Identification of Phytopathogenic Coryneform Bacteria Using the Biolog Automated Microbial Identification System

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ABSTRACT

Harris-Baldwin, A., and Gudmestad, N. C. 1996. Identification of phytopathogenic coryneform bacteria using the Biolog automated microbial identification system. Plant Dis. 80:874-878.

A carbon utilization microplate assay system developed by Biolog, Inc., has the potential to simplify the identification scheme of phytopathogenic bacteria. Carbon source utilizations by 140 plant-pathogenic coryneform bacterial strains were evaluated using the Biolog system. All *Curtobacterium* strains were identified correctly to the genus level. Correct identification of the *Clavibacter* strains to the genus level varied from 27 to 77% depending on the subspecies. After supplementing the Biolog database with newly derived data, identification of certain species improved. The greatest improvement was observed with *Rhodococcus fascians*, *Clavibacter michiganensis* subsp. *tessellarius,* and three pathovars of *Curtobacterium.* Of the organisms tested, *Clavibacter xyli* subsp. *cynodontis*, *C. toxicus*, *C. rathayi*, *C. tritici*, *C. iranicus, Arthrobacter ilicis,* and *R. fascians* were not identified using the original Biolog database. Eighty-three percent of the carbon sources on the Biolog plate are utilized by at least one of the phytopathogenic coryneforms. Only one carbon source, Tween 40, was used by all organisms in this study.

The classification of phytopathogenic coryneform bacteria was in disarray for many years. Initially, this diverse collection of microorganisms was grouped together in the genus *Corynebacterium*, mainly on the basis of cell morphology, staining properties, and respiratory metabolism (15). With developing technology, this nonhomogeneous group of microorganisms has undergone multiple revisions (5). Currently, the taxonomic scheme for phytopathogenic coryneform bacteria includes four genera: *Arthrobacter* (4), *Clavibacter* (6), *Curtobacterium* (3), and *Rhodococcus* (10).

Although the taxonomy of the phytopathogenic coryneform bacteria appears to be largely resolved at this time, difficulties still remain with regard to identification. The identity of a slow-growing gram-positive bacterium isolated from diseased plant material is confirmed by inoculation tests in plants (24,25). Depending on whether or not suitable plant material is available for inoculation, this procedure may take from a few weeks to a few months. Delineation of phytopathogenic coryneforms to species or subspecies can also be achieved using traditional biochemical and physiological tests (24,25) or by using fatty acid analysis (12). These types of analysis, however, are also time and labor intensive and are not conducive to large-scale testing. A need for rapid and reliable screening of strains exists for this group of organisms.

Studies were conducted to assess a rapid, commercial indicator-microplate-assay system developed by Biolog, Inc. (Hayward, CA). The Biolog automated identification system is based on the differential utilization of 95 carbon sources. A redox dye, tetrazolium violet, is used to visualize the increased respiration of bacteria while utilizing a carbon source (1). Each microorganism gives a metabolic fingerprint that is compared with those of known bacteria whose profiles are entered into a database. The Biolog phenotypic characterization may simplify the identification of phytopathogenic coryneform bacteria. The main objectives of this study were to evaluate the accuracy of the Biolog database for identification of phytopathogenic coryneform bacteria, and to evaluate a supplemented database of Biolog using profiles generated from our collection of phytopathogenic coryneform bacteria.

MATERIALS AND METHODS

Bacterial strains. The accuracy of the Biolog gram-positive (GP) system was evaluated from tests of 140 plant-pathogenic bacterial strains (Table 1). Of these strains, 96 were of the genus *Clavibacter* and 33 were of the genus *Curtobacterium.* The remainder were *Rhodococcus fascians* (10 strains) and *Arthrobacter ilicis* (1 strain). This array of organisms included both type strains and recently recovered strains. Most were previously identified by Henningson and Gudmestad (12) using standard biochemical and physiological tests. Each organism was Gram stained using standard protocol (21). *Clavibacter michiganensis* subsp. *sepedonicus* was also identified by an immunofluorescence assay (7).

