Differential Characterization of *Agrobacterium* Species Using Carbon-Source Utilization Patterns and Fatty Acid Profiles

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ABSTRACT

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Pathogenic and nonpathogenic strains of *Agrobacterium tumefaciens* (i.e., biovar 1), *A. rhizogenes* (i.e., biovar 2), *A. vitis* (i.e., biovar 3), and *A. rubi* were tested for their ability to utilize 95 different carbon sources available in the GN Microplate system and analyzed for fatty acid content using the MIDI gas-liquid chromatography system. Several carbon sources were identified as being of diagnostic value. With the exception of the *A. rubi* strain, hydroxy-L-proline and i-erythritol were exclusively metabolized by *A. tumefaciens* and *A. rhizogenes*, respectively. Analysis of fatty acid composition confirmed that *cis*-vaccenic acid ($18:1_{\omega}$ *cis*) is the predominant acid in all the *Agrobacterium* strains. The amount of 3-hydroxypalmitic acid (16:0 3-OH) differed in *A. rhizogenes, A. tumefaciens,* and *A. vitis,* providing a quantitative measure of differentiation; this acid was not detected in *A. rubi* ICPB TR2. Another fatty acid with diagnostic value was 15:0 iso 3-OH, which was found exclusively in *A. rhizogenes* cells. Cluster analyses based on fatty acid composition and metabolic fingerprints confirmed the phenotypic differences between members of the four *Agrobacterium* species investigated; both systems grouped the strains according to their respective species. These rapid and simple methods are major improvements over the classical methods of identification of *Agrobacterium* species and should prove useful for the rapid classification of large numbers of bacterial strains, such as those isolated in ecological studies.

In Bergey's Manual of Systematic Bacteriology, the genus *Agrobacterium* is subdivided into four species (25). Nonphytopathogenic (i.e., saprophytic) agrobacteria are assigned to *A. radiobacter* (Beijerinck and van Delden 1902) Conn 1942; tumorigenic agrobacteria, causing crown gall, are assigned to *A. tumefaciens* (Smith and Townsend 1907) Conn 1942; the agents of cane gall on *Rubus* are assigned to *A. rubi* (Hildebrand 1940) Starr and Weiss 1943; and bacteria that induce root proliferations are assigned to *A. rhizogenes* (Riker, Banfield, Wright, Keitt, and Sagen 1930) Conn 1942. Epithets in these species refer only to phytopathogenic properties, and it has been recognized that this classification is inadequate (6,17,25). Pathogenicity is controlled by genes carried on a plasmid that is transferable between strains (42);

thus, acquisition or loss of such a plasmid by a bacterium would change its species status accordingly. Also, there is no correlation between this nomenclature and the taxonomic structure of the genus based on the chromosomally encoded phenotypic and genetic characteristics. According to these more stable characteristics, the overwhelming majority of agrobacteria have been clustered into at least three well-defined chromosomal groups or biovars (12–14,16,17,23,24,26,40,45). *Biovar* is an infrasubspecific term that was used to distinguish physiological groups within each species (except *A. rubi*) at the time when *Agrobacterium* nomenclature was based on pathogenicity (reviewed in ref. 25). Later, it was proposed that biovars 1, 2, and 3 be raised to *A. tumefaciens*, *A. rhizogenes*, and *A. vitis*, respectively, regardless of pathogenicity (18,33). In this paper, the *Agrobacterium* nomenclature based on chromosomal groups will be adopted, because it better reflects the taxonomic structure of the genus.

Cellular fatty acid profiles have become a primary tool to support classification of bacteria (1,20,28,39,41,43,44). Analysis of cellular fatty acids relies on the commercially available MIDI automated capillary column gas chromatograph (Microbial ID, Inc., Newark, DE) (37). Another automated and commercially available identification technique is the Biolog system (Biolog, Inc., Hayward, CA), which is based on the simultaneous testing of metabolic activity of the test strain on 95 different carbon sources, including amino acids, carboxylic acids, and carbohydrates (3). With both systems, a computerized database can be built and numerical analysis of data performed, resulting in computer-generated dendrograms showing relationships among strains and clusters. The database of each system may be expanded to include newly identified strains. The Biolog and MIDI systems have proven useful for characterization and identification of bacteria (9,11,15,19–21,27,29,30,38,39,44). In this paper, we present information on the ability of the Biolog and MIDI systems to distinguish between *Agrobacterium* species by their differential ability to oxidize a diverse group of carbon compounds and their fatty acid composition, respectively.

