

Evaluation of the Biolog GN MicroPlate System for Identification of Some Plant-Pathogenic Bacteria

J. B. JONES, University of Florida, Gulf Coast Research & Education Center, 5007 60th Street East, Bradenton 34203; A. R. CHASE, University of Florida, Central Florida Research & Education Center, 2807 Binion Road, Apopka 32703; and G. K. HARRIS, University of Florida, Gulf Coast Research & Education Center, 5007 60th Street East, Bradenton 34203

ABSTRACT

Jones, J. B., Chase, A. R., and Harris, G. K. 1993. Evaluation of the Biolog GN MicroPlate system for identification of some plant-pathogenic bacteria. *Plant Dis.* 77:553-558.

The Biolog GN MicroPlate system was evaluated for accuracy in identifying gram-negative and gram-positive phytopathogenic bacteria to genus, species, and pathovar. Approximately 1,000 strains of *Agrobacterium*, *Clavibacter*, *Erwinia*, *Pseudomonas*, and *Xanthomonas* were compared. Of 79 cultures representing 77 *P. syringae* strains and eight pathovars, identification to species and pathovar was correct in 100 and 2% of cases, respectively. Of 603 cultures representing 537 *X. campestris* strains and 28 pathovars, identification to species and pathovar was correct in 97 and 20% of cases, respectively. With *E. carotovora* and *E. chrysanthemi*, 22 and 62% of the strains, respectively, were correctly identified to species. After the library was amended by the addition of data on these strains, identification to pathovar was improved for many of the strains in the *X. campestris* group.

Identification of plant-pathogenic bacteria relies to a large extent on biochemical and physiological methods for routine characterization (30). Confirmation of a bacterial species, in the case of many plant pathogens, necessitates that pathogenicity tests be performed. Since the 1970s, new methods have been employed to identify plant-pathogenic bacteria, including the use of monoclonal antibodies (1), DNA probes (6), and fatty acid analysis (28). These techniques may not be practical in situations where bacteria are routinely isolated from diverse plant species; however, Norman and Alvarez (26) used two monoclonal antibodies for rapid identification of strains of *Xanthomonas* to genus.

Since identification of plant-pathogenic bacteria by conventional procedures is time-consuming and costly in terms of materials, the need for more rapid, reliable, and inexpensive procedures has led to the development of techniques that can be adapted for diagnostic purposes. These include fatty acid analysis (28) and a series of test strips (API Systems, BioMerieux, La Balme-les-Grottes, France) in which bacteria are tested on various substrates to determine enzymatic activity. API Systems also has

strips for carbon utilization, which have been used in taxonomic studies (39). Hayward et al (17) differentiated between biovars of *Pseudomonas solanacearum* by using a range of tests, including carbon source utilization and acid production from carbohydrates in microtiter plates.

Recently, Biolog, Inc. (Hayward, CA), introduced the GN MicroPlate system for diagnosis of gram-negative bacteria based on reactions to a series of 95 carbon sources, including alcohols, polymeric chemicals, sugars, organic acids, and amino acids (2). Positive use of a carbon source is indicated by a color reaction, which can be assessed at the appropriate wavelength by measuring the optical density with a spectrophotometer. The color reaction results from oxidation of the carbon source and the resulting reduction of tetrazolium violet to a purple formazan. With 95 different carbon sources on the microplate, a reaction pattern of purple and colorless wells results, which has been called the organism's "metabolic fingerprint" (2). The ability to interface the microplate system with a database containing a large number of patterns offers considerable advantages in speed and accuracy of identification. This paper reports on the potential of the GN MicroPlate system, adapted for identification of aerobic gram-negative bacteria, as a rapid and accurate system for identification of some plant-pathogenic bacteria (*Agrobacterium*, *Clavibacter*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*).

MATERIALS AND METHODS

The bacterial strains used in these studies are listed in Table 1. Approximately 1,000 strains representing species of *Agrobacterium*, *Clavibacter*, *Erwinia*, *Pseudomonas*, and *Xanthomonas* were grown on trypticase soy agar (BBL Microbiology Systems, Cockeysville, MD) for 24 hr at 28 C. Many *X. campestris* pathovars used in this study were confirmed to be xanthomonads by the MIDI system for fatty acid cell wall analysis (28).

Bacterial cells were removed from plates with sterilized cotton swabs, suspended in sterilized saline (0.85% NaCl), and adjusted to match the Biolog GN MicroPlate system's turbidity standards at 590 nm on a spectrophotometer. A multichannel micropipetter was used to add 150 μ l of this suspension to each well of microplates supplied by Biolog. The plates were incubated for 4 and 24 hr at 28 C before a reading was taken with a 590-nm filter on a microplate reader (EAR400 AT, SLT-Labinstruments, Grödig, Austria). Results were analyzed with Biolog GN database version 2.00 to determine the identity of each strain.

After all strains had been processed at least once, a new database (Biolog GN version 3.00) was developed, incorporating our database (test strains only). All strain reaction patterns were reanalyzed a final time.

