase chain reaction-coupled ligase chain reaction assay (PCR-coupled LCR) (24,25), and pathogenicity on the susceptible sweet corn cultivar Jubilee. Bacteria were stored at -70°C . For carbohydrate utilization testing, strains were taken from frozen stock, transferred twice on trypticase soy agar (TSA) (Becton Dickinson, Cockeysville, MD), and prepared for inoculation onto the Biolog GN microplates.

Carbohydrate utilization. Bacterial cells were incubated for 24 h on TSA and suspended in sterile saline at A_{600} of between 0.285 and 0.330. A Biolog GN microplate was immediately inoculated with the suspension at 150 μl per well and incubated at 28°C for 24 h. Reactions in individual wells were scored visually as positive (purple color in well) or negative (no color) in reference to a negative control well containing no carbon source.

Results of 91 carbon tests were stored as a user database using the Biolog Microlog 3.5 software. The user database was analyzed with the Biolog MLCLUST program by an unweighted pair-group method analysis (UPGMA) to construct a dendrogram and by principal component analysis (PCA) for a three-dimensional picture.

Test error and reproducibility. Because of the possible variability of carbon source utilization due to sampling and growing of bacteria (18), we followed a standardized protocol similar to that suggested by Biolog Inc. (3). Test reproducibility was assessed by replicating 15% of the strains (1). Individual carbohydrate test variance ($S_i^2 = n/2t$) (1) was calculated from 18 replicated strains, where n = number of replicated strains with a discrepant

Corresponding author: W. J. Wilson
E-mail: wilson69@llnl.gov

Current address of W. J. Wilson: Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, L-452 P.O. Box 808, Livermore, CA 94551.

Accepted for publication 21 October 1998.

Publication no. D-1998-1130-04R
© 1999 The American Phytopathological Society

carbohydrate utilization result and t = total number of replicated strains. The pooled variance, S^2 , was calculated by averaging the individual test variances. The pooled variance was used to find the average probability of an erroneous test result ($P = 1/2[1 - 4(S^2)]$).

Numerical analyses. Carbohydrate utilization results were analyzed by UPGMA. This phenetic method uses a sequential clustering algorithm to identify each strain's metabolic profile in order of similarity (10). A dendrogram was constructed based on UPGMA information (Figs. 1 and 2).

A three-dimensional taxonomic map of the data was made using PCA (Fig. 3). PCA is an ordination method that simplifies taxonomic space to three dimensions so that relationships between bacterial strains can be displayed pictorially. The first dimension represents the greatest variability among the bacterial strains, and the next two dimensions account for the next two levels of greatest variability (10).

RESULTS

Test error and reproducibility. Metabolic profiles were obtained for 124 *E. stewartii* strains. Since *E. stewartii* was not included in the Biolog Microlog 3.5N database, *E. amylovora*, *E. herbicola*, and *Pseudomonas syringae* pv. *phaseolicola* strains were used as control organisms to verify the microplates were functioning properly. Microlog correctly identified the control organisms. Reproducibility of the test was assessed by replicating 15% of the strains (1). The pooled variance, S^2 , was calculated by averaging the individual test variances for an overall error estimate of 5%. The probability of an erroneous test result was 6% and resulted from high individual probability errors for four carbohydrate tests: Tween 40 (68%), dextrin (20%), inosine (17%), and glycogen (17%). Reproducible results are difficult to obtain with certain carbohydrate tests for some bacterial species. These tests, if included in analyses, could lead to experimental errors when compiling similarity values (1,20). Austin and Priest (1) suggest rejecting tests where the individual probability errors are over 10 to 15%. Thus, the four carbohydrate tests were deleted from the data set. The resulting average probability of an erroneous test result was 1.2%.

Carbon source utilization of *E. stewartii* strains. Twenty-six percent of the carbon sources were utilized by all *E. stewartii* strains tested. They were: L-serine, *N*-acetyl-D-glucosamine, L-arabinose, D-fructose, D-galactose, α -D-glucose, *M*-inositol, D-mannose, D-melibiose, D-psicose, D-raffinose, sucrose, D-trehalose, mono-methyl succinate, D-gluconic acid, succinic acid, bromo-succinic acid, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl-L-aspartic acid, gly-

cyl-L-glutamic acid, glycerol, glucose-6-phosphate, and glucose-1-phosphate.