Propagation. Each strain, excluding *Clavibacter xyli* subsp. *cynodontis,* was propagated on nutrient broth yeast extract agar medium (NBY medium [12]) at 23°C. For Biolog tests, all *Clavibacter* and *Curtobacterium* species were transferred to trypticase soy agar (TSA; Becton Dickinson Microbiology Systems, Cockeysville, Md.) and again incubated at 23°C. Incubation times varied from the recommended (Biolog Microstation Manual) 4 to 18 h for *Curtobacterium, Rhodococcus,* and *Arthrobacter,* to 7 to 10 days for *Clavibacter. Rhodococcus* and *Arthrobacter* were transferred to the recommended blood agar medium. The bacterial cells were removed from the surface of the agar with a sterile swab and resuspended to 10^8 cells per ml (21) in sterile physiological saline. Because *Clavibacter xyli* subsp. *cynodontis* could not sustain growth on TSA, it was propagated in NBY broth; the bacterial cells were pelleted by centrifugation at 3,000 × *g* and washed three times with sterile physiological saline; and the pellet was resuspended in sterile physiological saline to 10^8 cells per ml.

Microplate assay. Each well in Biolog GP microplates was inoculated with 150 µl of a bacterial suspension of an individual strain. Prior to use, each plate was stored at 4°C and used before the expiration date. Following 24 h of incubation at 25°C, the plate was evaluated on a microplate autoreader (Bio-tek EL311) at a wavelength of 590 nm, and outputs were compared by the Microlog Software (Biolog, release 3.5).

The supplemented Biolog database was constructed by saving the profile for each organism on diskette. The entire identification process was repeated for each organism as described above, evaluated by the supplemented database, and reported as trial 1 and trial 2. The Biolog system produces a printout of identification choices that includes the identification of the species followed by the next 10 closest species. In building a database, correct identification within the top five choices of closest species is considered reasonable.

RESULTS

All strains of *Clavibacter* and *Curtobacterium* were gram-positive, rod-shaped

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Accepted for publication 9 April 1996.