For comparisons of genera, species, and pathovars, all strains were analyzed with Biolog's cluster analysis program. Dendrograms based on cluster analysis were obtained, indicating relationships between various groups of bacteria.

RESULTS AND DISCUSSION

All data presented here were obtained from cultures incubated for 24 hr. Readings after a 4-hr incubation (data not shown) did not result in sufficient data for accurate analysis of most strains tested. For some of the bacteria tested, the percentages of strains correctly identified with the original database (Biolog GN version 2.00) are presented in Table 2. Many more groups of plant-pathogenic bacteria and a few bacteria commonly associated with plants but not

known to cause disease were also tested, but only those with a minimum of seven representative strains are included in Table 2.

Wide variation existed in the percent correct identification, from 96% for

Pseudomonas andropogonis and 94% for *P. cichorii* to 0% for several other bacteria tested (*P. fluorescens*, *P. syringae* pathovars, and *X. campestris* pv. *poinsettii*cola). However, when a revised database (Biolog GN version 3.00) was

developed from the original database with the addition of the data obtained in this study, the Biolog system was more effective in identification, even to the pathovar level for some of the *X. campestris* strains. For example, correct

Table 1. Source of bacterial strains used in this study for testing the Biolog GN MicroPlate system^a

Bacterium/No. of strains	Source/No. of strains ^b	Bacterium/No. of strains	Source/No. of strains ^b
<i>Agrobacterium tumefaciens</i> /20	DPI/3, JBJ/2, RES/15	<i>Xanthomonas campestris</i> pathovars	
<i>Clavibacter michiganensis</i>		(continued)	
subspecies		<i>carotae</i> /10	PDDCC/8, RES/1, ATCC/1
<i>insidiosus</i> /6	RES/6	<i>citrumelo</i> /30	DSE/30
<i>michiganensis</i> /37	RES/4, MDR/10, RDG/23	<i>cucurbitae</i> /4	PDDCC/4
<i>nebraskensis</i> /4	RES/4	<i>dieffenbachiae</i>	
<i>sepedonicus</i> /2	RES/2	<i>aglaonema</i> strains/10	AMA/4, ARC/2, PDD/4
<i>Erwinia amylovora</i> /3	JLN/3	<i>anthurium</i> strains/17	AMA/8, ARC/3, DPI/2, PDD/4
<i>E. carotovora</i> /23	ARC/8, DPI/5, PDD/7, RES/3	<i>caladium</i> strains/4	ARC/3, DPI/1
<i>E. chrysanthemi</i> /19	ARC/4, DPI/8, PDD/6, RES/1	<i>colocasia</i> strains/7	AMA/7
<i>E. herbicola</i> /7	RES/7	<i>dieffenbachia</i> strains/7	DPI/1, GWS/2, YOD/4
<i>Pseudomonas aeruginosa</i> /5	PDD/2, RES/3	<i>epipremnum</i> strains/6	AMA/2, ARC/3, DPI/1
<i>P. andropogonis</i> /29	ARC/2, GWS/3, RES/2, AKV/5, FLC/9, RDG/1, JBJ/3, NCPPB/4	<i>philodendron</i> strains/11	ARC/4, DPI/3, GWS/2, PDD/2
<i>P. avenae</i> /15	PDD/1, RES/14	<i>syngonium</i> strains/6	AMA/2, ARC/2, YOD/2
<i>Pseudomonas 'calathea'</i> /9	ARC/7, GWS/2	<i>xanthosoma</i> strains/11	KHP/11
<i>P. cattleyae</i> /4	ARC/1, PDD/1, RES/2	<i>fici</i> /21	ARC/1, DPI/6, GWS/8, PDD/4, RES/2
<i>P. cepacia</i> /11	RES/7, ATCC/3, AKV/1	' <i>fittonia</i> '/14	ARC/12, DPI/1, PDD/1
<i>P. cichorii</i> /30	ARC/10, DPI/12, JBJ/1, PDD/4, GWS/1, RES/1, ATCC/1	' <i>gardneri</i> '/1	RES/1
<i>P. pseudoalcaligenes</i>	JBJ/18, GCW/3, ATCC/1, RES/10	<i>hederae</i> /23	AMA/3, ARC/8, DPI/7, PDD/4, GWS/1
subsp. <i>citulli</i> /32	RES/13	<i>juglandis</i> /1	ATCC/1
<i>P. fluorescens</i> /13	RES/13	<i>maculifoliigardeniae</i> /14	ARC/2, DPI/7, PDDCC/2, PDD/2, GWS/1
<i>P. gladioli</i> /5	ATCC/2, DPI/3	<i>malvacearum</i> /20	ARC/2, DPI/9, GWS/7, PDD/2
<i>P. marginalis</i> /2	DPI/2	<i>manihotis</i> /6	PDDCC/4, RES/1, ATCC/1
<i>P. solanacearum</i> /13	PDD/2, RES/1, MDR/6, RDG/3, JBJ/1	<i>oryzae</i> /7	JBJ/7
<i>P. syringae</i> pathovars		<i>papavericola</i> /1	RES/1
<i>atofaciens</i> /2	RES/2	<i>pelargonii</i> /102	AMA/1, ARC/1, DPI/13, GWS/9, JBJ/13, MD/17, PDD/7, RES/17, ATCC/1, JWV/13, RSD/9, YOD/1
<i>hibisci</i> /6	ARC/3, JBJ/3	<i>phaseoli</i> /12	DPI/4, RES/5, RW/3
' <i>impatiens</i> '/15	ARC/1, DPI/1, JBJ/11, DAC/2	' <i>pilea</i> '/11	ARC/3, DPI/8
<i>morsprunorum</i> /4	GWS/4	<i>poinsettii</i> cola/21	AMA/3, ARC/1, DPI/4, PDDCC/3, GWS/1, PDD/1, RES/8
<i>phaseolicola</i> /2	RES/2	' <i>strelitzia</i> '/18	AMA/1, ARC/12, DPI/1, GWS/2, PDD/2
<i>syringae</i> /32	SMM/20, RDG/7, PDDCC/5	<i>syngonii</i> /9	AMA/2, ARC/5, MD/1, RSD/1
<i>tabaci</i> /1	RES/1	' <i>Ti</i> '/7	AMA/7
<i>tomato</i> /15	JBJ/8, CL/1, MDR/4, RDG/1, SMM/1	<i>vesicatoria</i> /20	RES/19, ATCC/1
<i>P. tolaasii</i> /1	RES/1	<i>vignicola</i> /5	RES/4, ATCC/1
<i>P. viridiflava</i> /25	RES/2, RDG/2, ATCC/4, JBJ/17	<i>vitians</i> /11	AMA/10, ATCC/1
Tissue culture contaminant/4	PDD/4	<i>zinniae</i> /22	ARC/14, DPI/2, GWS/2, PDDCC/2, RES/2
<i>Xanthomonas campestris</i> pathovars		<i>X. fragariae</i> /4	GWS/3, ATCC/1
<i>alfalfae</i> /5	RES/4, ATCC/1	<i>X. maltophilia</i> /15	MLE/9, RES/6
<i>begoniae</i> /25	ARC/6, DPI/13, PDD/4, GWS/1, RES/1	Opportunistic xanthomonads/21	RES/21
<i>campestris</i> /37	DPI/2, RES/14, RDG/20, ATCC/1		

