Fatty acid analysis of phytopathogenic coryneform bacteria

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The classification of plant-pathogenic coryneform bacteria has been revised many times. All plant-pathogenic coryneform bacteria were once classified in the genus *Corynebacterium*. Currently, all are classified in the genera *Arthrobacter*, *Clavibacter*, *Curtobacterium* or *Rhodococcus*. Difficulties still remain regarding the characterization of species and subspecies within these groups. Analysis of cellular fatty acids by high-resolution capillary gas chromatography has recently become a standard method of bacterial identification in many laboratories. In the studies reported here, cellular fatty acid analyses were performed to examine the plant-pathogenic coryneform bacteria as an autonomous group. A total of 189 bacterial strains were profiled. Results from these studies support the division of plant-pathogenic coryneforms into four major groups (genera). Further subdivision was not required for *Rhodococcus* and *Arthrobacter*, as each genus contains only one species of plant-pathogenic bacteria. Some, but not all, *Curtobacterium* pathovars could be differentiated from one another based on fatty acid analysis alone. Intrasubspecific classification of plant-pathogenic *Curtobacterium* organisms appears to be appropriate. Most of the species or subspecies belonging to the genus *Clavibacter* could be differentiated using fatty acid profiles. Other chemotaxonomic methods also differentiate organisms within *Clavibacter*. Reclassification is warranted within *Clavibacter michiganense*. *Clavibacter michiganense* subspecies should be returned to full species status.

Introduction

The taxonomic scheme of the plant-pathogenic coryneform bacteria has undergone several revisions, many occurring within the last 10 years. These changes have been reviewed in detail by Davis (1986).

Among the revisions was the proposal of a pathovar system (Dye & Kemp, 1977) retaining all of the species in the genus *Corynebacterium*; and the reclassification of certain species into *Arthrobacter* (Collins *et al.*, 1981), *Curtobacterium* (Collins & Jones, 1983) and *Rhodococcus* (Goodfellow, 1984). Davis *et al.* (1984) described a new genus, *Clavibacter*, and reclassified the remaining corynebacterial plant pathogens in this genus.

Revision of the plant-pathogenic coryneform bacteria has been based on a number of methods, e.g. morphology, physiology, biochemistry, numerical analysis, and most recently, chemotaxonomy. However, to date, no study has used fatty acid analysis to examine these organisms as an autonomous group. The use of fatty acid analysis in the identification and taxonomy of bacteria has been well-documented (Bousfield *et al.*, 1983; Bowie *et al.*, 1972; Collins, 1983; Minnikin *et al.*, 1978; Moss, 1981). In this paper, we present the results of a study using fatty acid analysis to differentiate the plant-pathogenic coryneform bacteria.

Methods

Identification of organisms. Organisms used in the study are listed in Table 1. Their identities were confirmed using standard biochemical and physiological tests, including: Gram stain, gross colony colour, utilization of organic acids, production of lipase and gelatinase, growth on semi-selective media, production of acid from carbohydrates, and motility. All tests were repeated for each individual strain to assess the reliability of the test results. Gram stains were made of each culture using standard staining procedure (Difco crystal violet, 60 s; Difco iodine, 60 s; 95% (v/v) ethanol, destain until clear; Difco safranin 20 s). Cultures on NBY medium (Difco Nutrient Broth, 8.0 g; Difco Yeast Extract, 2.0 g; K₂HPO₄, 2.0 g; KH₂PO₄, 0.5 g; glucose, 5.0 g; MgSO₄·7H₂O, 1.0 ml of a 1.0 M solution; Oxoid agar, 10.0 g; H₂O, 1.0 l) were examined for gross colour differences and the presence or absence of large amounts of mucopolysaccharide (Vidaver, 1980).

Utilization of fumaric acid and sodium acetate was determined by spot inoculation of semi-solid medium in Petri dishes (NH₄H₂PO₄, 0.5 g; K₂HPO₄, 0.5 g; MgSO₄·7H₂O, 0.2 g; NaCl, 5.0 g; Difco Yeast Extract, 0.8 g; Oxoid agar, 12.0 g; Brom Thymol Blue, 0.016 g; organic acid 2.0 g; H₂O, 1.0 l; pH, 6.8) (Dye & Kemp, 1977). Plates were examined daily for 5 d for colour change (green to blue). Utilization was assumed if the medium surrounding the colony turned blue, which indicated a pH change to a value above 7.4. Comparison was made with inoculated control plates which lacked the organic acid in question.

The method of Sierra (1957) was used to determine the presence of lipase, with Tween 80 (polyoxyethylene (20) sorbitan monooleate) as substrate. Medium (peptone, 10.0 g; NaCl, 5.0 g; CaCl₂·2H₂O, 0.1 g; Oxoid agar, 12.0 g; H₂O, 1.0 l; adjust pH to 7.4, autoclave, then add 10 ml sterile Tween 80 to cooled medium) in Petri dishes was spot

Table 1. *Strains investigated*

Strain	Source*	Host
<i>Clavibacter michiganense</i> subsp. <i>sepedonicum</i> (44 strains)		
9850	ATCC - New York	<i>Solanum tuberosum</i>
33111	ATCC - Canada	
R2	S. H. DeBoer - British Columbia	
R5	S. H. DeBoer - British Columbia	
R6	S. H. DeBoer - Idaho	
Cs13	S. H. DeBoer - New Brunswick	
Cs14	S. H. DeBoer - Montana	
Cs16	S. H. DeBoer - New Brunswick	
Cs106	S. H. DeBoer - West Virginia	
Cs118	S. H. DeBoer	
SDB20	S. H. DeBoer	
3NM	S. H. DeBoer - Variant	
Cs3R	S. H. DeBoer - Reisolate	
Cs3RC	S. H. DeBoer - Reisolate	
BRR7	S. H. DeBoer - British Columbia	
INM-1	R. G. Clarke - Idaho	
INM-B	R. G. Clarke - Idaho	
ME-1	F. E. Manzer - Maine	
SS-14	S. A. Slack - Wisconsin	
SS-44	S. A. Slack - Wisconsin	
CsCA	A. Vidaver - California	
A-34	G. A. Secor - Yugoslavia	
2531	NZPDDCC - Sweden	
2532	NZPDDCC - Canada	
2534	NZPDDCC - USA	
2535	NZPDDCC - Canada	
2537	NZPDDCC - USA	
BJ19	NDSU Collection - Oregon	<i>Beta vulgaris</i> (seed)
ULTRA	NDSU Collection - Oregon	
DIPPE2	NDSU Collection - Oregon	
MONOR	NDSU Collection - Oregon	
Olm17	NDSU Collection - North Dakota	<i>Beta vulgaris</i> (root)
Colo18	NDSU Collection - Colorado	
SB-65	NDSU Collection - North Dakota	
SB-101	NDSU Collection - North Dakota	
SB-109	NDSU Collection - North Dakota	
SB-136	NDSU Collection - North Dakota	
Pros16	NDSU Collection - North Dakota	
EA1-B	NDSU Collection - North Dakota	<i>Solanum tuberosum</i>
SD-1	NDSU Collection - South Dakota	
H1-B	NDSU Collection - Minnesota	
OFF	NDSU Collection - Minnesota	
As-1	NDSU Collection - Minnesota	
PI	NDSU Collection - Minnesota	
<i>Clavibacter michiganense</i> subsp. <i>michiganense</i> (21 strains)		
4450	ATCC	Unknown
7430	ATCC	<i>Cyphomandra fragrans</i>
10202	ATCC	<i>Lycopersicon esculentum</i>
14456	ATCC	<i>Lycopersicon esculentum</i>
Cm3	S. H. DeBoer	Unknown
Cm5	S. H. DeBoer	
Cm6	S. H. DeBoer	
Cm8	S. H. DeBoer	
Cm9	S. H. DeBoer	
1104	L. E. Claffin	<i>Lycopersicon esculentum</i>
549	NZPDDCC	
1436	NZPDDCC - New Zealand	
1808	NZPDDCC - New Zealand	
1811	NZPDDCC - New Zealand	
2355	NZPDDCC - New Zealand	<i>Cyphomandra betacea</i>
2541	NZPDDCC - United Kingdom	
2545	NZPDDCC - Sicily	
2550	NZPDDCC - Hungary	
2551	NZPDDCC - Brazil	
5026	NZPDDCC - USA	<i>Capsicum annuum</i>
6726	NZPDDCC - New Zealand	<i>Lycopersicon esculentum</i>