Carbon sources not utilized by any *E. stewartii* strain were: Tween 80, *N*-acetyl-D-glucosamine, adonitol, D-arabitol, cellobiose, gentibiose, L-rhamnose, turanose, xylitol, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, D,L- α -glycerol phosphate, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-pyrroglutamic acid, γ -aminobutyric acid, phenyl ethylamine, 2-aminoethanol, *p*-hydroxyphenylacetic acid, L-phenylalanine, D,L-carnitine, urocanic acid, putrescine, 2,3-butanediol, and L-threonine.

Variable carbon source utilization and percentage of *E. stewartii* strains utilizing those carbon sources are listed in Table 2.

Phenetic analysis. A dendrogram was constructed from the 131 bacterial strains tested (Figs. 1 and 2). One carbon test

difference between strains is equivalent to one hatch mark (1%) on the dendrogram. *E. stewartii* strains clustered together into one phenon at 93% similarity. One outlier, Es69, was present at 89% similarity to the other strains. *E. amylovora*, *E. herbicola*, and *P. syringae* pv. *phaseolicola* were 85, 78, and 72% similar, respectively. A taxonomic map of the data was made by three-dimensional principal component analysis (PCA) (Fig. 3). PCA of the 131 bacterial strains also revealed the homogeneity within the *E. stewartii* species. Four strains of bacteria proposed as a new subspecies of *E. stewartii* clustered with the *E. herbicola* strain in both the dendrogram and PCA.

DISCUSSION

The Biolog system provided a robust, standardized method to gather a large set of individual carbohydrate utilization data (3). Both UPGMA and PCA of the carbon utilization data successfully differentiated two other *Erwinia* sp. and *P. syringae* pv.

Table 1. Bacterial strains and geographical origin

Strain(s) ^a , source	Origin
<i>Erwinia stewartii</i>	
Es61-92	Delaware, 1994, CFB ^b
Es93-95	Delaware, 1994, corn
Es4,8,9	Genesee Co., NY, 1991, corn
Es1,51-53	Wayne Co., NY, 1991, corn
Es5,32,33,35,49,50,54	Ontario Co., NY, 1991, corn
Es12-14	Onondaga Co., NY, 1991, corn
Es2,3,10,11	Cayuga Co., NY, 1991, corn
Es29-31,34,36	Yates Co., NY, 1991, corn
Es37,38	Seneca Co., NY, 1991, corn
Es7	Saratoga Co., NY, 1991, corn
Es17-21	Schenactady Co., NY, 1991, corn
Es26-28	Rensselaer Co., NY, 1991, corn
Es16,22-25	Columbia Co., NY, 1991, corn
Es15,56-60	Ulster Co., NY 1991, corn
Es39-48	Suffolk Co., NY, 1991, corn
Es100-106 (CUCPB 0176-0182)	New York, 1975, corn
Es6	Illinois, 1984, corn
Es109-114	Illinois, 1993, corn
Es55	Maryland, 1991, corn
Es95 (CUCPB 2993)	Ohio, 1974, corn
Es96 (CUCPB 2994)	Indiana, 1975, corn
Es97 (CUCPB 2995)	Kentucky, 1975, corn
Es98 (CUCPB 2996)	Tennessee, 1975, corn
Es99 (CUCPB 2997)	Missouri, 1975, corn
Es115 (NCPBP 449)	USA, 1957, corn
Es116 (Z05)	USA, corn
Es117 (CUCPB 1374)	USA, 1932, corn
Es118 (CUCPB 1375)	New York, 1965, corn
Es119,120 (DC116,DC122)	Missouri, 1976, corn
Es121-123 (ATCC 29227-29229)	Connecticut, 1975, CFB
Es124	Illinois, corn
<i>Pantoea stewartii</i> subsp. <i>indologenes</i>	
NCPBP 1845	Pineapple, Hawaii, 1948
NCPBP 1562	Pearl millet, India, 1961
NCPBP 2275	Pearl millet, India, 1966
NCPBP 2280	Foxtail millet, India, 1960
<i>Erwinia amylovora</i> (CUCPB Ea321)	France, Hawthorne
<i>E. herbicola</i> 1	New York, 1993, corn
<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	New York, bean

^a Strain names and numbers correspond to those in unweighted pair-group method analysis (UPGMA). Other designation, if any, is in parentheses: CUCPB, Cornell University Collection of Phytopathogenic Bacteria; NCPBP, National Collection of Plant Pathogenic Bacteria; ATCC, American Type Culture Collection; DC, supplied by Dave Coplin, Ohio State University.