Publication no. D-1996-0502-04R

Table 1. Bacterial strains tested by the Biolog GP Micro Plate System

Strain	Source[a]	Host
Clavibacter michiganensis subsp. *sepedonicus* (22 strains)		
33111	ATCC - Canada	*Solanum tuberosum*
33113	ATCC - Canada	
Cs5	New York	
Cs12	New Brunswick	
Cs14	S.H. DeBoer - Montana	
Cs106	S.H. DeBoer - West Virginia	
INM-1	R.G. Clarke - Idaho	
CsCA	A. Vidaver - California	
2531	NZPDDCC - Sweden	
2537	NZPDDCC - USA	
BJ19	NDSU Collection - Oregon	*Beta vulgaris* (seed)
DIPPE2	NDSU Collection - Oregon	
MONOR	NDSU Collection - Oregon	
SB-109	NDSU Collection - North Dakota	*B. vulgaris* (root)
Ak14	NDSU Collection - Alaska	*S. tuberosum*
As-1	NDSU Collection - Minnesota	
BC-P45	British Columbia	
Florida	NDSU Collection - Florida	
OFF	NDSU Collection - Minnesota	
RR1a	S.H. DeBoer - Alberta	
SD-1	NDSU Collection - South Dakota	
Wi2	NDSU Collection - Wisconsin	
Clavibacter michiganensis subsp. *michiganensis* (15 strains)		
4450	ATCC	Unknown
7430	ATCC	*Cyphomandra fragranis*
10202	ATCC	*Lycopersicon esculentum*
14456	ATCC	*L. esculentum*
1104	L.E. Clafin	Unknown
549	NZPDDCC	*L. esculentum*
1436	NZPDDCC - New Zealand	*L. esculentum*
1808	NZPDDCC	
1811	NZPDDCC - New Zealand	*Cyphomandra betacea*
2355	NZPDDCC - New Zealand	
2539	NZPDDCC	*L. esculentum*
2541	NZPDDCC - United Kingdom	
2545	NZPDDCC - Sicily	
2550	NZPDDCC - Hungary	
2551	NZPDDCC - Brazil	
Clavibacter michiganensis subsp. *insidiosus* (14 strains)		
239	A. Vidaver	Unknown
10253	ATCC	Unknown
Ci4	S.H. DeBoer	Unknown
Ci16	S.H. DeBoer	Unknown
CiN53	S.H. DeBoer	*Medicago sativa*
Ci102B	S.H. DeBoer	Unknown
CiLeth	S.H. DeBoer	*M. sativa* (seed)
CiAW81	S.H. DeBoer	*M. sativa*
2621	NZPDDCC - USA	
2611	NZPDDCC - United Kingdom	
2948	NZPDDCC - New Zealand	
3567	NZPDDCC - Australia	
3983	NZPDDCC - USA	
6565	NZPDDCC - Florida	
Clavibacter michiganensis subsp. *nebraskensis* (15 strains)		
27794	ATCC	*Zea mays*
27822	ATCC	
1105	L.E. Clafin - Colorado	
1106	L.E. Clafin - Kansas	
1118	L.E. Clafin - Kansas	
1119	L.E. Clafin - Kansas	
3294	NZPDDCC	
3299	NZPDDCC - USA	
3300	NZPDDCC - USA	
3303	NZPDDCC - USA	
5366	NZPDDCC - USA	
5367	NZPDDCC - USA	
5368	NZPDDCC - USA	
CNEB	J. Venette - North Dakota	
5369	NZPDDCC - USA	
Clavibacter michiganensis subsp. *tessellarius* (12 strains)		
1122	L.E. Clafin - Nebraska	Unknown
7219	NZPDDCC - USA	*Triticum aestivum*
7220	NZPDDCC - USA	
7221	NZPDDCC - USA	
7222	NZPDDCC - USA	
7223	NZPDDCC - USA	
7224	NZPDDCC - USA	
7225	NZPDDCC - USA	*T. aestivum*
7226	NZPDDCC - USA	
7227	NZPDDCC - USA	
7228	NZPDDCC - USA	
7229	NZPDDCC - USA	
Clavibacter xyli subsp. *cynodontis* (4 strains)		
TB1-A	M.J. Davis - China	*Cynodon dactylon*
FB-1	M.J. Davis - China	
33973	ATCC - China	
TB2B	M.J. Davis - China	
Clavibacter toxicus (2 strains)		
49908	NZPDDCC - Australia	*Lolium* sp.
49909	NZPDDCC - Australia	
Clavibacter rathayi (5 strains)		
13659	ATCC	*Dactylis glomerata*
2572	NZPDDCC - New Zealand	
2573	NZPDDCC - New Zealand	
2574	NZPDDCC - New Zealand	
2575	NZPDDCC - New Zealand	
Clavibacter tritici (5 strains)		
2623	NZPDDCC - India	*T. aestivum*
2624	NZPDDCC - Egypt	
2626	NZPDDCC - Egypt	
2627	NZPDDCC - India	
2628	NZPDDCC - Iran	
Clavibacter iranicus (1 strain)		
3496	NZPDDCC	
Curtobacterium flaccumfaciens pv. *poinsettiae* (9 strains)		
2561	NZPDDCC - USA	*Euphorbia pulcherrima*
2563	NZPDDCC - USA	
2564	NZPDDCC - USA	
2565	NZPDDCC - USA	
2567	NZPDDCC - USA	
2568	NZPDDCC - USA	
2569	NZPDDCC - USA	
2570	NZPDDCC - USA	
3500	NZPDDCC - USA	
Curtobacterium flaccumfaciens pv. *flaccumfaciens* (10 strains)		
2580	NZPDDCC - USA	*Phaseolus* sp.
2581	NZPDDCC - USA	
2582	NZPDDCC - Romania	*Phaseolus vulgaris*
2583	NZPDDCC	
2584	NZPDDCC - Hungary	
2585	NZPDDCC - D. R. of Germany	
2588	NZPDDCC - USA	*Vigna angularis*
2590	NZPDDCC - USA	*Vigna radiata*
3495	NZPDDCC - Netherlands	*P. vulgaris*
5370	NZPDDCC - USA	*P. vulgaris*
Curtobacterium flaccumfaciens pv. *betae* (9 strains)		
13437	ATCC	Unknown
2591	NZPDDCC - United Kingdom	*B. vulgaris*
2592	NZPDDCC - United Kingdom	
2593	NZPDDCC - United Kingdom	
2595	NZPDDCC - United Kingdom	
4735	NZPDDCC - United Kingdom	
4736	NZPDDCC - United Kingdom	
4737	NZPDDCC - United Kingdom	
7458	NZPDDCC - Brazil	
Curtobacterium flaccumfaciens pv. *oortii* (5 strains)		
6-83-70-A	G. A. Secor - Netherlands	Unknown
2632	NZPDDCC - Netherlands	*Tulipa gesneriana*
3497	NZPDDCC - Netherlands	
3498	NZPDDCC - United Kingdom	
3499	NZPDDCC - United Kingdom	
Rhodococcus fascians (10 strains)		
13000	ATCC	*Lathyrus odoratus*
2596	NZPDDCC - United Kingdom	*Fragaria* × *ananassa*
2597	NZPDDCC - United Kingdom	
2598	NZPDDCC - United Kingdom	
2599	NZPDDCC - United Kingdom	
2600	NZPDDCC - United Kingdom	*Chrysanthemum* × *morifolium*
2601	NZPDDCC - United Kingdom	*Justicia brandegeana*
2605	NZPDDCC - USA	Unknown
7109	NZPDDCC - Netherlands	*Gladiolus* sp.
7364	NZPDDCC	*Dahlia* sp.
Arthrobacter ilicis (1 strain)		
2609	NZPDDCC - USA	*Ilex opaca*