Table 1 (continued)

Strain	Source*	Host
<i>Clavibacter michiganense</i> subsp. <i>nebraskense</i> (17 strains)		
27794	ATCC	Zea mays
27822	ATCC	
ValCo	J. R. Venette	
1-1	C. Ash	
1105	L. E. Claflin - Colorado	
1106	L. E. Claflin - Kansas	
1118	L. E. Claflin - Kansas	
1119	L. E. Claflin - Kansas	
1120	L. E. Claflin - Kansas	
3294	NZPDDCC	
3299	NZPDDCC - USA	
3300	NZPDDCC - USA	
3303	NZPDDCC - USA	
5366	NZPDDCC - USA	
5367	NZPDDCC - USA	
5368	NZPDDCC - USA	
5369	NZPDDCC - USA	
<i>Clavibacter michiganense</i> subsp. <i>insidiosum</i> (18 strains)		
239	A. Vidaver	Unknown
10253	ATCC	Unknown
33114	ATCC	<i>Medicago sativa</i>
Ci4	S. H. DeBoer	Unknown
Ci16	S. H. DeBoer	Unknown
CiN	S. H. DeBoer	<i>Medicago sativa</i>
CiN53	S. H. DeBoer	<i>Medicago sativa</i>
Ci102B	S. H. DeBoer	Unknown
CiLeth	S. H. DeBoer	<i>Medicago sativa</i> (seed)
CiAW81	S. H. DeBoer	Medicago sativa
2611	NZPDDCC - United Kingdom	
2621	NZPDDCC - USA	
2948	NZPDDCC - New Zealand	
3567	NZPDDCC - Australia	
3619	NZPDDCC - Australia	
3983	NZPDDCC - USA	
4191	NZPDDCC - New Zealand	
4543	NZPDDCC - reisolate	
6565	NZPDDCC - Florida	
<i>Clavibacter michiganense</i> subsp. <i>tessellarius</i> (12 strains)		
1122	L. E. Claflin - Nebraska	Unknown
7219	NZPDDCC - USA	
7220	NZPDDCC - USA	
7221	NZPDDCC - USA	
7222	NZPDDCC - USA	
7223	NZPDDCC - USA	
7224	NZPDDCC - USA	
7225	NZPDDCC - USA	
7226	NZPDDCC - USA	
7227	NZPDDCC - USA	
7228	NZPDDCC - USA	
7229	NZPDDCC - USA	
<i>Clavibacter xyli</i> subsp. <i>cynodontis</i> (3 strains)		
TB1-A	M. J. Davis - China	Cynodon dactylon
FB-1	M. J. Davis - China	
33973	ATCC - China	
<i>Clavibacter rathayi</i> (7 strains)		
2572	NZPDDCC - New Zealand	Dactylis glomerata
2573	NZPDDCC - New Zealand	
2574	NZPDDCC - New Zealand	
2575	NZPDDCC - New Zealand	
2576	NZPDDCC - New Zealand	
2577	NZPDDCC - New Zealand	
2579	NZPDDCC - Scotland	

Table 1 (continued)

Strain	Source*	Host
<i>Clavibacter tritici</i> (6 strains)		
11402	ATCC - India	Unknown <i>Triticum aestivum</i>
2623	NZPDDCC - India	
2624	NZPDDCC - Egypt	
2626	NZPDDCC - Egypt	
2627	NZPDDCC - India	
2628	NZPDDCC - Iran	
<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> (17 strains)		
1101	M. L. Schuster - Nebraska	Unknown
6887	ATCC	<i>Phaseolus</i> sp.
2A-DK	A. Vidaver	Unknown
CV3	A. Vidaver	Unknown
2580	NZPDDCC - USA	<i>Phaseolus</i> sp.
2581	NZPDDCC - USA	<i>Phaseolus</i> sp.
2582	NZPDDCC - Romania	<i>Phaseolus vulgaris</i>
2583	NZPDDCC	
2584	NZPDDCC - Hungary	
2585	NZPDDCC - Dem. Rep. of Germany	
2588	NZPDDCC - USA	<i>Vigna angularis</i>
2590	NZPDDCC - USA	<i>Vigna radiata</i>
3495	NZPDDCC - Netherlands	<i>Phaseolus vulgaris</i>
5370	NZPDDCC - USA	
5371	NZPDDCC - USA	
5372	NZPDDCC - USA	
5373	NZPDDCC - USA	
<i>Curtobacterium flaccumfaciens</i> pv. <i>betae</i> (10 strains)		
13437	ATCC	Unknown
2591	NZPDDCC - United Kingdom	<i>Beta vulgaris</i>
2592	NZPDDCC - United Kingdom	
2593	NZPDDCC - United Kingdom	
2594	NZPDDCC - United Kingdom	
2595	NZPDDCC - United Kingdom	
4735	NZPDDCC - United Kingdom	
4736	NZPDDCC - United Kingdom	
4737	NZPDDCC - United Kingdom	
7458	NZPDDCC - Brazil	
<i>Curtobacterium flaccumfaciens</i> pv. <i>oortii</i> (5 strains)		
6-83-70-A	G. A. Secor - Netherlands	Unknown
2632	NZPDDCC - Netherlands	<i>Tulipa gesneriana</i>
3497	NZPDDCC - Netherlands	
3498	NZPDDCC - United Kingdom	
3499	NZPDDCC - United Kingdom	
<i>Curtobacterium flaccumfaciens</i> pv. <i>poinsettiae</i> (10 strains)		
1136	L. E. Claffin	Unknown
2561	NZPDDCC - USA	<i>Euphorbia pulcherrima</i>
2563	NZPDDCC - USA	
2564	NZPDDCC - USA	
2565	NZPDDCC - USA	
2567	NZPDDCC - USA	
2568	NZPDDCC - USA	
2569	NZPDDCC - USA	
2570	NZPDDCC - USA	
3500	NZPDDCC - USA	
<i>Arthrobacter ilicis</i> (2 strains)		
14264	ATCC	<i>Ilex opaca</i>
2609	NZPDDCC - USA	<i>Ilex opaca</i>
<i>Rhodococcus fascians</i> (16 strains)		
13000	ATCC	<i>Lathyrus odoratus</i>
2596	NZPDDCC - United Kingdom	<i>Fragaria</i> × <i>ananassa</i>
2597	NZPDDCC - United Kingdom	
2598	NZPDDCC - United Kingdom	
2599	NZPDDCC - United Kingdom	
2600	NZPDDCC - United Kingdom	
<i>Chrysanthemum</i> × <i>morifolium</i>		