^b Corn flea beetle.

phaseolicola as distinct taxonomic units from *E. stewartii*. *E. amylovora* was 85% similar and *E. herbicola* was 78% similar to *E. stewartii* (Fig. 1). These results were consistent with those of a previous study by Verdonck et al. (22) that analyzed numerically the genus *Erwinia* with 72 phenotypic tests using API 20E and API 50CHE (API Systems, bioMérieux Vitek, Inc., Hazelwood, MO). The four *E. stewartii* strains tested in that study clustered at 96% similarity; our extensive collection of *E. stewartii* strains confirmed the homogeneous nature of *E. stewartii*. Generally, phenetic groups formed at about 80% similarity are equivalent to a bacterial species (19).

There was some variation in the ability of *E. stewartii* strains to utilize carbon sources. This variation separated 83 strains into 18 groups of two or more isolates with the same metabolic profile (phenotype) (Figs. 1 and 2, Table 1). Ten phenotypes consisted solely of isolates from a certain year, source (corn flea beetle or sweet corn), and/or location (e.g., corn flea beetles, Delaware, 1994), but there were also phenotypes containing isolates from diverse sources.

One phenotype consisted of 18 isolates (beginning at Es28 on the dendrogram pictured in Fig. 1), 12 of which were collected from New York State during a 1991 outbreak of Stewart's bacterial wilt. Other members of this phenotype were collected from Tennessee (Es98), Ohio (Es108), Illinois (Es124), and Connecticut (Es122) from 1974-75 and from Delaware (Es68) in 1994. The isolates from Connecticut and Delaware came from corn flea beetles. Another phenotype (beginning with Es16, Fig. 2) contained nine isolates from the NYS 1991 outbreak and one Ohio isolate collected in 1967. It has been shown that other bacterial species may persist for long periods of time and achieve widespread geographic distributions (23). This seems to be true of several *E. stewartii* phenotypes, assuming their metabolic profiles reflect their genotype.

A corn flea beetle isolate (Es75) and a sweet corn isolate (Es93), from adjacent fields in Delaware, shared identical metabolic profiles, suggesting a single pathogenic phenotype's association in vector and host. Evidence for the geographical stability of a phenotype is shown in Figure 2 by the identical metabolic profiles of an Illinois phenotype isolated from corn in 1984 (Es6) and in 1993 (Es111).

E. stewartii was isolated from two separate lesions on each of 11 single corn plants collected from different counties in the 1991 NYS outbreak. One plant contained two isolates representing the same phenotype (Es29 and Es30) (Fig. 2); the other plants contained isolates with different metabolic profiles. In 1991, there were high populations of corn flea beetles, and it was common to see more than one beetle

on a single corn plant. A plant infested by more than one beetle could contain several different phenotypes. Corn flea beetles may also have had more than one phenotype in their intestinal tract.

In a study by Mergaert et al. (11), results from DNA-DNA hybridization experiments suggested that *E. stewartii* was more similar to *E. herbicola* (*Pantoea agglomerans*) than to *E. amylovora*, and proposed that *E. stewartii* be transferred to the genus *Pantoea*. Furthermore, based on similar DNA-DNA hybridization experiments, 10 strains formally identified as *E. herbicola*

and *E. ananas* were found to be borderline for inclusion in the new species *P. stewartii* (Smith) Mergaert. However, electrophoretic protein profiles and other phenotypic tests discriminated between the 10 strains and *P. stewartii*; the strains were transferred to the new subspecies *P. stewartii* subsp. *indologenes*.

Four representative strains (NCPPB 1845, 1562, 2275, and 2280), one from each protein profile group of this proposed subspecies, were included in our carbohydrate utilization studies. All four strains clustered with the *E. herbicola* strain and

Fig. 1. First half of the dendrogram of *Erwinia stewartii* strains assembled by unweighted pair-group method analysis (UPGMA). Each hatch mark on the dendrogram is equivalent to one carbon test difference between strains. Refer to Table 1 for the key to the *Erwinia* strains (Es1 = *E. stewartii*-1).

were only 78% similar to *E. stewartii*. They were not pathogenic on sweet corn cultivar Jubilee. Three of the strains showed typical *E. herbicola* reactions for O/F and motility. They were also LCR(-) when tested using a PCR-coupled LCR assay specific for *E. stewartii* (24).