MATERIALS AND METHODS

Bacterial strains. The list and taxonomic affiliation of 59 *Agrobacterium* strains used in this study are presented in Table 1; the list includes B6 (ATCC 23308), the type strain of *A. tumefaciens*. This panel of strains represents members of the four *Agrobacterium* species (i.e., biovars), which were isolated from different hosts grown in diverse geographical regions. The bacteria were stored in 15% glycerol at −80 C. Before testing, strains were streaked for purity, and single colonies were selected for culture on mannitol-glutamate medium (31). To confirm species affiliation, the strains were tested for oxidation of lactose to 3-ketolactose (2) and production of acid on potato-dextrose agar (PDA) (Difco Laboratories, Detroit) supplemented with $CaCO_3$ (5). These tests are used to characterize *A. tumefaciens* (i.e., biovar 1) and *A. rhizogenes* (i.e., biovar 2), respectively. Strains with unusual reaction patterns were tested twice to confirm their metabolic fingerprint or fatty acid profile.

Utilization of carbon substrates. Bacterial strains were tested for their ability to oxidize carbon sources using Biolog's automated identification system. Substrate utilization is detected by the concomitant reduction of the tetrazolium dye, which results in the formation of a purple formazan. Bacteria were initially grown on PDA supplemented with 0.08% $CaCO_3$ before being transferred to trypticase soy broth agar (TSBA) prepared by mixing trypticase soy broth (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) with 1.5% agar. Inoculated TSBA plates were incubated for 18 h at 28 C, and the bacteria were harvested and suspended in sterilized 0.85% (w/v) NaCl to an optical density of A_{590nm} = 0.17 to 0.19 (approximately 4.5×10^8 cfu/ml). The 96 wells of each plate in the GN Microplate system (Biolog, Inc.) were inoculated with a bacterial suspension (150 µl per well) and incubated for 24 h at 28 C. The resulting utilization patterns were read against a substrate blank well at A_{590nm} with an automated plate reader (EAR 400 AT, SLT-Labinstruments, A-5082

TABLE 1. *Agrobacterium* strains analyzed for carbon-source utilization and fatty acid profiles

Species[a] Strain	Pathogenicity[b]	Origin	Location	Source[c]
A. tumefaciens				
1.52	−	Ficus	Florida	1
1.101	−	Ficus	Florida	1
3.1	−	Ficus	Florida	1
3.51	−	Ficus	Florida	1
ANT-4	+	Chrysanthemum	France	4
AT3	+	Chrysanthemum	Florida	2
AT5	+	Chrysanthemum	Florida	2
AT7	+	Chrysanthemum	Florida	2
ATCC 15955	+	...[d]	...	3
B6	+	Apple	Iowa	3
C5/73	+	Mountain ash	Oregon	3
C58	+	Cherry	New York	3
D44	+	Soil	Algeria	1
D46	+	Soil	Algeria	1
D72	−	Soil	Algeria	1
FACH	+	Grape	Missouri	5
H4	−	Soil	Algeria	1
H25	−	Soil	Algeria	1
K13/73	−	Willow	Oregon	3
K15/73	−	Willow	Oregon	3
K16	+	Peach	Australia	3
K26	+	Apple	Australia	3
RR5	+	Raspberry	Oregon	3
S2/73	−	*Lippia*	Arizona	3
T28/73	+	Rose	Missouri	3
T37	+	Walnut	...	3
A. rhizogenes				
AB2/73	+	*Lippia*	Arizona	3
AR5K/71	+	Apple	Oregon	3
B3/73	+	Norway maple	Oregon	3
B49c/83	+	Apple	Washington	3
B224/85	+	Blackberry	Oregon	3
D10b/87	+	Apple	Washington	3
D21/91	+	Pear	Oregon	3
D31/91	+	Pear	Oregon	3
D84	−	Soil	Algeria	1
H5	−	Soil	Algeria	1
H8	−	Soil	Algeria	1
H30	−	Soil	Algeria	1
JB3	−	Grape	Algeria	1
K27	+	Peach	Australia	3
K32	+	Almond	Australia	3
K84	−	Soil	Australia	3
M2/73	+	Birch	Oregon	3
A. rubi				
ICPB TR2	−	*Rubus*	United States	3
A. vitis				
3/2	+	Grape	Hungary	3
AG63	+	Grape	Greece	5
AG162	+	Grape	Greece	5
CG48	+	Grape	New York	3
CG49	+	Grape	New York	6
CG62	+	Grape	New York	3
CG64	+	Grape	New York	3
CG472	−	Grape	Washington	6
CG481	−	Grape	New York	6
CG482	−	Grape	Washington	6
CG487	−	Grape	Washington	6
CG660	+	Grape	New York	6
CG957	+	Grape	Afghanistan	6
CG964	+	Grape	Italy	6
CG1005	+	Grape	Australia	6

[a] *A. tumefaciens* = biovar 1; *A. rhizogenes* = biovar 2; *A. vitis* = biovar 3.
[b] + = Disease; − = no disease.
[c] 1 = Authors; 2 = R. E. Stall, University of Florida, Gainesville; 3 = L. W. Moore, Oregon State University, Corvallis; 4 = Y. Dessaux, ISV, CNRS, Gif-sur-Yvette, France; 5 = R. N. Goodman, University of Missouri, Columbia; 6 = T. J. Burr, New York State Agricultural Experiment Station, Cornell University, Geneva.
[d] Information not available.