[a] ATCC, American Type Culture Collection; NDSU, North Dakota State University; NZPDDCC, New Zealand Plant Disease Division Culture Collection.

bacteria. *R. fascians,* also gram positive, formed branched hyphae that fragmented into rods and cocci. *A. ilicis* stained gram positive and formed rods upon transfer to fresh medium and coccoid cells during the stationary phase.

Correct identification to genus, species, subspecies, and pathovar varied widely among species and subspecies of *Clavibacter* and *Curtobacterium.* All strains of *Curtobacterium* were identified correctly (100%) to the genus level, excluding trial 2 for *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (90%) (Table 2). *Curtobacterium flaccumfaciens* pv. *oortii* was the only *Curtobacterium* organism to be correctly identified to the species level (33% of the time). *C. f.* pv. *flaccumfaciens* and *Curtobacterium flaccumfaciens* pv. *betae* were identified to pathovar; however, each organism was correctly identified only 10 and 20% of the time, respectively.

Strains of *Clavibacter* spp. were correctly identified less frequently than those of *Curtobacterium.* Correct genus identification varied from 27 to 77% depending on the subspecies. Within the *Clavibacter* genus, subspecies *sepedonicus* was correctly identified 59% (trial 1) and 36% (trial 2) of the time; subspecies *michiganensis* was identified correctly 20% (trial 1) and 0% (trial 2) of the time; and subspecies *insidiosus* was identified correctly 13% (trials 1 and 2) of the time. The Biolog database does not include the subspecies *nebraskensis* and *tessellarius* for the genus *Clavibacter.* Even so, 20% (trial 1) and 33% (trial 2) of subspecies *nebraskensis* strains, and 33% (trial 1) and 25% (trial 2) of subspecies *tessellarius* strains, were correctly identified at the species level. The Biolog system provides an identification and also lists 10 other possibilities. However, the similarity factor on which identification is partially based can be below the recommended 0.500 value for identification. A high percentage of *Clavibacter* strains was identified correctly to the subspecies level in the top five choices. More specifically, in trial 1, *Clavibacter michiganensis* subsp. *sepedonicus*, subsp. *michiganensis,* and subsp. *insidiosus* were identified in the top five choices at 68, 93, and 53%, respectively (data not shown).

All strains of *Clavibacter xyli* subsp. *cynodontis*, *C. toxicus*, *C. rathayi*, *C. tritici,* and *C. iranicus* were unable to sustain growth on the Biolog microplate and resulted in an "insufficient growth" profile. Hence, no identification could be made. The single *Arthrobacter* strain produced a profile of "too many borderline reactions" and therefore could not be identified at any time. *R. fascians* did not have a matching profile in the database.

R. fascians could be identified using the supplemented database. In fact, when the supplemented database was compiled by propagating *R. fascians* on TSA rather than on blood agar, a greater increase in identification was noted. *R. fascians* was identified to the genus level 33% (trial 1) and 67% (trial 2) (data not shown), and to the species level 33% (trials 1 and 2) of the time (Table 3).

Within the genus *Curtobacterium*, identification with the supplemented database to the species and pathovar levels increased considerably for three pathovars: *poinsettiae*, *flaccumfaciens,* and *betae.* Species identification nearly doubled, and all were identified to the pathovar level 40% of the time, excluding trial 1 for *Curtobacterium flaccumfaciens* pv. *betae* at 20%. This represents an approximate increase in correct identification of 30% over the Biolog database. *Curtobacterium flaccumfaciens* pv. *oortii* identification at the pathovar level did not improve with the supplemented database. Using the supplemented database, identification of *Clavibacter michiganensis* subsp. *tessellarius* was improved from 42% (trial 1) and 33% (trial 2) to 80% (trials 1 and 2) at the genus level (data not shown), and to 80% (trial 1) and 60% (trial 2) at the species level. However, neither *Clavibacter michiganensis* subsp. *nebraskensis* nor *Clavibacter michiganensis* subsp. *tessellarius* can be identified to the subspecies level with the supplemented database.