One strain in our study, NCPPB 1845, showed characteristics of both *E. stewartii*

and *E. herbicola*. It was O/F(+) and nonmotile like *E. stewartii*, but it had a carbohydrate utilization profile similar to *E. herbicola*. It was also identified as LCR(+) by the PCR-coupled LCR assay specific for *E. stewartii* (24). This strain was isolated from pineapple in Hawaii and was classified originally as *E. ananas*. It seems that this strain may be

an intermediate between *E. herbicola* and *E. stewartii*.

From UPGMA and PCA, none of the proposed *P. stewartii* subsp. *indologenes* strains appeared similar to *E. stewartii*. Furthermore, the subspecies *indologenes* strains were found on different hosts in geographically isolated regions, from grasses in India and pineapple in Hawaii. *E. stewartii* has never been reported from these regions, where its main plant host, corn, is not native. Since these strains were borderline for inclusion in *E. stewartii* by DNA-DNA hybridization analysis and have obvious differences in electrophoretic protein and carbohydrate utilization profiles, it may be more relevant biologically to treat them as separate species. The definitive test of this taxonomic and ultimately phylogenetic question would be a study of the 16S rDNA of the *Erwinia* group (26).

In our study, *E. stewartii* was phenotypically most similar to *E. amylovora*. The two plant pathogens share significant homologies in the hypersensitive response and pathogenicity (*hrp*) and water-soaking (*wts*) gene clusters, respectively (9). Perhaps the carbohydrate utilization data reflect the parasitic lifestyle of these bacteria (11).

Numerical taxonomy of phenotypic characters is one measure of the variability of an organism. It is possible that the lack of phenotypic diversity in our *E. stewartii* isolates is because most samples were collected in eastern North America, where sweet corn is widely grown. However, the

Fig. 2. Second half of dendrogram of *Erwinia stewartii* strains assembled by unweighted pair-group method analysis (UPGMA). Refer to Table 1 for the key to the *Erwinia* strains.

Table 2. Variable carbon compound utilization and percentage of *Erwinia stewartii* strains using the carbon source, as determined by the standard BIOLOG GN microplate test

Compound	% strains
Aspartic acid	99
β-Methyl-D-glucoside	99
L-Proline	98
Methyl pyruvate	97
Thymidine	95
D-Mannitol	93
Lactic acid	87
D-Alanine	81
Acetic acid	50
Succinamic acid	40
Sorbitol	27
Lactulose	24
Maltose	17
α-D-Serine	15
Uridine	6
L-Fucose	5
Propionic acid	3
α-Cyclodextrin	2
Glucuronamide	2
Citric acid	1
Alaninamide	1
L-Erythritol	1
cis-Aconitic acid	1
Hydroxybutyric acid	1
L-Threonine	1
α-Keto valeric acid	1
α-D-Lactose	1

Fig. 3. Principal component analysis (PCA) of *Erwinia stewartii* strains. Number-letter combinations refer to groups and strains in the dendrogram. A1 = *Pseudomonas syringae*; E5 = member of *E. herbicola* cluster; AP131 = *E. amylovora*; G9 = member of *E. stewartii* cluster. Circled numbers 1, 2, and 3 represent the three dimensions of PCA.

phenotypic homogeneity may imply that the pathogen has been streamlined to exist in particular hosts; one might expect greater diversity in an organism surviving more ubiquitously in the environment (16).

An appreciation of the phenotypic diversity of *E. stewartii* may be useful in understanding the epidemiology of Stewart's bacterial wilt and the biology of the pathogen. Poos (17) showed that *E. stewartii* overwinters only in adult corn flea beetles and in no other insect vectors. In another study, no *E. stewartii* was isolated from weed sources in New York State or Delaware (24), and therefore weed reservoirs could not be confirmed for Stewart's wilt. Recent research reports indicate there is negligible evidence of seed transmission of *E. stewartii* in plants with less than 50% diseased leaf area (2,8). From these findings, we hypothesize that *E. stewartii* is a specialist surviving in two main habitats, corn flea beetles and *Z. mays*. In occupying these particular niches, the relatively predictable environments may have selected for the homogeneous metabolic profile seen in this pathogen (13). In contrast, nonspecialist erwinias like *E. herbicola* and *E. carotovora* are ubiquitous in nature (16). Studies by Dye (7) revealed these to be phenotypically diverse species.

ACKNOWLEDGMENTS

This work was supported by a grant from the New York State Sweet Corn Research Association.