Table 1 (continued)

Strain	Source*	Host
2601	NZPDDCC - United Kingdom	<i>Justicia brandegeana</i>
2602	NZPDDCC - USA	Unknown
2603	NZPDDCC - Canada	Unknown
2604	NZPDDCC - USA	<i>Lathyrus odoratus</i>
2605	NZPDDCC - USA	Unknown
5339	NZPDDCC - United Kingdom	} <i>Lathyrus odoratus</i>
5340	NZPDDCC - United Kingdom	
5833	NZPDDCC - USA	
7109	NZPDDCC - Netherlands	<i>Gladiolus</i> sp.
7113	NZPDDCC - Netherlands	<i>Begonia</i> sp.

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

inoculated and examined after 5 d for the presence of a cloudy precipitate surrounding the colony.

Bacteria were tested for growth on CNS and TTC media. CNS medium consisted of 1.01 NBY; nalidixic acid (freshly solubilized in 0.1 M-NaOH), 25.0 mg; polymyxin B sulphate (8000 US Pharmacopeia units mg⁻¹), 32.0 mg; cycloheximide, 40.0 mg; LiCl, 10.0 g; Bravo 6F (tetrachloroisophthalonitrile), 0.0625 ml; filter-sterilized ingredients were added to cooled NBY. TTC medium consisted of glucose, 10.0 g; peptone, 10.0 g; Casamino acids, 1.0 g; Difco agar, 18.0 g; H₂O, 1.0 l; 5.0 ml of 1.0% (w/v) solution of 2,3,5-triphenyltetrazolium chloride, autoclaved separately, was added to cooled medium (Vidaver, 1980). Suspensions of bacteria were made in test tubes containing 3.0 ml 0.1 M-phosphate-buffered saline. The solution was thoroughly vortexed, then 100 µl volumes were placed on the medium and spread with a sterile L-shaped glass rod. Plates were examined for growth after 5 d. Control plates which lacked the growth-inhibiting chemicals were inoculated for comparison.

Two types of media, Medium C and Difco Purple Broth Base were used to test for the production of acid from lactose, trehalose, mannitol, rhamnose and sucrose. Medium C consisted of NH₄H₂PO₄, 0.5 g; K₂HPO₄, 0.5 g; MgSO₄·7H₂O, 0.2 g; NaCl, 5.0 g; Difco Yeast Extract, 1.0 g; Brom Cresol Purple, 0.7 ml (1.6%, w/v, alcoholic solution); Oxoid agar, 10.0 g; H₂O, 1.0 l; carbohydrate, 5.0 g (Dye & Kemp, 1977). All the substrates were filter sterilized and added to cooled medium at a concentration of 0.5%, then placed in sterile test tubes. Tubes were examined daily for 5 d. Acid production was indicated by a colour change from purple to yellow.

The presence of gelatinase was tested for by stab-inoculating tubes of gelatin (Difco Nutrient Gelatin, 120.0 g; H₂O, 1.0 l) (Dye & Kemp, 1977). Tubes were incubated for 10 d at 23 ± 1 °C.

Motility was determined by stab-inoculating tubes of soft medium (Difco Nutrient Broth, 8.0 g; Difco agar, 5.0 g) (Krieg & Gerhardt, 1981). A heavy, cloudy transverse growth pattern from the area of inoculation after 10 d incubation at 23 ± 1 °C was determined to be due to a motile culture.

Fatty acid analysis. Bacteria were stored at -83 °C in a freezing solution (1.0 g yeast extract, 2.28 g K₂HPO₄, 0.68 g KH₂PO₄, 70.0 ml H₂O, 30.0 ml glycerol; mix equal volumes of freezing solution and broth) until needed. Bacteria were prepared for fatty acid analysis as previously described (Gudmestad *et al.*, 1988). Each strain was analysed once. Studies assessing the precision of fatty acid analysis were performed prior to the previous studies (Gudmestad *et al.*, 1988). FAMES (fatty acid methyl esters) were analysed using a gas chromatograph equipped with a flame ionization detector. A 30 m × 0.25 mm i.d. bonded-phase fused silica capillary column (Supelco SPB-1, film thickness 0.25 µm) was used with helium as the carrier gas.

The temperature programme was initiated at 150 °C and increased to 250 °C at 4 °C min⁻¹. FAME profiles were integrated and recorded using a Shimadzu C-R4A Chromatopac data processor. FAMES were identified as previously described (Gudmestad *et al.*, 1988; Henningson *et al.*, 1988).

Ratios of fatty acids were calculated in all possible combinations to evaluate relative quantitative fatty acid differences. Only fatty acid ratios that allowed for the differentiation of bacterial subspecies were used for comparison and separation of the bacterial strains.

Cluster analysis of fatty acids was performed using the NTSYS-pc computer programs (Rohlf, 1988). The SIMQUAL (similarity for qualitative data) program with the Dice coefficient was chosen for data analysis. This program was derived from the statistics developed by Sneath & Sokal (1973). Data were input in a 14 column × 189 row matrix. Fatty acids were assigned the values 0 or 1 for the program based on the following scheme: 0 = fatty acid was absent, 1 = fatty acid was present for i14:0, a15:1, a15:0, i16:0, 16:1t, X1 (unknown), 18:2 and 10me19:0. Fatty acids 14:0, i15:0, a17:0 and i18:0 present in amounts <1% = 0 and >1% = 1. For 16:0, amounts <10% = 0, >10% = 1. For 18:1c, amounts <5% = 0 and >5% = 1.