Both the Biolog database and the supplemented database poorly distinguished *Clavibacter* from *Curtobacterium* (Table 4). When *Clavibacter michiganensis* subsp. *michiganensis* subsp. *nebraskensis,* and subsp. *tessellarius* were misidentified, a *Curtobacterium* species was indicated approximately half of the time. Identification of these organisms with the supplemented database did not differ significantly from the original Biolog database (Table 4).

Specific carbon utilization was evaluated with a computer program developed in our laboratory (data not shown). Of the 95 carbon sources, 79 were utilized by at least

Table 2. Correctly[a] identified strains using the Biolog database system

Organism (no. of strains tested)	Strains correctly identified (%) Genus Trial 1/trial 2	Species Trial 1/trial 2	Subsp./pathovar Trial 1/trial 2
Clavibacter michiganensis subsp. *sepedonicus* (22)	77/45	68/41	59/36
C. m. subsp. *michiganensis* (15)	60/47	33/27	20/0
C. m. subsp. *insidiosus* (15)	40/53	40/47	13/13
C. m. subsp. *nebraskensis* (15)	27/40	20/33	0/0
C. m. subsp. *tessellarius* (12)	42/33	33/25	0/0
Curtobacterium flaccumfaciens pv. *poinsettiae* (9)	100/100	33/33	0/0
C. f. pv. *flaccumfaciens* (10)	100/90	20/20	10/20
C. f. pv. *betae* (9)	100/100	20/33	11/0
C. f. pv. *oortii* (5)	100/100	60/80	0/0
Rhodococcus fascians (10)	0/0	0/0	NA[b]

[a] Compared to described identifications, generally made by traditional methods.
[b] NA = not applicable.

Table 3. Correctly identified strains determined with a supplemented Biolog database

Organism (no. of strains tested)	Strains correct to species level (%) (trial 1/trial 2)[a]
Clavibacter michiganensis subsp. *sepedonicus* (19)	52/79
C. m. subsp. *michiganensis* (5)	20/20
C. m. subsp. *insidiosus* (5)	40/60
C. m. subsp. *nebraskensis* (5)	40/80
C. m. subsp. *tessellarius* (5)	80/60
Curtobacterium flaccumfaciens pv. *poinsettiae* (5)	40/80
C. f. pv. *flaccumfaciens* (5)	40/80
C. f. pv. *betae* (5)	20/60
C. f. pv. *oortii* (5)	0/60
Rhodococcus fascians (5)	33/33[b]

[a] Top choice in top 10 choices.
[b] *R. fascians* only to species level.

Table 4. Percent misidentified *Clavibacter* strains identified as *Curtobacterium* from the Biolog database[a]

Organism	Misidentified (%) (trial 1/trial 2)
Clavibacter michiganensis subsp. *sepedonicus*	22/13
C. m. subsp. *michiganensis*	47/40
C. m. subsp. *insidiosus*	27/13
C. m. subsp. *nebraskensis*	67/53
C. m. subsp. *tessellarius*	67/42

[a] Misidentification percentages with the supplemented database was ±1% of the results shown.

one of the phytopathogenic coryneform organisms tested. Only one carbon source, Tween 40, was used by all of the coryneforms. In fact, Tween 40 was the only carbon source on the Biolog plate used by *Clavibacter xyli* subsp. *cynodontis*, *C. toxicus*, *C. rathayi*, *C. tritici*, and *C. iranicus*. Sixteen carbon sources were not utilized by any of the organisms tested during this study. These include L-rhamnose, *y*-hydroxybutyric acid, *p*-hydroxybutyric acid, lactamide, propionic acid, succinamic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, L-serine, uridine, adenosine-5-monophosphate, thymidine-5-monophosphate, uridine-5-monophosphate, glucose-1-phosphate, glucose-6-phosphate, and D-1-a-glycerol phosphate.