^aOriginal strain designations used in this study to generate the Biolog GN database version 3.00 can be obtained from Biolog, Inc. (B. Bochner, personal communication) or from the first two authors.

^bAKV = A. K. Vidaver, University of Nebraska, Lincoln. AMA = A. M. Alvarez, University of Hawaii at Manoa, Honolulu. ARC = A. R. Chase, University of Florida, Apopka. ATCC = American Type Culture Collection, Rockville, MD. CL = C. Leben, Ohio State University, Wooster. DAC = D. A. Cooksey, University of California, Riverside. DPI = Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville. DSE = D. S. Egel, University of Florida, Gainesville. FLC = F. L. Caruso, Cranberry Experiment Station, East Wareham, MA. GCW = G. C. Wall, University of Guam, Mangilao. GWS = G. W. Simone, University of Florida, Gainesville. JBJ = J. B. Jones, University of Florida, Bradenton. JLN = J. L. Norelli, New York State Agricultural Experiment Station, Cornell University, Geneva. JWV = J. W. L. van Vuurde, Institute for Plant Protection, Wageningen, Netherlands. KHP = K. H. Pohronezny, University of Florida, Belle Glade. MD = M. Daughtrey, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, NY. MDR = M. Ricker, Heinz USA, Bowling Green, OH. MLE = M. L. Elliott, University of Florida, Ft. Lauderdale. NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, England. PDD = Plant Disease Diagnostics, Apopka, FL. PDDCC = Plant Diseases Division Culture Collection, Auckland, New Zealand. RDG = R. D. Gitaitis, University of Georgia, Tifton. RES = R. E. Stall, University of Florida, Gainesville. RSD = R. S. Dickey, Cornell University, Ithaca, NY. RW = R. Wilkinson, Cornell University, Ithaca, NY. SMM = S. M. McCarter, University of Georgia, Athens. YOD = Yoder Brothers, Inc., Alva, FL.

identification of *X. campestris* pv. *begoniae* rose from 6 to 71%, and that of *X. campestris* pv. *pelargonii* rose slightly from 69 to 76% (Table 2). Thus, the *X. c. begoniae* database improved dramatically by the addition of our 32 strains, while the *X. c. pelargonii* database did not improve even when 130 additional strain patterns were included. This may indicate that the number or diversity of strains in the original Biolog GN database (version 2.00) better represented the population of *X. c. pelargonii* strains than *X. c. begoniae* strains, or it may indicate that the latter organism is more diverse.