Grödig/Salzburg, Austria). Metabolic profiles were downloaded to a computer for compilation of a database, which was then subjected to cluster analysis using the MLCLUST program (Biolog, Inc.) to identify strain relationships.

Identification of cellular fatty acid composition. Bacteria were grown for 24 ± 2 h at 28 C on TSBA, harvested, and processed according to the procedure described by Sasser (37). Approximately 40 mg of bacteria (wet weight) were mixed with 1.0 ml of 50% aqueous methanol solution containing 15% NaOH, and cellular fatty acids were saponified at 100 C for 30 min. Treatment of the fatty acids with 2.0 ml of 6 N HCl in 50% aqueous methanol resulted in the formation of fatty acid methyl esters (FAMEs). FAMEs were solvent-extracted from the aqueous phase by mixing with 1.25 ml of a 1:1 (v/v) mixture of hexane and methyl-*tert*-butyl ether; the organic phase was washed with 3 ml of 1.2% NaOH in distilled water. FAMEs were separated by the MIDI Microbial Identification System (Microbial ID, Inc.) utilizing a Hewlett-Packard 5890 gas-liquid chromatograph fitted with a fused silica capillary column (25 m × 0.2 mm i.d.) and coated with 5% phenyl methyl silicone. The initial oven temperature was 170 C and was increased by 5 C/min to a final temperature of 270 C; the carrier gas was hydrogen. After flame ionization, FAME peaks were measured by a Hewlett-Packard 3392 integrator and expressed as percentages of the total fatty acid profile. Profiles were compared to those stored in the MIDI TSBA library (ver. 3.60). The MIDI Library Generation System software and dendrogram programs were used for cluster analysis to show relationships among strains.

RESULTS

Carbon-source utilization. The differences in carbon utilization by the 59 strains of *Agrobacterium* are illustrated in a dendrogram (Fig. 1). All *A. tumefaciens* (i.e., biovar 1) strains clustered together (cluster 1). The same held true for the *A. rhizogenes* (i.e., biovar 2) strains, even though strain AB2/73 appeared to be phenotypically distinct from other members of this phenon (cluster 2). With the exception of strain CG487, all *A. vitis* (i.e., biovar 3) strains formed a distinct group (cluster 3); CG48 was the least similar of the strains within the cluster. According to the location of CG487 on the dendrograms, this outlying strain is more closely related to *A. tumefaciens* than to *A. vitis*. Interestingly, CG487 produced detectable quantities of 3-ketolactose (data not shown), a characteristic of *A. tumefaciens* strains, and formed colonies on PDA-$CaCO_3$ that were similar to those of *A. tumefaciens*. The only *A. rubi* strain tested (ICPB TR2) was different enough to be segregated from the three clusters.

All *Agrobacterium* strains oxidized L-arabinose, D-arabitol, cellobiose, dextrin, D-fructose, D-galactose, α-D-glucose, maltose, D-mannitol, D-mannose, psicose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, bromosuccinic acid, and methyl pyruvate. With the exception of the *A. rubi* strain, α-lactose was utilized by all agrobacteria tested. Adonitol was oxidized by all agrobacteria except *A. vitis* AG63. D-Melibiose was oxidized by all strains except *A. vitis* CG487 and *A. rubi* ICPB TR2. AG63 and CG487 were the only strains not to utilize L-glutamic acid. L-Fucose and gentiobiose were metabolized by all agrobacteria except strains CG48 and CG49 of *A. vitis*. Glycerol was degraded by all but three strains (K27, CG487, and ICPB TR2), each from a different species.

None of the *Agrobacterium* strains metabolized α-cyclodextrin, itaconic acid, α-ketovaleric acid, sebacic acid, L-phenylalanine, D-serine, phenylethylamine, putrescine, 2-aminoethanol, and D,L-α-glycerol phosphate. *A. vitis* AG63 and *A. rubi* ICPB TR2 were the only strains that oxidized urocanic acid. *A. rhizogenes* AB2/73 was the only strain that oxidized thymidine; this strain also differed from other *A. rhizogenes* strains by its ability to oxidize L-threonine, acetic acid, and *p*-hydroxyphenylacetic acid.