DISCUSSION

Many diagnostic, regulatory, and research laboratories could benefit from a rapid identification system for bacterial strains. The usefulness of such a system would include traditional bacterial identification as well as verifying the identification of strains from other sources, identifying bacteria recovered during research studies, and corroborating classification of previously described organisms. The adaptation of a Biolog system for the rapid identification of plant-pathogenic coryneforms would be useful, as demonstrated by similar studies using other phytopathogenic bacteria (13,16,22).

The Biolog protocol for propagation of coryneform organisms recommends TSA medium for *Curtobacterium* and *Clavibacter* species prior to inoculating a microplate. Both *Rhodococcus* and *Arthrobacter* are to be grown on blood agar medium. We found that all 10 of the *Rhodococcus* strains and the single strain of *Arthrobacter* evaluated in this paper grew well on TSA medium. Also, *R. fascians* was correctly identified approximately 10% more frequently when propagated on TSA medium using a supplemented database (Table 3). We believe that TSA medium should be used for all plant coryneforms. However, until the Biolog manual states differently, it is important not to deviate from the recommended growth media, because the Biolog database is categorized by medium type.

The single strain of *Arthrobacter* could not be identified at any time. Optimally, multiple strains are needed for a better assessment of the Biolog system. The Biolog database contains only *Arthrobacter histidinolovorans*, hence its inability to correctly identify a plant-pathogenic *Arthrobacter* species. Growth on a Biolog gram-positive microplate did not occur for five *Clavibacter* species (*Clavibacter xyli* subsp. *cynodontis*, *C. toxicus*, *C. rathayi*, *C. tritici,* and *C. iranicus*). This insufficient growth may be due to inadequate nutrients in the microplate for maintaining metabolic processes. As a result, none of these *Clavibacter* species will be correctly identified by the Biolog system.

The Biolog manual lists the carbon source for each well of the microtiter plate. Vidaver (23) observed an increase in growth of *Clavibacter michiganensis* subsp. *nebraskensis* and *C. m.* subsp. *tessellarius* when the propagation medium was supplemented with biotin, nicotinic acid, and thiamine. She also noted a requirement of L-methionine. *C. m.* subsp. *michiganensis*, *insidiosus,* and *sepedonicus* were reported to require histidine and one or more of adenine, guanine, and uracil (17). All three species were also reported to require biotin, nicotinic acid, and thiamine (14,20). *C. m.* subsp. *sepedonicus* may also require L-asparagine and L-methionine, and be stimulated by histidine and leucine (14). A number of these may be needed to supplement the Biolog system for use with plant-pathogenic coryneforms.

The minimal nutritional requirements of *Clavibacter rathayi*, *C. tritici*, and *C. iranicus* are unknown. Possibly, if some or all of the nutrients mentioned above were added or increased in the Biolog microplate, growth may occur for the five *Clavibacter* species not identified. The microplate well of water, A1, would remain as the reference for background reactions.

A new genus, *Rathayibacter*, has been proposed to accommodate *Clavibacter rathayi*, *C. tritici,* and *C. iranicus* (26). The new genus proposal was partially based on menaquinone and whole-cell sugar compositions, cell wall composition, carbon utilization, and resistance to bacteriocins. These three species have also been grouped on the basis of their allelic profiles (18), distinguishing them from *Clavibacter michiganensis* and its subspecies. Colorimetric indicator plate assay systems can be reliable sources for metabolic and taxonomic studies (2,11,19). If the Biolog system could accommodate *Clavibacter rathayi*, *C. tritici,* and *C. iranicus* nutritional requirements, possibly a phenotypic pattern from carbon utilization could validate the new genus *Rathayibacter*.

The data presented here demonstrate that the Biolog database cannot identify all phytopathogenic coryneform bacteria with high accuracy. We feel the low identification rate may indicate that the database is not comprised of profiles from ample strains or a significant variety of strains. Biolog does not claim any accuracy for identifying phytopathogenic coryneforms. However, the Biolog system can reliably identify to genus *Curtobacterium*. In 1983, Collins and Jones reclassified *Corynebacterium flaccumfaciens*, *C. beta*, *C. oortii,* and *C. poinsettiae* in the genus *Curtobacterium*, as *Curtobacterium flaccumfaciens* comb. nov. (3). This proposal was supported by DNA–DNA homology studies (8) indicating that all four strains were genetically similar, and biochemical and physiological data showed a close relationship (9). Our tests indicate that carbon utilization within the Biolog system does not differentiate the species of the above taxa, and the tests therefore support the Collins and Jones reclassification scheme.