The relationships of all strains tested were evaluated by dendrogram analysis (Fig. 1). Biolog results showed that strains within a pathovar or species did fit into fairly tight groups. Two examples are *X. c. begoniae* (Fig. 2) and *P. andropogonis* (Fig. 3).

The clustering of strains from many pathovars of *X. campestris* in the Biolog analysis indicates the high degree of relatedness between the various pathovars and agrees with previous reports that separation of pathovars based on phenotypic uniqueness is difficult (3,10,16,37). DNA homology studies have been useful for identifying taxonomic uniqueness among the *X. campestris* pathovars (19,25,36), but groups exist within the pathovars that are difficult to separate. Although *X. fragariae* is considered a distinct species from *X. campestris*, the four strains we tested were very similar to *X. campestris* pv. *dieffenbachiae* isolated from *Epipremnum*. Further examination revealed that these *X. fragariae* strains were atypical and more likely belong to the group of opportunistic xanthomonads (15). Typical *X. fragariae* (20 strains) did not react in the Biolog GN MicroPlate system (A. R. Chase et al, unpublished data). Pathogenicity tests confirmed that the four strains that we used in the Biolog system were not pathogens of strawberry, while the three fastidious strains tested caused severe symptoms of angular leaf spot on strawberry, typical of those caused by *X. fragariae*.

Several xanthomonads were more distantly related according to cluster analysis. These included *X. campestris* pv. *oryzae* and *X. maltophilia* and several others. This corroborates previous studies in which these bacteria were determined to be quite distinct from other xanthomonads. In a study of 266 strains from many pathovars of *X. campestris*, using 295 morphological, biochemical, and physiological tests, van den Mooter and Swings (33) were able to differentiate to a limited extent some entities by means of phenotypic properties. They showed that *X. c. oryzae* was phenotypically distinct from many of the other xanthomonads, and it was proposed that the bacterium be reclassified

as a new species, *X. oryzae* (33). *X. maltophilia* has been classified as a xanthomonad (32), but it is distinct from other xanthomonads, with the exception of a group isolated from *Syngonium* and another group isolated from *Xanthosoma*. Recently it was proposed that *X. maltophilia* be placed in a new genus but be kept in a more general group with the other xanthomonads (34). The bacterial strains isolated from *Xanthosoma* appear to form a unique group according to fatty acid analysis (4), and strains isolated from *Syngonium* and identified as *X. campestris* pv. *syngonii* were phenotypically distinct from other *X. campestris* pathovars studied (9). With the complex nature of this genus existing, it is apparent that a polyphasic approach must be undertaken for more precise taxonomic structure within this group (36). Biolog tests have helped to confirm the uniqueness of these organisms, compared with other xanthomonads (4).

In the *Pseudomonas syringae* group, many strains were not correctly identified to pathovar with the MicroLog database (version 2.00); however, the strains from the pathovars of *P. syringae* clustered fairly close together. The paucity of

correct identity may have resulted from the low number of strains in the library or from the lack of accurate differentiation of *P. syringae* pathovars by carbon utilization patterns. Other researchers have substantiated the difficulty in discriminating between these pathovars or nomenspecies by physiological and biochemical tests (18,23,24,29), although nonrandom variation in phenotypic characteristics has been alluded to with the fluorescent pseudomonads (18,20). In fact, DNA homology studies indicate significant similarities between some of the pathovars and suggest that grouping of similar strains may be merited (27). When more strains were added to the Biolog database, 100% of *P. syringae* pv. *tomato* strains were identified correctly to pathovar, whereas only 65% of *P. syringae* pv. *syringae* strains were identified correctly after revision of the library. Thus, definitive phenotypic differentiation of many of the pathovars by physiological tests may require the use of more than the 95 tests available in the Biolog GN MicroPlate system. In addition, some groups may be inherently too diverse to differentiate at this time.

P. solanacearum was separated from most of the other pseudomonads tested

Table 2. Bacterial species with seven or more strains tested with the Biolog GN MicroPlate system and percentage of strains correctly identified by Biolog GN database version 2.00 and version 3.00

Bacterium	Original database (version 2.00)		Revised database (version 3.00)	
	No. of strains ^a	Percentage correctly identified	No. of strains	Percentage correctly identified
<i>Agrobacterium tumefaciens</i>	21	24	21	81
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	37	49	23	... ^b
<i>Erwinia carotovora</i>	23	52	16	96
<i>E. chrysanthemi</i>	16	87	31	88
<i>Pseudomonas</i> species				
<i>andropogonis</i>	31	96	31	100
<i>cepacia</i>	12	59	12	100
<i>cichorii</i>	52	94	52	100
<i>fluorescens</i>	13	0	13	100
<i>solanacearum</i>	13	31	13	100
<i>syringae</i> pv. <i>syringae</i>	32	0	32	65
<i>syringae</i> pv. <i>tomato</i>	15	0	15	100
<i>viridiflava</i>	25	8	25	87
<i>Xanthomonas campestris</i> pathovars				
<i>begoniae</i>	32	6	32	71
<i>campestris</i>	37	3	34	70
<i>carotae</i>	10	40	6	60
<i>citrumelo</i>	30	13	30	60
<i>dieffenbachiae</i>	7	43	4	50
'fittonia'	13	84	12	42
<i>hederae</i>	22	5	22	84
<i>malvacearum</i>	23	4	23	63
<i>pelargonii</i>	89	69	89	76
<i>phaseoli</i>	12	8	11	91
<i>poinsetticola</i>	21	0	21	29
'strelitzia'	18	11	15	27
<i>syngonii</i>	14	50	11	82
<i>vesicatoria</i>	20	10	24	50
<i>vitians</i>	11	9	11	50