Carbon substrates that were differentially metabolized by the different agrobacteria facilitated discrimination between the species (Table 2). With the exception of the *A. rubi* strain, hydroxy-L-proline and i-erythritol were metabolized exclusively by *A. tumefaciens* and *A. rhizogenes*, respectively. Propionic acid, D-glucuronic acid, glucose-1-phosphate, glucose-6-phosphate, glycyl-L-aspartic acid, and acetic acid were utilized almost exclusively by *A. tumefaciens*. D-Glucosaminic acid and D,L-carnitine were metabolized by the majority of *A. rhizogenes* strains, and they were not oxidized by other agrobacteria, with the exception

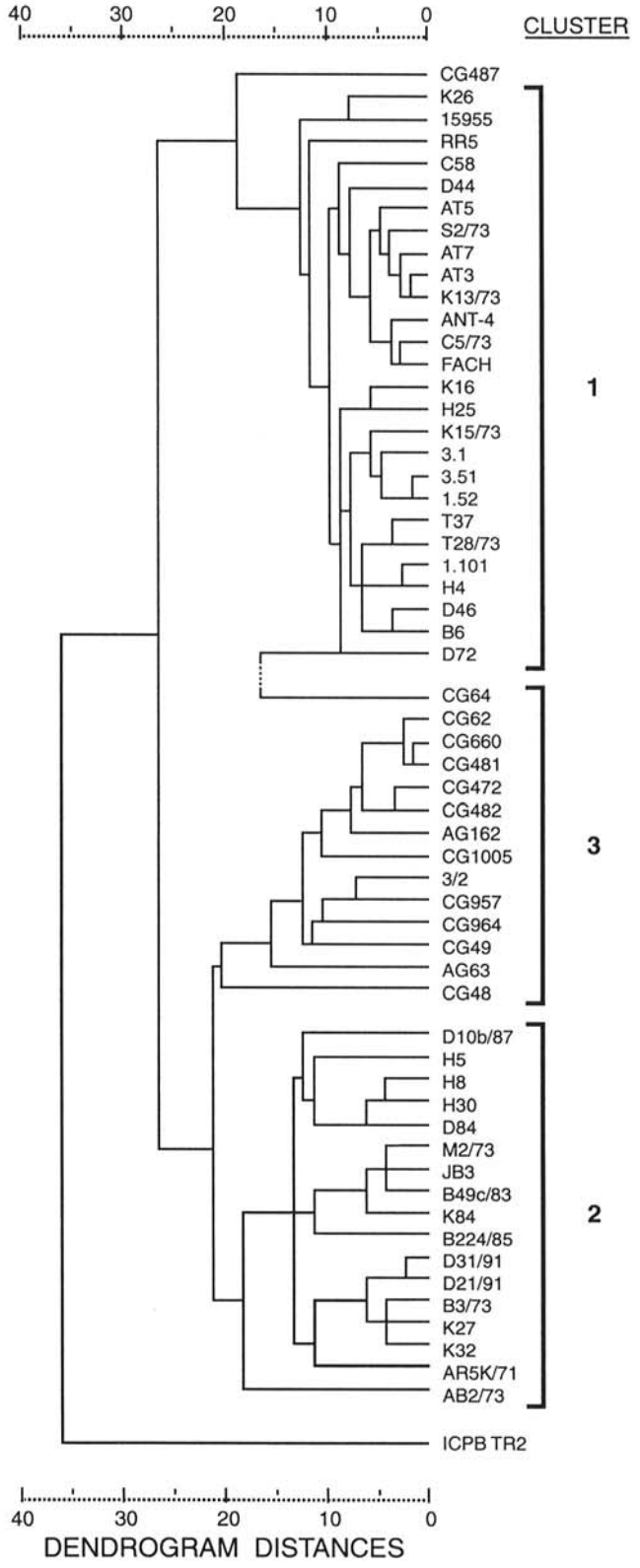

Fig. 1. Dendrogram showing the relationships among *Agrobacterium* strains based on differential utilization of the 95 carbon substrates available in the Biolog GN Microplate. Strains in clusters 1, 2, and 3 are *A. tumefaciens* (i.e., biovar 1), *A. rhizogenes* (i.e., biovar 2), and *A. vitis* (i.e., biovar 3), respectively. *A rubi* is represented by strain ICPB TR2.

of D-glucosaminic acid metabolized by ICPB TR2 and CG487. Xylitol, D-galactonic acid lactone, and D-gluconic acid were degraded by all *A. tumefaciens* and most *A. rhizogenes* strains but rarely by *A. vitis* strains. On the other hand, malonic acid was utilized by all *A. vitis* strains and by most *A. rhizogenes* strains but not by any *A. tumefaciens* strains or the *A. rubi* strain; *p*-hydroxyphenylacetic acid was utilized exclusively by *A. vitis* strains and *A. rhizogenes* strain AB2/73.