Results

Cultural and biochemical characteristics

All cultures tested were Gram-positive. Cells from all species and subspecies were small and generally pleomorphic, with the exception of *Rhodococcus fascians*. Many of the *R. fascians* strains were long and straight, and had a small number of cells that appeared to be joined end-to-end in pairs. On NBY medium certain species exhibited very characteristic colony colours that could be used tentatively to identify the organisms. For example, *Clavibacter michiganense* subsp. *insidiosum* produced indigoidine (a deep blue and indigo mix) on the colony edges (Dye & Kemp, 1977). Cultures of *Cl. michiganense* subsp. *tessellarius*, *Cl. michiganense* subsp. *nebraskense* and *Curtobacterium flaccumfaciens* pv. *poinsettiae* were orange. *Clavibacter rathayi* and *Cl. tritici* were bright yellow while strains of *Cur. flaccumfaciens*

Table 2. Biochemical and cultural characteristics of some plant-pathogenic coryneform bacteria*

Bacterial species	Acid from carbohydrate using:																
	Organic acids		Enzymes		Selective medium		Mot	Purple Broth plus			Medium C plus				Colony characteristics		
	Fm	Na	T80	Gel	CNS	TTC		Man	Rha	Suc	Lac	Tre	Man	Rha	Ind	Ep	Colour†
<i>A. ilicis</i>	v	+	—	+	+	+	v	+	+	—	v	+	+	+	—	—	Y;W/Be
<i>Cl. michiganense</i> subsp. <i>insidiosum</i>	—	—	—	—	—	—	—	+	—	—	v	v	+	—	+	+	DY
<i>Cl. m.</i> subsp. <i>michiganense</i>	+	+	—	—	+	+	—	—	—	v	v	+	+	—	—	—	Y;DY
<i>Cl. m.</i> subsp. <i>nebraskense</i>	+	v	—	—	+	—	—	—	—	—	v	+	v	—	—	—	O
<i>Cl. rathayi</i>	+	—	—	v	—	—	—	+	—	—	—	+	+	—	—	—	BrY
<i>Cl. m.</i> subsp. <i>sepedonicum</i>	v	v	—	—	—	—	—	+	—	+	—	—	+	—	—	—	W/Be
<i>Cl. m.</i> subsp. <i>tessellarius</i>	+	+	+	—	+	+	—	—	—	v	+	+	+	—	—	—	O
<i>Cl. tritici</i>	+	v	+	—	+	—	—	+	—	+	—	v	+	—	—	—	BrY
<i>Cur. flaccumfaciens</i> pv. <i>betae</i>	—	+	+	v	+	+	+	—	+	v	+	+	+	+	—	—	Y;DY
<i>Cur. fl.</i> pv. <i>flaccumfaciens</i>	+	v	+	v	+	+	v	—	v	v	v	+	+	+	—	v	Y;V
<i>Cur. fl.</i> pv. <i>oortii</i>	—	+	+	+	+	+	+	—	+	v	+	+	+	+	—	—	Y;V
<i>Cur. fl.</i> pv. <i>poinsettiae</i>	—	+	+	v	+	+	v	—	—	v	+	+	+	—	—	—	O;Y/O
<i>R. fascians</i>	+	+	+	—	—	+	—	—	—	—	—	+	+	—	—	—	Y/O;BrY

+, 80–100% of organisms positive; —, 80–100% of organisms negative; v, organisms were variable either between strains or when test was repeated.

* Abbreviations: Fm, fumaric acid; Na, sodium acetate; T80, lipase for Tween 80; Gel, gelatinase; CNS, growth on CNS; TTC, growth on TTC; Mot, motility; Man, mannitol; Rha, rhamnose; Suc, sucrose; Lac, lactose; Tre, trehalose; Ind, presence of indigoidine; Ep, presence of extracellular pigments.

† Colony colour on NBY: DY, dull yellow; Y, yellow; O, orange; BrY, bright yellow; Y/O, yellow-orange; W/Be, white/beige; V, variable.

pv. *oortii* were yellow. Most cultures of *Cl. michiganense* subsp. *sepedonicum* were a pale white/beige colour and formed a distinct pattern on the medium. A deep blue extracellular pigment was found in two cultures of *Cur. flaccumfaciens* pv. *flaccumfaciens*. Purple-coloured strains of *Cur. flaccumfaciens* have been described and named *Cur. flaccumfaciens* var. *violaceum* (Shuster *et al.*, 1968). The vast majority of species and subspecies of *Clavibacter* produced small to moderate amounts of mucopolysaccharide in culture, in contrast to *Curtobacterium* strains, which were nearly all non-mucoid.

Results from the biochemical tests are presented in Table 2. Average test scores for each test for each species were determined by the number of strains that were positive for that particular trait. Percent positive scores were transcribed to + (80–100%), — (0–20%) or v (variable among strains or when the test was repeated with conflicting results).

No cultures produced acid from lactose or trehalose using the Difco Purple Broth Base test. All cultures were positive for acid production from sucrose on Medium C.

Fatty acid composition

A total of 24 fatty acids were detected from bacterial samples, of which 19 were in quantities large enough to be identified (Table 3). Ranges and means of fatty acid amounts for each genus are listed in Table 3. Means for each genus were calculated using mean values of each species within that genus.

Rhodococcus can be separated from the other genera by

the presence of 14:0, 15:0, 16:1t, 16:0, X1, 18:1c and 10me19:0 fatty acids (Table 3). These fatty acids are absent or greatly diminished in *Clavibacter*, *Curtobacterium* and *Arthrobacter*. *Rhodococcus* can also be separated by the absence or small amounts of i14:0, i15:0, i16:0, a17:0 and i18:0 compared to greater amounts of these fatty acids in the *Clavibacter*, *Curtobacterium* and *Arthrobacter* species.

Arthrobacter can be separated from *Clavibacter* by comparing mean amounts of 14:0, a15:1, a15:0, i16:0, a17:0 and i18:0 (Table 3), and from *Curtobacterium* by comparing mean amounts of i14:0, i15:0, a15:0, a17:0 and i18:0. *Clavibacter* and *Curtobacter* species can be differentiated by comparing mean amounts of a15:1, i15:0, i16:0, a17:0 and i18:0.

Values are given for minimum, maximum, mean and standard deviation of each major fatty acid (>1% of total) for each species and subspecies in the genus *Clavibacter* in Table 4. Fatty acids a15:0, i16:0 and a17:0 constitute approximately 90% of the total fatty acid content in this genus.