^aIncludes strains run through the Biolog system more than once.

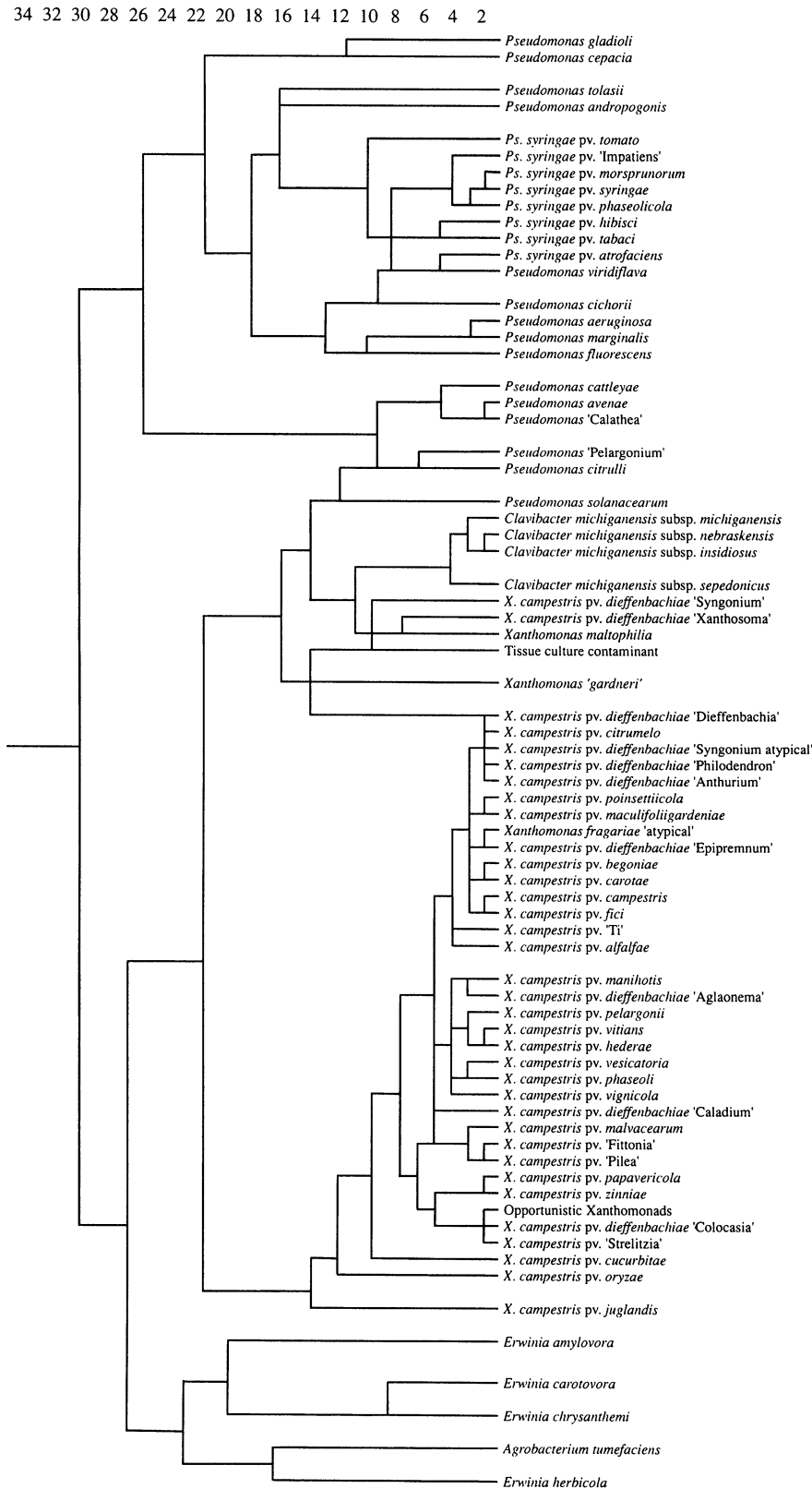
^bNot determined.

with Biolog but was placed closest to strains in the *P. acidovorans* complex. In DNA homology and DNA-rRNA hybridization studies, *P. solanacearum* was reported to be unique, compared with the other pseudomonads (5,8). De

Ley (5) placed *P. solanacearum* in superfamily 3, away from other plant-pathogenic bacteria, while Stakebrandt et al (31) placed it in the β group. In both instances it was in a large group that also contained the *P. acidovorans* complex.