The current Biolog library (Microloge GN, release 3.00) accurately identified all but two of the test strains to the genus *Agrobacterium*. Twenty *A. tumefaciens* strains were identified as *A. tumefaciens* B, and the remaining six strains were identified as *A. radiobacter* B. All *A. rhizogenes* strains were identified as *A. tumefaciens* A. Nine *A. vitis* strains were identified as *A. tumefaciens* biovar 3; five of the remaining grape strains were identified as *A. tumefaciens* A. The only strains not identified correctly to genus were CG487 and ICPB TR2; the first choices proposed were *Enterobacter agglomerans* B and *Ochrobactrum anthropi*, respectively.

Fatty acid analyses. Most of the *Agrobacterium* strains used in this study separated into four clusters at Euclidian distances (E.D.) ranging from 6 to 20 (Fig. 2). Most *A. tumefaciens* strains fell into one of two clusters (Fig. 2, clusters 1A and 1B), primarily according to their percentage of 18:1$_{\omega}$ *cis*, which varied over a wide range. Strains C58 and K15/73 did not fit into either *A. tumefaciens* cluster and linked more closely to *A. rhizogenes* strains (cluster 2). Analyses based on fatty acid composition indicate that *A. tumefaciens* strains in cluster 1A are apparently more closely related to *A. vitis* than to those of cluster 1B. All *A. vitis* strains, except strain CG487, were linked in the same cluster (Fig. 2, cluster 3) at a low E.D., indicating homogeneous biosynthesis of fatty acids by members of that species. With the exception of AB2/73, strains of *A. rhizogenes* formed a fairly tight cluster (Fig. 2), which indicates that they are likely to be of the same species. *A. rubi* ICPB TR2 had a distinct fatty acid composition that resulted in its segregation from the other agrobacteria.

The average fatty acid compositions of strains in each cluster were calculated and sorted by chemical structure (Table 3). The predominant acid in these bacteria was 18:1$_{\omega}$ *cis* (i.e., *cis*-vaccenic or *cis*-11-octadecenoic acid), which comprised between 50 and 75% of the total fatty acid content. Fatty acids 16:0 (i.e., palmitic or hexadecanoic acid), 19:0 cyclo$_{\omega}$ *cis* (i.e., lactobacillic or *cis*-11,12-methyleneoctadecanoic acid), and 14:0 3-OH (i.e., 3-hydroxymyristic or 3-hydroxytetradecanoic acid) were also shared by all agrobacteria, although their presence was detected in much lower concentrations.

There were qualitative differences in fatty acid composition. One acid, 15:0 iso 3-OH, was detected exclusively in strains of *A. rhizogenes*, including AB2/73. Some strains of this species contained small amounts of 10:0 3-OH (i.e., 3-hydroxycapric or 3-hydroxydecanoic acid). This acid was not detected in strains of other *Agrobacterium* species. With the exception of *A. vitis* CG487, 18:0 (i.e., stearic or octadecanoic acid) and 18:1 2-OH (i.e., 2-hydroxyoctadecenoic acid) were shared by *A. rhizogenes*, *A. rubi*, and *A. vitis* strains. Similarly, 20:3$_{\omega}$ *cis* (i.e, *cis*-8,11,14-eicosatrienoic or dihomo-γ-linoleic acid) was shared by *A. rhizogenes* and *A. vitis* strains, except CG487. This unusual strain was the only *Agrobacterium* strain to contain 17:1$_{\omega}$ *cis* (i.e., *cis*-9-heptadecenoic acid). *A. rhizogenes* AB2/73 and *A. rubi* ICPB TR2 were the only *Agrobacterium* strains not to contain 19:0 10 methyl.