LITERATURE CITED

1. Austin, B., and Priest, F. 1986. Modern Bacterial Taxonomy. Van Nostrand Reinhold, Workingham, Berkshire, UK.
2. Block, C. C., Hill, J. H., and McGee, D. C. 1998. Seed transmission of *Pantoea stewartii* in field and sweet corn. *Plant Dis.* 82:775-780.
3. Bochner, B. R. 1989. Sleuthing out bacterial identities. *Nature* 339:157-158.
4. Boewe, G. H. 1959. Stewart's disease prospects for 1950. *Plant Dis. Rep.* 34:155.
5. Castor, L. L., Ayers, J. E., MacNab, A. A., and Krause, R. A. 1975. Computerized forecast system for Stewart's bacterial disease on corn. *Plant Dis. Rep.* 59:533-536.
6. Dillard, H. R., and Kline, W. L. 1989. An outbreak of Stewart's bacterial wilt of corn in New York State. *Plant Dis.* 73:273.
7. Dye, D. W. 1981. A numerical taxonomic study of the genus *Erwinia*. *N.Z. J. Agric. Res.* 24:223-229.
8. Khan, A., Ries, S. M., and Pataky, J. K. 1996. Transmission of *Erwinia stewartii* through seed of resistant and susceptible field and sweet corn. *Plant Dis.* 80:398-403.
9. Laby, R. J., and Beer, S. V. 1992. Hybridization and functional complementation of the *hrp* gene cluster from *Erwinia amylovora* strain Ea321 with DNA of other bacteria. *Mol. Plant-Microbe Interact.* 4:412-419.
10. Li, W. H., and Graur, D. 1991. Fundamentals

of Molecular Evolution. Sinauer Associates, Inc., Sunderland, MA.

11. Mergaert, J., Verdonck, L., and Kersters, K. 1993. Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartii* to genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb. nov., respectively, and description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. *Int. J. Syst. Bacteriol.* 43:162-173.
12. 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.
13. Nevo, E., and Cleve, H. 1978. Genetic differentiation during speciation. *Nature* 275:125.
14. Pataky, J. K., and Eastburn, D. M. 1993. Using hybrid disease nurseries and yield loss studies to evaluate levels of resistance in sweet corn. *Plant Dis.* 77:760-765.
15. Pepper, E. H. 1967. Stewart's bacterial wilt of corn. Monograph no. 4. American Phytopathological Society, St. Paul, MN.
16. Perombelon, M. C. M. 1992. The genus *Erwinia*. Pages 2899-2921 in: *The Prokaryotes*. Vol. III. 2nd ed. A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K. H. Schleifer, eds. Springer-Verlag, New York.
17. Poos, F. W. 1955. Studies of certain species of *Chaetocnema*. *J. Econ. Entomol.* 48:555-563.
18. Scortichini, M., and Rossi, M. P. 1995. Influence of subculturing some phytopathogenic bacteria on their carbohydrate utilization profile. *Lett. Appl. Microbiol.* 21:237-241.
19. Sneath, P. H. A. 1984. Numerical taxonomy. Pages 5-7 in: *Bergey's Manual of Systematic Bacteriology*. Vol. 1. N. R. Krieg and J. G. Holt, eds. Williams and Wilkins, Baltimore, MD.
20. Sneath, P. H. A., and Johnson, R. 1972. The influence on numerical taxonomic similarities of errors in microbiological tests. *J. Gen. Microbiol.* 72:372-391.
21. Stevens, N. E. 1934. Stewart's disease in relation to winter temperatures. *Plant Dis. Rep.* 18:141-149.
22. Verdonck, L., Mergaert, J., Rijckaert, C., Swings, J., Kersters, K., and De Ley, J. 1987. Genus *Erwinia*: Numerical analysis of phenotypic features. *Int. J. Syst. Bacteriol.* 37:4-18.
23. Whittam, T. S. 1989. Clonal dynamics of *Escherichia coli* in its natural habitat. *Antonie van Leeuwenhoek* 55:23-32.
24. Wilson, W. J. 1995. Detection and characterization of *Erwinia stewartii*, the cause of Stewart's bacterial wilt of maize. Ph.D. thesis. Cornell University, Ithaca, NY.
25. Wilson, W. J., Wiedmann, M., Dillard, H. R., and Batt, C. A. 1994. Identification of *Erwinia stewartii* by a ligase chain reaction assay. *Appl. Environ. Microbiol.* 60:278-284.
26. Woese, C. R. 1987. Bacterial evolution. *Microbiol. Rev.* 51:221-271.