Ratios of various fatty acids were calculated to differentiate species and subspecies within the genus *Clavibacter* (Table 5). The mean for each species listed is the mean of ratios calculated for each strain of the species. Ratios calculated were a15:0/i16:0, a17:0/i16:0, a15:0/a17:0, i16:0/a15:1 and a15:1/i16:0. Not all strains of *Cl. rathayi* and *Cl. michiganense* subsp. *tessellarius*, *Cl. michiganense* subsp. *michiganense* and *Cl. michiganense* subsp. *nebraskense* contained a15:1. There-

Table 3. Minimum, maximum, mean and standard deviation values of fatty acids for *Clavibacter*, *Curtobacterium*, *Arthrobacter* and *Rhodococcus*

Genus	Fatty acid (%) [*]																				
	i14:0	14:0	a15:0	i15:0	a15:0	15:0	i16:0	a16:0	16:1c	16:1t	16:0	i17:0	a17:0	X1	17:0	18:2	i18:0	18:1c	18:0	m19:0	
<i>Clavibacter</i>																					
Minimum	0.00	0.00	0.00	0.00	33.23	0.00	4.57	0.00	0.00	0.00	0.79	0.00	7.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Maximum	5.25	0.37	10.84	2.87	70.16	0.77	40.15	0.00	0.21	0.00	8.27	1.24	44.75	0.00	0.47	1.54	1.63	2.49	1.42	0.00	
Mean†	0.93	0.03	1.89	0.27	46.30	0.07	19.44	0.00	0.00	0.00	3.65	0.04	25.93	0.00	0.01	0.11	0.03	0.53	0.45	0.00	
SD	1.07	0.04	1.75	0.16	3.79	0.07	7.11	0.00	0.00	0.00	1.00	0.04	8.96	0.00	0.02	0.15	0.05	0.30	0.29	0.00	
<i>Curtobacterium</i>																					
Minimum	0.00	0.00	0.00	1.13	36.91	0.00	4.62	0.00	0.00	0.00	0.28	0.00	21.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Maximum	1.30	1.58	0.63	5.18	64.28	0.59	13.56	0.00	0.00	0.00	7.39	1.05	43.46	0.00	0.12	0.00	18.15	0.60	0.94	0.00	
Mean†	0.68	0.32	0.01	2.24	47.98	0.03	9.80	0.00	0.00	0.00	2.37	0.49	31.80	0.00	0.00	0.00	3.75	0.14	0.34	0.00	
SD	0.16	0.25	0.02	0.09	2.44	0.03	0.28	0.00	0.00	0.00	1.00	0.10	4.85	0.00	0.00	0.00	2.38	0.08	0.06	0.00	
<i>Arthrobacter</i>																					
Minimum	0.24	0.00	0.00	0.46	36.05	0.00	5.43	0.00	0.00	0.00	1.55	0.00	10.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Maximum	2.39	1.30	0.00	1.47	71.49	0.25	9.62	0.00	0.00	0.00	6.07	0.00	19.84	0.00	0.00	0.00	30.94	0.47	0.71	0.00	
Mean†	1.32	0.65	0.00	0.97	53.77	0.13	7.53	0.00	0.00	0.00	3.81	0.00	14.93	0.00	0.00	0.00	15.47	0.24	0.36	0.00	
SD	0.00	0.00	0.00	0.00	17.72	0.00	0.00	0.00	0.00	0.00	2.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
<i>Rhodococcus</i>																					
Minimum	0.00	5.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.55	24.56	0.00	0.00	0.00	0.00	0.00	0.00	23.91	0.00	8.59	
Maximum	0.00	8.75	0.00	0.00	0.00	1.72	0.00	1.05	0.85	16.75	34.63	0.00	0.42	4.05	0.47	0.00	0.00	36.70	1.80	21.57	
Mean†	0.00	7.56	0.00	0.00	0.00	0.69	0.00	0.26	0.17	12.47	30.70	0.00	0.04	1.36	0.05	0.00	0.00	29.85	0.75	14.36	
SD	0.00	0.00	0.00	0.00	0.00	0.48	0.00	0.38	0.00	0.00	3.16	0.00	0.00	0.00	0.13	0.00	0.00	3.74	0.00	0.00	

SD, Standard deviation.

^{*} i14:0, 12-methyltridecanoic acid; 14:0, tetradecanoic acid; a15:1, 12-methyl-*cis*-4-tetradecenoic acid; i15:0, 13-methyltetradecanoic acid; a15:0, 12-methyltetradecanoic acid; 15:0, pentadecanoic acid; i16:0, 14-methylpentadecanoic acid; a16:0, 13-methylpentadecanoic acid; 16:1c, *cis*-9-hexadecenoic acid; 16:1t, *trans*-9-hexadecenoic acid; 16:0, hexadecanoic acid; i17:0, 15-methylhexadecanoic acid; a17:0, 14-methylhexadecanoic acid; X1, unknown; 17:0, heptadecanoic acid; 18:2, 9,11-octadecadienoic acid; i18:0, 16-methylheptadecanoic acid; 18:1c, *cis*-9-octadecenoic acid; 18:0, octadecanoic acid; m19:0, 10-methyloctadecanoic acid.

† Calculated using mean values of each species within that genus.

Table 4. Minimum, maximum, mean and standard deviation of major fatty acids in *Clavibacter* species

Bacterial species	Fatty acids (%)*						
	i14:0	a15:1	a15:0	i16:0	16:0	a17:0	18:1c
<i>Cl. xyli</i> subsp. <i>cynodontis</i>							
Minimum	0.00	0.00	40.79	9.17	4.17	39.69	0.00
Maximum	0.14	0.00	42.55	10.66	6.31	44.75	0.32
Mean	0.05	0.00	41.46	9.75	5.33	42.73	0.11
SD	0.07	0.00	0.78	0.65	0.88	2.19	0.15
<i>Cl. michiganense</i> subsp. <i>michiganense</i>							
Minimum	0.00	0.00	45.78	8.36	1.07	13.54	0.00
Maximum	1.43	7.35	65.53	28.89	8.27	30.57	0.97
Mean	0.66	1.70	50.88	20.06	4.00	20.78	0.57
SD	0.32	1.42	4.35	4.90	1.87	3.86	0.32
<i>Cl. m.</i> subsp. <i>sepedonicum</i>							
Minimum	0.00	1.48	33.23	7.22	0.97	21.94	0.00
Maximum	0.67	10.84	50.32	20.70	6.18	41.04	2.49
Mean	0.15	5.84	42.86	15.50	3.02	29.67	0.83
SD	0.20	2.39	4.24	3.11	1.19	4.58	0.70
<i>Cl. m.</i> subsp. <i>nebraskense</i>							
Minimum	0.31	0.00	42.95	12.20	1.76	14.68	0.00
Maximum	1.20	3.98	52.77	28.01	6.22	34.60	0.74
Mean	0.67	1.47	48.14	20.38	4.22	23.75	0.24
SD	0.22	1.02	2.51	3.22	1.37	4.62	0.26
<i>Cl. m.</i> subsp. <i>insidiosum</i>							
Minimum	0.00	0.51	45.11	4.57	0.79	23.81	0.00
Maximum	0.84	3.76	70.16	17.82	3.59	41.85	1.38
Mean	0.17	1.61	48.87	11.46	1.89	34.32	0.39
SD	0.22	0.96	5.21	3.22	0.71	4.64	0.45
<i>Cl. m.</i> subsp. <i>tessellarius</i>							
Minimum	0.73	0.00	45.74	20.61	2.83	16.04	0.26
Maximum	1.43	1.23	49.12	28.39	4.47	25.25	0.91
Mean	0.94	0.74	47.49	23.86	3.76	21.99	0.59
SD	0.19	0.36	0.91	2.14	0.43	2.26	0.16
<i>Cl. rathayi</i>							
Minimum	0.80	0.00	47.62	18.05	2.38	20.65	0.00
Maximum	1.54	0.95	51.88	26.58	3.11	24.31	0.94
Mean	1.26	0.51	50.15	20.62	2.74	23.15	0.42
SD	0.23	0.36	1.21	3.01	0.26	1.22	0.38
<i>Cl. tritici</i>							
Minimum	2.41	1.01	37.88	29.10	3.59	7.64	0.00
Maximum	5.25	4.99	43.36	40.15	4.92	14.51	2.17
Mean	3.56	3.26	40.58	33.90	4.27	11.08	1.12
SD	0.86	1.51	1.60	3.53	0.50	2.06	0.73