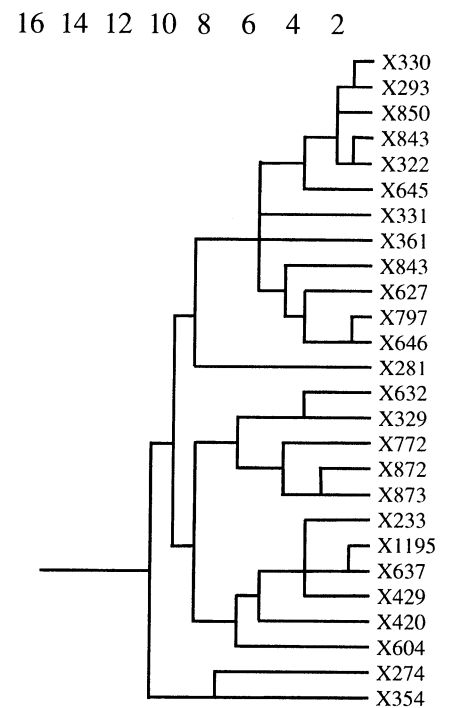
In another instance, Biolog placed *P. cattleyae*, *P. avenae*, and *P. pseudoalcaligenes* subsp. *citrulli* in a fairly tight group, which was substantially separate from other phytopathogenic pseudomonads. The close relationship between these three bacterial species has been shown in other studies (35,38,39). Willems et al (38) proposed that these organisms be placed in the new family Comamonadaceae. Using DNA-DNA hybridizations, DNA-rRNA hybridizations, and other physiological tests, Willems et al (39) proposed that *P. cattleyae*, *P. avenae*, and *P. p. citrulli* be transferred to the new genus *Acidovorax*. A bacterium isolated in our laboratory from geranium and identified by fatty acid analysis as either *P. facilis* or *P. delafieldii* (J. B. Jones et al, unpublished data) was placed in that same group by the Biolog system.

Although *Erwinia* species were not studied to the same extent as the xanthomonads and pseudomonads, the Biolog system grouped these strains in a predictable manner. *E. carotovora* and *E. chrysanthemi* strains grouped most closely, whereas *E. amylovora* and *E. herbicola* were considerably less closely related to the former species according to the dendrogram analysis. These groupings conformed to reports in the literature (11-14,22). Within *Erwinia* there appear to be at least four groups, as determined by analysis by Dye (11-14). The first is composed of



34 32 30 28 26 24 22 20 18 16 14 12 10 8 6 4 2

Fig. 1. Dendrogram showing the distances of all species and pathovars tested with the Biolog GN MicroPlate system in this study.



16 14 12 10 8 6 4 2

Fig. 2. Dendrogram showing the distances of all strains of *Xanthomonas campestris* pv. *begoniae* tested with the Biolog GN MicroPlate system.

pectolytic bacteria, such as *E. carotovora* and *E. chrysanthemi*, and has been labeled the carotovora group; the others have been labeled the herbicola group, the amylovora group, and the atypical erwinias. Dendrogram analysis showed similar relationships for the first three groups studied.

With *Agrobacterium*, the 23 strains formed a fairly isolated group, having most similarity to *E. herbicola*. According to the work of De Smedt and De Ley (7), *Agrobacterium* is more closely related to *Rhizobium* than to any of the other plant-pathogenic bacteria. Recently, in a study in which a large number of *Agrobacterium* strains representative of the three biovars were compared for utilization patterns in the GN MicroPlate system, all three biovars could be readily distinguished (H. Bouzar and J. B. Jones, unpublished data).

The efficiency of the GN MicroPlate system was clearly improved as the database was enlarged. The currently available Biolog library for many pathogens of *X. campestris* and *P. syringae* is limited in the number of strains and the diversity of their sources (unreported data), but it will continue to be improved as strains are added to the library. As a research tool, this system can be used in combination with DNA techniques (21), fatty acid profiles (21), serological assays (1,21,26), and pathogenicity tests to greatly improve the existing taxonomic structure of many of the plant-

pathogenic bacteria and to place newly described pathogens in the existing structure.

The Biolog GN MicroPlate system offers promise as a routine diagnostic procedure. It may be of limited value for a variety of moderately to highly fastidious bacteria, such as *Clavibacter* spp., *X. fragariae*, and a few *X. campestris* pathovars, which either would not grow on the trypticase soy agar medium or did not utilize the carbon sources employed in the plates. The latter possibility may be due to the binder compounds used in the wells or the choice of carbon sources themselves. The recently developed GP MicroPlate system for gram-positive bacteria was not tested, but for identification of *Clavibacter* it may prove superior to the GN MicroPlate system.