There were also quantitative differences in the amounts of fatty acids shared by agrobacteria. 3-Hydroxypalmitic acid is the only one present in all *A. tumefaciens*, *A. rhizogenes*, and *A. vitis* strains and at significantly different concentrations. This acid was present in larger amounts in *A. rhizogenes* and in smaller amounts in *A. vitis*; it was not detected in *A. rubi* ICPB TR2. *A. tumefaciens* strains had twice as much palmitic acid as did members of the other two species. Differences in amounts of fatty acids between clusters 1A and 1B of *A. tumefaciens* ranged from very small to insignificant, except for *cis*-vaccenic acid. This acid was the basis for the designation of the two subgroups (1A and 1B) for that species. *A. rhizogenes* strains contained the least 16:$_{\omega}$ *cis* (i.e., *cis*-9-hexadecenoic or palmitoleic acid), whereas *A. vitis* strains had the most 19:0 10 methyl and the least 19:0 cyclo$_{\omega}$ *cis*. The latter acid was the second most common acid found in *A. rubi* ICPB TR2. Except for trace amounts of 17:0 (i.e., heptadecanoic or margaric acid) detected in *A. rhizogenes* D10b/87 (not shown), this acid was found in a significant amount only in *A. rubi* ICPB TR2.

The current MIDI library (TSBA, rev. 3.60) identified *A. tumefaciens* and *A. vitis* strains correctly to genus; all were identified as *A. radiobacter*. *A. rhizogenes* strains were identified as *Phyllobacterium rubiacearum* and *A. rubi* ICPB TR2 as related to either *Xanthobacter agilis* or *Ochrobactrum anthropi*.

TABLE 2. Differential utilization of substrates as sole carbon source by strains of different *Agrobacterium* clusters and strains

Carbon source	Percentage of strains oxiding carbon sources in:[a] 1	2	3	Oxidation of carbon sources by:[b] *A. vitis* CG487	*A. rubi* ICPB TR2
i-Erythritol	0	100	0	0	100
α-Lactose	100	100	100	100	0
D-Melibiose	100	100	100	0	0
Xylitol	100	100	7	100	0
Acetic acid	100	6[c]	7	100	100
Citric acid	0	88	93	0	0
D-Galactonic acid lactone	100	94	7	100	100
D-Gluconic acid	100	76	0	100	100
D-Glucosaminic acid	0	88	0	100	100
D-Glucuronic acid	92	0	7	0	0
p-Hydroxyphenylacetic acid	0	6[c]	71	0	0
Malonic acid	0	94	100	0	0
Propionic acid	92	0	7	100	100
D-Saccharic acid	0	94	79	0	0
Alaninamide	0	76	71	0	0
Glycyl-L-aspartic acid	92	12	7	100	100
Hydroxy-L-proline	100	0	0	0	100
L-Threonine	100	6[c]	64	0	100
D,L-Carnitine	0	65	0	0	100
Glucose-1-phosphate	100	12	0	100	0
Glucose-6-phosphate	92	12	0	100	0

[a] Dendrogram clusters 1 (26 *A. tumefaciens* strains), 2 (17 *A. rhizogenes* strains), and 3 (14 *A. vitis* strains).
[b] 100 = Oxidation of carbon source; 0 = no oxidation.
[c] Outlier strain, not included in cluster 3.

DISCUSSION

Analyses based on fatty acid composition and metabolic fingerprints confirm previous reports (12–14,17,23,24,26,45) that the genus *Agrobacterium* consists of at least four distinct clusters and lend support to the elevation of biovars to species. With few exceptions (i.e., CG487, AB2/73, K15/73, and C58), both systems grouped the strains according to their respective species; the presence or absence of an oncogenic plasmid did not seem to impact the clustering of strains. However, the fatty acid dendrogram segregated *A. tumefaciens* strains into two clusters and suggests that members of cluster 1A are more closely related to *A. vitis*. In contrast, the dendrogram of metabolic fingerprints did not segregate these strains into two groups. It is interesting to note that in a DNA homology study, De Ley (12) found two major clusters within *A. tumefaciens*; however, their relationship to *A. vitis* was not examined, because this group of agrobacteria had not been isolated at the time of that study. Fatty acid and Biolog analyses segregated strains AB2/73 and CG487 from *A. rhizogenes* and *A. vitis*, respectively, which suggests that the two strains may belong to different *Agrobacterium* taxons are rarely encountered.

Two carbon sources, hydroxy-L-proline and i-erythritol, were metabolized almost exclusively by *A. tumefaciens* and *A. rhizogenes*, respectively. Erythritol has long been recognized as a specific substrate for *A. rhizogenes* and is used in isolation media selective for this species (7,32). Similarly, hydroxy-L-proline should prove useful for the selective isolation of *A. tumefaciens*. None of the substrates tested was oxidized exclusively by *A. vitis*; however, *p*-hydroxyphenylacetic acid was oxidized by a majority of the members of this species but not by other agrobacteria. Adonitol is the carbon source currently used in a medium selective for *A. vitis* strains (35); however, our results confirm that this substrate is oxidized by other members of the genus (17,25). This nonspecific utilization of adonitol by a broad spectrum of agrobacteria would explain the recovery of *A. tumefaciens* strains on this medium (4,8).