SD, Standard deviation.

* See Table 3 for abbreviations.

fore, ratios generated for these organisms using a value of zero for the amount of a15:1 as the denominator were not useful in differentiating them from other organisms.

A dichotomous key for differentiation of species and subspecies of *Clavibacter* was developed using ratio amounts (Table 6). The species and subspecies could be differentiated when ranges of fatty acid ratios did not overlap. Further differentiation could be accomplished when the means of fatty acid ratios were widely divergent (Table 5).

Values for the minimum, maximum, mean and standard deviation for major fatty acids for each *Curtobacterium flaccumfaciens* pathovar are given in Table 7. Among the *Curtobacterium flaccumfaciens*

pathovars, pv. *poinsettiae* could be differentiated from pv. *flaccumfaciens* and pv. *betae* by comparing amounts of a17:0. *Cur. flaccumfaciens* pv. *poinsettiae* could be separated from pv. *oortii* and pv. *betae* by i18:0. No other direct fatty acid comparisons were useful in differentiating the *Curtobacterium* pathovars. Ratios developed from pairs of fatty acids were not useful in distinguishing the *Cur. flaccumfaciens* pathovars.

The *Rhodococcus* and *Arthrobacter* genera each contain only one plant-pathogenic species, therefore no differentiation was necessary (Table 3).

In calculating similarity coefficients, fatty acid data were converted to a binary, weighted statistical method that treated fatty acids as being either present or absent

Table 5. Fatty acid ratios in *Clavibacter* species

Bacterial species	Fatty acid ratios*				
	a15:0/ i16:0	a17:0/ i16:0	a15:0/ a17:0	i16:0/ a15:1	a15:1/ 16:0
<i>Cl. xyli</i> subsp. <i>cynodontis</i>					
Minimum	3.99	3.72	0.92	—†	0.00
Maximum	4.45	4.77	1.07	—	0.00
Mean	4.27	4.42	0.97	—	0.00
SD	0.20	0.49	0.07	—	0.00
<i>Cl. michiganense</i> subsp. <i>tessellarius</i>					
Minimum	1.67	0.56	1.90	19.43	0.00
Maximum	2.35	1.17	2.95	34.59	0.31
Mean	2.01	0.94	2.19	27.55	0.19
SD	0.20	0.16	0.26	5.04	0.10
<i>Cl. m.</i> subsp. <i>insidiosum</i>					
Minimum	2.59	1.60	1.15	3.43	0.25
Maximum	15.35	6.23	2.95	27.61	1.99
Mean	4.88	3.34	1.47	9.89	0.86
SD	2.72	1.28	0.38	6.68	0.43
<i>Cl. m.</i> subsp. <i>michiganense</i>					
Minimum	1.58	0.54	1.85	2.29	0.00
Maximum	6.77	3.66	4.35	36.69	2.69
Mean	2.79	1.16	2.54	14.40	0.55
SD	1.16	0.63	0.61	6.88	0.67
<i>Cl. m.</i> subsp. <i>nebraskense</i>					
Minimum	1.70	0.69	1.34	5.34	0.00
Maximum	4.33	2.37	3.57	45.72	0.72
Mean	2.44	1.23	2.12	18.16	0.34
SD	0.55	0.45	0.52	9.72	0.19
<i>Cl. m.</i> subsp. <i>sepedonicum</i>					
Minimum	1.90	1.10	0.81	1.29	0.58
Maximum	6.54	5.42	2.19	11.11	8.21
Mean	2.92	2.08	1.48	3.24	2.37
SD	0.90	0.89	0.29	1.91	1.69
<i>Cl. rathayi</i>					
Minimum	1.79	0.78	2.06	19.08	0.00
Maximum	2.87	1.34	2.31	39.88	0.33
Mean	2.49	1.15	2.17	29.05	0.18
SD	0.37	0.20	0.08	9.08	0.12
<i>Cl. tritici</i>					
Minimum	1.02	0.19	2.79	5.83	0.28
Maximum	1.39	0.50	5.37	39.75	1.12
Mean	1.21	0.34	3.81	15.52	0.74
SD	0.12	0.09	0.80	12.07	0.30

SD, Standard deviation.

* See Table 3 for abbreviations.

† —, These values could not be calculated because a15:1 = 0.

(1 or 0). Some fatty acids present in minor amounts were disregarded, i.e. treated as if they were absent. Therefore, genera initially having qualitatively similar fatty acid profiles resulted in similarity coefficients from 0 to 100%. For example, the genus *Rhodococcus* linked at 0% similarity to the three remaining genera (Fig. 1).

Curtobacterium, *Clavibacter* and *Arthrobacter* species linked at the 70% level of similarity. Four *Clavibacter* strains (two *Cl. xyli* subsp. *cynodontis*, one *Cl. michiganense* subsp. *michiganense* and one *Cl. michiganense* subsp. *nebraskense*) clustered with the *Curtobacter* and *Arthro-*

bacter genera. The remaining *Clavibacter* strains formed a homogeneous group and were linked together at the 84% level of similarity. *Curtobacter* and *Arthrobacter* strains linked at the 73% level, with the vast majority linked at the 88% similarity level. No definite trends were observed in subspecies relationships beyond the 88% level of similarity.