LITERATURE CITED

1. Alvarez, A. M., Benedict, A. A., and Mizumoto, C. Y. 1985. Identification of xanthomonads and grouping of strains of *Xanthomonas campestris* pv. *campestris* with monoclonal antibodies. *Phytopathology* 75:722-728.
2. Bochner, B. 1989. Sleuthing out bacterial identities. *Nature* 339:157-158.
3. Burkholder, W. H., and Starr, M. P. 1948. The generic and specific characters of phytopathogenic species of *Pseudomonas* and *Xanthomonas*. *Phytopathology* 38:494-502.
4. Chase, A. R., Stall, R. E., Hodge, N. C., and Jones, J. B. 1992. Characterization of *Xanthomonas campestris* strains from aroids using physiological, pathological, and fatty acid analyses. *Phytopathology* 82:754-759.
5. De Ley, J. 1978. Modern molecular methods in bacterial taxonomy: Evaluation, application, prospects. Pages 347-357 in: *Proc. Int. Conf. Plant Pathog. Bact.*, 4th.
6. DeParasis, J., and Roth, D. A. 1990. Nucleic acid probes for identification of phyto bacteria: Identification of genus-specific 16s rRNA sequences. *Phytopathology* 80:618-621.
7. De Smedt, J., and De Ley, J. 1977. Intra- and intergeneric similarities of *Agrobacterium* ribosomal ribonucleic acid cistrons. *Int. J. Syst. Bacteriol.* 27:222-240.
8. De Vos, P., and De Ley, J. 1983. Intra- and intergeneric similarities of *Pseudomonas* and *Xanthomonas* ribosomal ribonucleic acid cistrons. *Int. J. Syst. Bacteriol.* 33:487-509.
9. Dickey, R. S., and Zumoff, C. H. 1987. Bacterial leaf blight of *Syngonium* caused by a pathovar of *Xanthomonas campestris*. *Phytopathology* 77:1257-1262.
10. Dye, D. W. 1962. The inadequacy of the usual determinative tests for identification of *Xanthomonas* spp. *N.Z. J. Sci.* 4:393-416.
11. Dye, D. W. 1968. A taxonomic study of the genus *Erwinia*. I. The "amylovora" group. *N.Z. J. Sci.* 11:590-607.
12. Dye, D. W. 1969. A taxonomic study of the genus *Erwinia*. II. The "carotovora" group. *N.Z. J. Sci.* 12:81-97.
13. Dye, D. W. 1969. A taxonomic study of the genus *Erwinia*. III. The "herbicola" group. *N.Z. J. Sci.* 12:223-236.
14. Dye, D. W. 1969. A taxonomic study of the genus *Erwinia*. IV. "Atypical" erwinias. *N.Z. J. Sci.* 12:833-839.
15. Gitaitis, R. D., Sasser, M. J., Beaver, R. W., McInnes, T. B., and Stall, R. E. 1987. Pectolytic xanthomonads in mixed infections with *Pseudomonas syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper transplants. *Phytopathology* 77:611-615.
16. Hartung, J. S., and Civerolo, E. L. 1991. Variation among strains of *Xanthomonas campestris* causing citrus bacterial spot. *Plant Dis.* 75:622-626.