The fatty acid profiles in this study are in agreement with a previous report (38), and several fatty acids look promising for differentiating the different *Agrobacterium* species. The major fatty acids identified in this work also are similar to those previously reported using different methods (10,22,36). Palmitic acid was predominant among straight-chain saturated fatty acids, whereas *cis*-vaccenic acid predominated among the unsaturated fatty acids; the latter acid has been reported as the characteristically predominant cellular fatty acid in agrobacteria (37,38). This acid is absent from the lipopolysaccharides, which are made up of 3-hydroxymyristic and 3-hydroxypalmitic acids (36). Both hydroxy acids were present in most of the strains tested. Different amounts of 3-hydroxypalmitic acid were characteristic of the different species, and this acid is of diagnostic value. Other acids of diagnostic value are 15:0 iso 3-OH, which was present exclusively in *A. rhizogenes* strains, and 17:0, which was present in significant amount only in *A. rubi* ICPB TR2. Roy (34) and Sasser (37) suggested that *Agrobacterium* "biovars" (i.e, species) could be distinguished by relative amounts of cyclopropane fatty acids. Although this was true for most strains, several strains did not contain the amount of 19:0 cyclo$_{\omega}$ *cis* that can be expected for their respective species. Cluster analysis of fatty acid profiles showed a closer relationship between *A. vitis* and *A. tumefaciens*, even though these two species had fewer acids in common than did *A. vitis* and *A. rhizogenes*. The latter two species shared three acids (18:0, 20:3$_{\omega}$ *cis*, and 18:1 2-OH) that were not detected in *A. tumefaciens*. The fact that strains clustered according to their chromosomal grouping, and not to their phytopathogenic trait, agrees with previous studies (34,37); the presence or absence of an oncogenic plasmid does not seem to affect the fatty acid composition of agrobacteria.

The consistent ability of both systems in grouping most strains of the same species into discrete clusters demonstrates the value of these automated systems for rapid identification of agrobacteria. Both are major improvements over the classical methods of identification for *Agrobacterium* species, which rely on a battery of time-consuming and labor-intensive cultural, biochemical, and

TABLE 3. Mean fatty acid compositions[a] of *Agrobacterium* clusters and outlier strains[b]

		A. tumefaciens				*A. rhizogenes*		*A. vitis*		
Fatty acid	ECL[c]	Cluster 1A (n[d] = 10)	Cluster 1B (n = 14)	Strain K15/73	Strain C58	Cluster 2 (n = 16)	Strain AB2/73	Cluster 3 (n = 14)	Strain CG487	*A. rubi* strain ICPB TR2
Saturated acid										
14:0	14.000	...	tr[e]	tr	...	...	...	...	tr	...
16:0	16.000	8.1 ± 1.4	9.2 ± 1.0	9.4	8.3	4.8 ± 0.8	5.6	4.6 ± 0.7	7.6	3.8
17:0	17.000	...	...	...	...	...	...	...	...	1.2
18:0	18.000	...	...	...	...	1.5 ± 0.4	2.1	1.5 ± 0.7	...	3.4
Unsaturated acid										
16:1$_{\omega 7}$ *cis*	15.817	2.8 ± 0.8	3.8 ± 0.9	8.6	5.4	tr	...	4.9 ± 0.7	10.1	1.3
17:1$_{\omega 8}$ *cis*	16.792	...	...	...	...	...	...	...	tr	...
18:1$_{\omega 7}$ *cis*[f]	17.822	75.2 ± 3.1	67.4 ± 1.9	51.5	58.6	62.6 ± 2.2	53.2	74.4 ± 2.3	64.2	63.6
20:3$_{\omega 6}$ *cis*	19.551	tr	tr	...	...	2.1 ± 0.3	2.8	1.5 ± 0.5	...	...
Branched acid										
15:0 iso 3-OH	16.135	...	...	...	...	3.6 ± 0.5	3.5	...	...	...
19:0 10 methyl	19.368	tr	tr	1.4	1.3	tr	...	2.5 ± 0.4	1.8	...
Cyclopropane										
17:0 cyclo$_{\omega 7}$ *cis*	16.888	tr	1.3 ± 0.6	5.6	3.6	tr	...	...	1.6	...
19:0 cyclo$_{\omega 8}$ *cis*	18.900	2.3 ± 0.9	5.0 ± 1.3	11.0	12.0	8.5 ± 2.2	18.0	tr	4.5	22.7
Hydroxy acid										
10:0 3-OH	11.423	...	...	...	...	tr	...	...	...	...
14:0 3-OH[g]	15.490	6.5 ± 1.0	7.8 ± 1.2	8.0	6.5	5.5 ± 0.8	5.2	6.6 ± 0.9	6.3	tr
16:0 3-OH	17.520	4.1 ± 0.7	4.6 ± 0.7	4.1	4.3	6.6 ± 1.0	5.9	1.9 ± 0.4	2.4	...
18:1 2-OH	19.088	...	...	...	...	2.0 ± 0.5	3.9	1.7 ± 0.8	...	2.0