The dendrogram contains 129 *Clavibacter* strains. Four major groups were noted above the 84% level, containing 23, 61, 16 and 19 strains, respectively. Strains within groups were linked at 100%. The first group

Fig. 1. Dendrogram obtained by cluster analysis of similarity coefficients derived from 14 fatty acids of 189 strains of phytopathogenic coryneform bacteria. Numbers before strain abbreviations indicate the number of strains of each type located in the cluster. Ai, *Arthrobacter ilicis*; Rf, *Rhodococcus fascians*; Cfo, *Cur. flaccumfaciens* pv. *oortii*; Cff, *Cur. flaccumfaciens* pv. *flaccumfaciens*; Cfb, *Cur. flaccumfaciens* pv. *betae*; Cfp, *Cur. flaccumfaciens* pv. *poinsettiae*; Ct, *Cl. tritici*; Cr, *Cl. rathayi*; Cmm, *Cl. michiganense* subsp. *michiganense*; Cmn, *Cl. michiganense* subsp. *nebraskense*; Cmi, *Cl. michiganense* subsp. *insidiosum*; Cms, *Cl. michiganense* subsp. *sepedonicum*; Cmt, *Cl. michiganense* subsp. *tessellarius*; Cxc, *Cl. xyli* subsp. *cynodontis*.

containing 23 strains was primarily composed of *Cl. michiganense* subsp. *sepedonicum* (15 strains) along with 8 strains of various *Clavibacter* subspecies. The largest group containing 61 strains was a mixture of *Cl. michiganense* subsp. *nebraskense* (15), *Cl. tritici* (4), *Cl. michiganense* subsp. *sepedonicum* (4), *Cl. michiganense* subsp. *insidiosum* (6), *Cl. michiganense* subsp. *tessellarius* (9), *Cl. rathayi* (5) and *Cl. michiganense* subsp. *michiganense* (18). This group contained the majority (85%) of *Cl. michiganense* subsp. *michiganense* strains and the major-

ity (88%) of *Cl. michiganense* subsp. *nebraskense* strains. The group containing 16 strains was composed of approximately one half *Cl. michiganense* subsp. *sepedonicum* and one half *Cl. michiganense* subsp. *insidiosum*. The last major group was composed of *Cl. michiganense* subsp. *sepedonicum* (89%) and *Cl. michiganense* subsp. *insidiosum* (11%).

Two small groups containing 1 and 5 strains (1 strain *Cl. michiganense* subsp. *insidiosum*, and 1 strain *Cl. xyli* subsp. *cynodontis*, 2 strains *Cl. rathayi* and 2 strains *Cl.*

Table 6. Key for the separation of *Clavibacter* species and subspecies using fatty acid ratios

A1: a15:0/i16:0 < 139*	<i>Cl. tritici</i>
A2: a15:0/i16:0 > 139	B
B1: a17:0/i16:0 < 134	C
B2: a17:0/i16:0 > 134	D
C1: i16:0/a15:1 < 1111	E
C2: i16:0/a15:1 > 1111	F
D1: a15:0/a17:0 < 115	G
D2: a15:0/a17:0 > 115	E
E1: a15:1/i16:0 ≥ 237	<i>Cl. m. subsp. sepedonicum</i>
E2: a15:1/i16:0 ≥ 45-86	H
F1: i16:0/a15:1 ≥ 2755-2905	<i>Cl. rathayi</i> or <i>Cl. m. subsp. tessellarius</i>
F2: i16:0/a15:1 ≥ 1440-1816	<i>Cl. m. subsp. michiganense</i> or subsp. <i>nebraskense</i>
G1: a15:1/i16:0 > 58	<i>Cl. m. subsp. sepedonicum</i>
G2: a15:1/i16:0 = 0	<i>Cl. x. subsp. cynodontis</i>
H1: a15:0/i16:0 ≥ 488 and a17:0/i16:0 ≥ 334	<i>Cl. m. subsp. insidiosum</i>
H2: a15:0/i16:0 ≥ 261 and a17:0/i16:0 ≥ 120	<i>Cl. m. subsp. michiganense</i> or <i>Cl. m. subsp. nebraskense</i>

* Values have been multiplied by 100.

Table 7. Minimum, maximum, mean and standard deviation of major fatty acids of *Curtobacterium flaccumfaciens* pathovars

<i>Curtobacterium flaccumfaciens</i> pathovar	Fatty acid (%)*					
	i15:0	a15:0	i16:0	16:0	a17:0	i18:0
<i>flaccumfaciens</i>						
Minimum	1.13	40.41	4.62	0.28	22.68	0.00
Maximum	5.18	63.19	13.56	5.16	33.78	14.95
Mean	2.37	50.96	9.50	1.96	29.49	3.39
SD	0.98	6.37	2.57	1.30	3.22	3.75
<i>betae</i>						
Minimum	1.40	36.91	6.49	2.42	21.74	0.84
Maximum	2.81	64.28	11.40	7.39	31.45	18.15
Mean	2.17	49.80	9.81	4.04	26.16	5.64
SD	0.37	7.76	1.40	1.86	2.85	5.52
<i>oortii</i>						
Minimum	1.66	40.27	8.73	1.33	25.91	2.57
Maximum	3.25	50.65	10.79	4.36	34.97	8.90
Mean	2.28	45.26	10.24	2.09	32.21	5.97
SD	0.53	3.73	0.78	1.15	3.24	2.01
<i>poinsettiae</i>						
Minimum	1.87	43.44	8.57	0.80	33.86	0.00
Maximum	2.66	51.91	12.02	3.52	43.46	0.00
Mean	2.15	45.90	9.66	1.40	39.35	0.00
SD	0.24	2.53	1.03	0.74	3.12	0.00

* See Table 3 for abbreviations.

michiganense subsp. *tessellarius*, respectively) were found.

Discussion

Reclassification of the plant-pathogenic coryneform bacteria into four groups was not proposed by a single

research group, but was the cumulative result of several individual research groups (Davis *et al.*, 1984; Collins *et al.*, 1981; Goodfellow, 1984; Collins & Jones, 1983). The research that resulted in the reclassification used a variety of criteria such as serology, biochemistry, physiology, cell wall sugars, cell wall proteins and peptidoglycans, menaquinones, DNA studies and lipid compositions. This is the first report using fatty acid

profiles to compare and contrast the plant-pathogenic corynebacteria as a distinct group.

The results of the studies reported here show that the plant-pathogenic coryneform bacteria can be separated into four distinct groups based on fatty acid analysis, and support the current classification of the plant-pathogenic coryneform bacteria at the genus level. *Rhodococcus fascians* has large amounts of straight-chain saturated and unsaturated fatty acids and is also distinguished by the presence of tuberculostearic acid. These compounds are either not found or are present in very small quantities among species in the genera *Clavibacter*, *Arthrobacter* and *Curtobacterium*, which are characterized by relatively large amounts of iso- and anteiso-branched chain fatty acids that can be used to differentiate the three genera. Comparison of ranges and averages of 14:0, a15:1, i15:0, a15:0, i16:0, a17:0 and i18:0 can be used to delineate *Arthrobacter* from *Clavibacter*. Comparison of ranges and averages of 14:0, a15:1, i15:0, i16:0, i17:0, a17:0 and i18:0 can be used to delineate *Clavibacter* from *Curtobacterium*.