The *Clavibacter* species evaluated in this study were identified with low accuracy by the Biolog database. We did, however, see a higher proportion of *Clavibacter* strains identified correctly to the subspecies level in the top choices provided by the system. It is evident that the five *Clavibacter michiganensis* subspecies (*sepedonicus*, *michiganensis*, *insidiosus*, *nebraskensis,* and *tessellarius*) can develop usable carbon utilization patterns, or metabolic fingerprints, on the Biolog microplate. Misidentified *Clavibacter* were identified as *Curtobacterium* approximately half of the time in the Biolog database (Table 4). Apparently, the Biolog database should be supplemented with profiles from more *Clavibacter* strains to more reliably distinguish *Clavibacter* from *Curtobacterium*.

The supplemented Biolog database improved identification accuracy. However, inputting carefully derived profiles can be costly and time-consuming. The greatest improvement in accuracy was observed with *R. fascians*, *Clavibacter michiganensis* subsp. *tessellarius,* and three pathovars of *Curtobacterium*. Perhaps the number or diversity of strains in the Biolog database does not adequately represent the organisms in this study. Verniere et al. (22) saw 63.2% increase in accuracy of identification of *Xanthomonas campestris* pv. *citri* with a supplemented database to the Biolog system (release 2.0).

Most of the carbon sources (83%) on the Biolog plate could be utilized by at least one of the phytopathogenic coryneforms. Zgurskaya et al. (26) indicated that *Clavibacter rathayi*, *C. tritici*, and *C. iranicus* utilize inositol, melibiose, L-rhamnose, and tagatose. All four of these carbon sources are on the Biolog plate; however, none were catabolized. This again leads us to believe that a higher concentration of these nutrients may lead to improved metabolic process, resulting in utilization of the carbon sources. Also, feasible additions to the Biolog microplate might include levan, potato starch, and gelatin. *Clavibacter tritici* and *C. rathayi* utilize levan; *R. fascians* and *C. rathayi* hydrolyze potato starch; and *C. rathayi* hydrolyzes gelatin (9).

Performance of diagnostic systems may not meet the expectations of all laboratories. The Biolog automated system has provided excellent identification of other organisms (13,22), primarily gram-negative bacterial species. In these studies, a supplemented database improved accuracy in identifying plant-pathogenic coryneform bacteria, making the Biolog system more reliable, convenient, and time-saving.