[a] Values represent percent of total fatty acid composition.
[b] Clusters and outlier strains according to fatty acid dendrogram (Fig. 2).
[c] Equivalent carbon chain length.
[d] Number of strains included in a cluster (see Fig. 2).
[e] Trace amounts (<1.0%) detected.
[f] Identified as "Summed Feature 7" of the MIDI library.
[g] Identified as "Summed Feature 3" of the MIDI library.

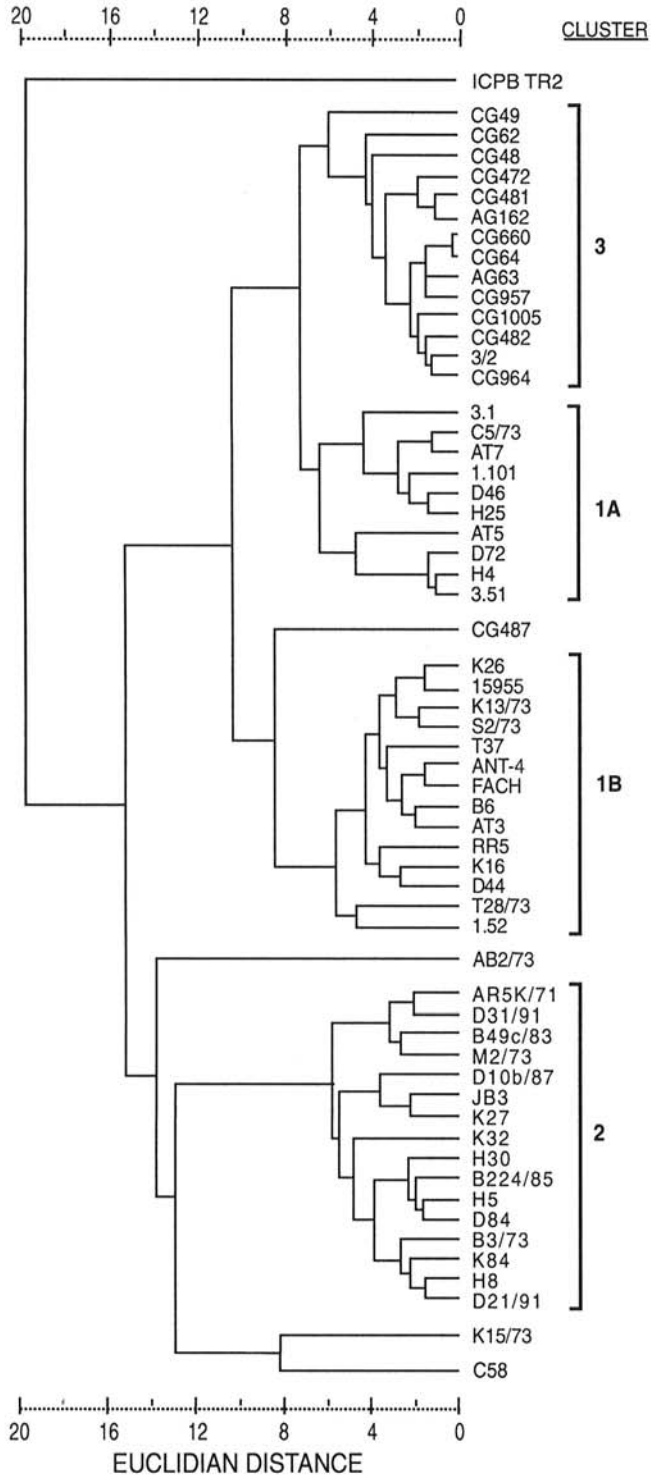

Fig. 2. Dendrogram showing the relationships among strains of *Agrobacterium* based on fatty acids composition data. Strains in clusters 1 (1A and 1B), 2, and 3 are *A. tumefaciens* (i.e., biovar 1), *A. rhizogenes* (i.e., biovar 2), and *A. vitis* (i.e., biovar 3), respectively. *A rubi* is represented by strain ICPB TR2.

physiological tests (6,31), and should prove useful for classification of large numbers of bacterial strains such as those isolated in ecological studies.

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