17. Hayward, A. C., El-Nashaar, H. M., De Lindo, L., and Nydegger, U. 1989. The use of microtiter plates in the phenotypic characterization of phytopathogenic pseudomonads. Pages 593-598 in: *Plant Pathogenic Bacteria. Proc. Int. Conf. Plant Pathog. Bact.*, 7th. Z. Klement, ed. Akadémiai Kiadó, Budapest.
18. Hildebrand, D. C., Huisman, O. C., Portis, A. M., and Schroth, M. N. 1987. The nonrandom variation in the distribution of phenotypic properties within a DNA homology matrix of fluorescent pseudomonads. Pages 291-297 in: *Plant Pathogenic Bacteria. E. L. Civerolo, A. Colmer, R. E. Davis, and A. G. Gillaspie, eds. Martinus Nijhoff, Dordrecht, Netherlands.*
19. Hildebrand, D. C., Palleroni, N. J., and Schroth, M. N. 1990. Deoxyribonucleic acid relatedness of 24 xanthomonad strains representing 23 *Xanthomonas campestris* pathovars and *Xanthomonas fragariae*. *J. Appl. Bacteriol.* 68:263-269.
20. Hildebrand, D. C., Schroth, M. N., and Huisman, O. C. 1982. The DNA homology matrix and non-random variation concepts as the basis for the taxonomic treatment of plant pathogenic and other bacteria. *Annu. Rev. Phytopathol.* 20:235-256.
21. Klement, Z., Rudolph, K., and Sands, D. C., eds. 1990. *Methods in Phytobacteriology.* Akadémiai Kiadó, Budapest.
22. Lelliott, R. A. 1974. Genus XII. *Erwinia*. Pages 332-340 in: *Bergey's Manual of Determinative Bacteriology*, 8th ed. R. E. Buchanan and N. E. Gibbons, eds. Williams & Wilkins, Baltimore.
23. Lelliott, R. A., Billing, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. *J. Appl. Bacteriol.* 29:470-489.
24. Misaghi, I., and Grogan, R. G. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonads. *Phytopathology* 59:1436-1450.
25. Murata, N., and Starr, M. P. 1973. A concept of the genus *Xanthomonas* and its species in the light of segmental homology of deoxyribonucleic acids. *Phytopathol. Z.* 77:285-323.
26. Norman, D., and Alvarez, A. 1989. A rapid method for presumptive identification of *Xanthomonas campestris* pv. *dieffenbachiae* and other xanthomonads. *Plant Dis.* 73:654-658.
27. Pecknold, P. C., and Grogan, R. G. 1973. Deoxyribonucleic acid homology groups among phytopathogenic *Pseudomonas* species. *Int. J. Syst. Bacteriol.* 23:111-121.
28. Roy, M. A. 1988. Use of fatty acids for the identification of phytopathogenic bacteria. *Plant Dis.* 72:460.
29. Sands, D. C., Schroth, M. N., and Hildebrand, D. C. 1970. Taxonomy of phytopathogenic pseudomonads. *J. Bacteriol.* 110:9-23.
30. Schaad, N. W., ed. 1988. *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. 2nd ed. American Phytopathological Society, St. Paul, MN.
31. Stakebrandt, E., Murray, R. G. E., and Truper, H. G. 1988. *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the "purple bacteria and their relatives." *Int. J. Syst. Bacteriol.* 38:321-325.
32. Swings, J., De Vos, P., Van den Mooter, M., and De Ley, J. 1983. Transfer of *Pseudomonas maltophilia* Hugh 1981 to the genus *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov. *Int. J. Syst. Bacteriol.* 33:409-413.
33. Van den Mooter, M., and Swings, J. 1990. Numerical analysis of 295 phenotypic features of 266 *Xanthomonas* strains and related strains and an improved taxonomy of the genus. *Int. J. Syst. Bacteriol.* 40:348-369.
34. Van Zyl, E., and Steyn, P. L. 1992. Reinterpretation of the taxonomic position of *Xanthomonas maltophilia* and taxonomic criteria in this genus. Request for an opinion. *Int. J. Syst. Bacteriol.* 42:193-198.
35. Van Zyl, E., and Steyn, P. L. 1991. Taxonomy of the phytopathogenic *Pseudomonas* species belonging to the acidovorans rRNA complex. *Syst. Appl. Microbiol.* 14:165-168.

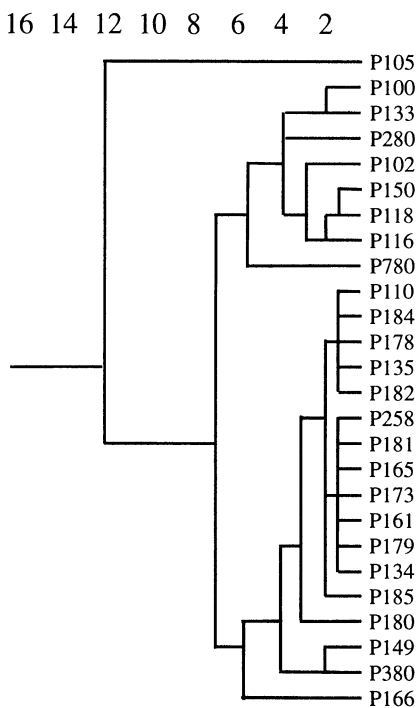


Fig. 3. Dendrogram showing the distances of all strains of *Pseudomonas andropogonis* tested with the Biolog GN MicroPlate system.

36. Vauterin, L., Swings, J., Kersters, K., Gillis, M., Mew, T. W., Schroth, M. N., Palleroni, N. J., Hildebrand, D. C., Stead, D. E., Civerolo, E. L., Hayward, A. C., Maraite, H., Stall, R. E., Vidaver, A. K., and Bradbury, J. F. 1990. Towards an improved taxonomy of *Xanthomonas*. Int. J. Syst. Bacteriol. 40:312-316.
37. Wernham, C. C. 1948. The species value of pathogenicity in the genus *Xanthomonas*. Phytopathology 38:283-291.
38. Willems, A., De Ley, J., Gillis, M., and Kersters, K. 1991. *Comamonadaceae*, a new family encompassing the acidovorans rRNA complex, including *Variovorax paradoxus* gen. nov., comb. nov., for *Alcaligenes paradoxus* (Davis 1969). Int. J. Syst. Bacteriol. 41:445-450.
39. Willems, A., Goor, M., Thielemans, S., Gillis, M., Kersters, K., and De Ley, J. 1992. Transfer of several phytopathogenic *Pseudomonas* species to *Acidovorax avenae* subsp. *avenae* subsp. nov., comb. nov., *Acidovorax avenae* subsp. *citrulli*, *Acidovorax avenae* subsp. *cattleyae*, and *Acidovorax konjaci*. Int. J. Syst. Bacteriol. 42:107-119.