LITERATURE CITED

1. Bochner, B. R. 1989. Sleuthing out bacterial identities. Nature (London) 339:157-158.
2. Bochner, B. R., and Savageau, M. A. 1977. Generalized indicator plate for genetic, metabolic, and taxonomic studies with microorganisms. Appl. Environ. Microbiol. 33:434-444.
3. Collins, M. D., and Jones, D. 1983. Reclassification of *Corynebacterium flaccumfaciens*, *Corynebacterium betae*, *Corynebacterium oortii* and *Corynebacterium poinsettiae* in the genus *Curtobacterium*, as *Curtobacterium flaccumfaciens* comb. nov. J. Gen. Microbiol. 129:3545-3548.
4. Collins, M. D., Jones, D., and Kroppenstedt, R. M. 1981. Reclassification of *Corynebacterium ilicis* (Mandel, Guba and Litsky) in the genus *Arthrobacter*, as *Arthrobacter ilicis* comb. nov. Zentralbl. Bakteriol., Mikrobiol. Hyg. 2:318-323.
5. Davis, M. J. 1986. Taxonomy of plant-pathogenic coryneform bacteria. Annu. Rev. Phytopathol. 24:115-140.
6. Davis, M. J., Gillaspie, A. G., Jr., Vidaver, A. K., and Harris, R. W. 1984. *Clavibacter*: A new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause ratoon stunting disease of sugarcane and bermudagrass stunting disease. Int. J. Syst. Bacteriol. 34:107-117.
7. De Boer, S. H. 1990. Immunofluorescence for bacteria. Pages 295-298 in: Serological methods of detection and identification of viral and bacterial plant pathogens. R. Hampton, E. Ball, and S. De Boer, eds. American Phytopathological Society, St. Paul, MN.
8. Dofper, H., Stackebrandt, E., and Fiedler, F. 1982. Nucleic acid hybridization studies on *Microbacterium*, *Curtobacterium*, *Agromyces* and related taxa. J. Gen. Microbiol. 28:1697-1708.
9. Dye, D. E., and Kemp, W. J. 1977. A taxonomic study of plant pathogenic *Corynebacterium* species. N.Z. J. Agric. Res. 20:563-582.
10. Goodfellow, M. 1984. Reclassification of *Corynebacterium fascians* (Tilford) Dowson in the Genus *Rhodococcus*, as *Rhodococcus fascians* comb. nov. Syst. Appl. Microbiol. 5:225-229.
11. Grimes, D. J., Jacobs, D., Swartz, D. G., Brayton, P. R., and Colwell, R. R. 1993. Numerical taxonomy of gram-negative, oxidase-positive rods from carcharhinid sharks. Int. J. Syst. Bacteriol. 43:88-98.
12. Henningson, P. J., and Gudmestad, N. C. 1991. Fatty acid analysis of phytopathogenic coryneform bacteria. J. Gen. Microbiol. 137:427-440.
13. Kilingler, J. M., Stowe, R. P., Oberhober, D. C., Groves, T. O., Mishra, S. K., and Pierson, D. L. 1992. Evaluation of the Biolog automated microbial identification system. Appl. Environ. Microbiol. 58:2089-2092.
14. Lachane, R. A. 1960. The vitamin requirements of *Corynebacterium sepedonicum* (Spieck. et Kott.) Skapt. et Burkh. Can. J. Microbiol. 6:171-174.
15. Liebel, W. 1992. The genus *Corynebacterium* - nonmedical. Pages 1157-1171 in: The Prokaryotes, Vol. 2. A. Balows, H. Truper, M. Dworkin, W. Harder, and K. Heinz Schleifer, eds. Springer-Verlag, New York.
16. Miller, M. J. 1991. Evaluating biochemical identification systems. J. Clin. Microbiol. 29:1559-1561.
17. Ramamurthi, C. S. 1959. Comparative studies of some Gram-positive phytopathogenic bacteria and their relationship to the corynebacteria. Mem. Cornell Univ. Exp. Stn. No. 366.
18. Riley, I. A., Reardon, T. B., and McKay, A. C. 1988. Genetic analysis of plant pathogenic bacteria in the genus *Clavibacter* using allozyme electrophoresis. J. Gen. Microbiol. 134:3025-3030.
19. Roslev, P., and King, G. M. 1993. Application of a tetrazolium salt with a water-soluble formazan as an indicator of viability in respiring bacteria. Appl. Environ. Microbiol. 59:2891-2896.
20. Starr, M. P. 1949. The nutrition of phytopathogenic bacteria. III. The Gram-positive phytopathogenic Corynebacterium species. J. Bacteriol. 57:253-258.
21. Tortora, G. J., Funke, B. R., and Case, C. L. 1992. Microbiology, an Introduction. 4th ed. Benjamin/Cummings Publishing Co., Inc., Redwood City, CA.
22. Verniere, C., Pruvost, O., Civerolo, E. L., Gambin, O., Jacquemoud-Collet, J. P., and Luisetti, J. 1993. Evaluation of the Biolog substrate utilization system to identify and assess metabolic variation among strains of *Xanthomonas campestris* pv. citri. Appl. Environ. Microbiol. 59:243-249.
23. Vidaver, A. K. 1982. The plant pathogenic corynebacteria. Annu. Rev. Microbiol. 36:495-517.
24. Vidaver, A. K., and Davis, M. J. 1988. Gram-Positive bacteria: Coryneform plant pathogens. Pages 104-113 in: Laboratory Guide for the Identification of Plant Pathogenic Bacteria. 2nd ed. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN.
25. Vidaver, A. K., and Starr, M. P. 1981. Phytopathogenic coryneform and related bacteria. Pages 1879-1887 in: The Prokaryotes, Vol. 2. A. Balows, H. Truper, M. Dworkin, W. Harder, and K. Heinz Schleifer, eds. Springer-Verlag, New York.
26. Zgurskaya, H. I., Evtushenko, L. I., Akimov, V. N., and Kalakoutskii, L. V. 1993. *Rathayibacter* gen. nov., including the species *Rathayibacter rathayi* comb. nov., *Rathayibacter tritici* comb. nov., *Rathayibacter iranicus* comb. nov., and six strains from annual grasses. Int. J. Syst. Bacteriol. 43:143-149.