Cluster analysis separated the four genera into three major groups: group A, *Arthrobacter* and *Curtobacterium*; group B, *Clavibacter*; and group C, *Rhodococcus*. In group A, no predictions were possible at any level regarding the placement of an unknown *Curtobacterium* or *Arthrobacter* strain. Any cluster within group A at the 100% level was heterogeneous, i.e. it consisted of a variety of *Curtobacterium* or *Arthrobacter* species or pathovars.

Group B contained six clusters of strains, none of which were completely homogeneous. Two major clusters were linked at the 91% level, and most others above the 85% level. No definite trends were observed, with the exception of *Cl. michiganense* subsp. *michiganense*, in which 18 (85%) strains were found in one cluster. This cluster also contained 15 (88%) of the *Cl. michiganense* subsp. *nebraskense* strains. It is apparent that on the basis of fatty acid analysis, *Cl. michiganense* subsp. *michiganense* and subsp. *nebraskense* are closely related. However, these two subspecies can be separated from one another by biochemical and cultural characteristics (Table 2) and by other chemotaxonomic methods (Riley *et al.* 1988). Other species and subspecies of *Clavibacter* were distributed equally among the clusters.

Group C contained two clusters of 4 and 12 *Rhodococcus fascians* strains linked at the 91% level. The *Rhodococcus* group appeared to be completely unrelated to all other bacterial species examined. It was linked to all others at zero percent similarity. No *Rhodococcus* strains were found in any other cluster or group.

The fatty acid profiles of *Curtobacterium flaccumfaciens* pathovars were found to have a very high degree of similarity, and few comparisons of fatty acid profiles

were useful in separating these pathovars from one another. Pathovars could be differentiated by biochemical tests; however, very few of these were useful. All pathovars of *Cur. flaccumfaciens* were essentially non-mucoid and of the same general colour. The results of this study indicate that the *Cur. flaccumfaciens* pathovars comprise a very homogeneous group and have few discernible differences. According to Dye *et al.* (1980), at the infrasubspecific level, host specificity has greater taxonomic significance than minor differences in results of biochemical or serological tests. Fatty acid analysis is inadequate for differentiating the four pathovars of *Cur. flaccumfaciens*. Therefore, host-specific pathogenicity may be the only differentiating characteristic. A pathovar has been defined as a strain or set of strains with the same or similar characteristics which can be differentiated at infrasubspecific level from other strains of the same species or subspecies on the basis of distinctive pathogenicity to one or more plant hosts (Dye *et al.*, 1980). On this basis, pathovars *oortii*, *flaccumfaciens*, *betae* and *poinsettiae* should remain in the current classification and nomenclatural system.

Davis *et al.* (1984) proposed the transfer of *Corynebacterium iranicum*, *Cor. rathayi*, *Cor. tritici* and the *Cor. michiganense* subspecies to a new genus, *Clavibacter*. Results presented here on fatty acid analysis and biochemical tests raise questions regarding the current classification of the *Clavibacter michiganense* subspecies. Cowan (1971) has stressed the importance of using multiple taxonomic characters and the importance of objectivity in making a classification, supported by the use of statistical or computational methods. The reclassification of *Cor. michiganense*, *Cor. insidiosum*, *Cor. nebraskense*, *Cor. sepedonicum* and *Cor. tessellarius* as subspecies of *Cor. michiganense* was proposed on the basis of a single taxonomic character, polyacrylamide gel electrophoresis (PAGE) of cell wall proteins (Carlson & Vidaver, 1982). As little as one year prior to the reclassification, it was stated that the identity of most phytopathogenic coryneform and related bacteria could be determined only by inoculation tests in plants (Vidaver & Starr, 1981). These authors further concluded that recent taxonomic studies have not shown any easy or certain means of identification of these bacteria below the genus level – other than their ability to cause particular plant diseases. In variance with these authors (Carlson & Vidaver, 1982; Vidaver & Starr, 1981), chemotaxonomic and other data existed that clearly differentiated many of the *Clavibacter michiganense* subspecies. Studies using serology (Lazar, 1968), cell wall amino acids (Yamada & Komagata, 1972), DNA homology (Starr *et al.*, 1975), numerical taxonomy (Jones, 1975) and lipid analysis (Collins & Jones, 1980) all clearly differentiate a number of the *Cl. michiganense*

subspecies from one another.

Staley & Krieg (1984) define bacterial species as a collection of strains that share many features in common and differ considerably from other strains. Considerable information exists on the differentiation of *Clavibacter* species. *Clavibacter* species and subspecies can be differentiated by standard biochemical and physiological tests (Vidaver, 1980; Moffett *et al.*, 1983; this manuscript), pathogenicity tests, cultural characteristics, pigmentation and growth rate (Moffett *et al.*, 1983). Principal cell wall amino acids can be used to differentiate *Cl. michiganense* subsp. *insidiosum* and subsp. *sepedonicum* from subsp. *michiganense* (Yamada & Komagata, 1972). Starr *et al.* (1975) differentiated *Cl. michiganense* subsp. *insidiosum* from other *Cl. michiganense* subspecies using DNA homology. *Cl. michiganense* subsp. *sepedonicum* had a higher degree of homology to subsp. *insidiosum* than any other *Cl. michiganense* subspecies. Lazar (1968) separated certain *Clavibacter* species and subspecies using serological techniques (gel diffusion of nucleoprotein antigens). Jones (1975) found many *Clavibacter* species and subspecies well separated from one another in her numerical taxonomic study. The fatty acid profiles of *Clavibacter* species and subspecies generated during the course of the studies reported here were useful in delineating these organisms from one another. In most cases, species or subspecies could be differentiated from one another by comparing ratios of fatty acids. It is apparent from these studies that fatty acid analysis of coryneform bacteria recovered from diseased plants can be a useful technique in determining the identity of a bacterium. Fatty acid analysis has been used previously in our laboratory for this purpose (Gudmestad *et al.*, 1988).

Clavibacter michiganense subsp. *sepedonicum* and subsp. *insidiosum* each contain many copies of a unique repeated DNA sequence not found in any other *Clavibacter* species or subspecies (A. E. Oleson, personal communication; Mogen & Oleson, 1987), which is in agreement with the data from Starr *et al.* (1975). On this basis, these subspecies are closely related to one another, but not closely related to any other *Cl. michiganense* subspecies. Riley *et al.* (1988), using allozyme electrophoresis, found large differences between *Cl. michiganense* subsp. *michiganense*, subsp. *insidiosum* and subsp. *nebraskense*. These differences were large enough, in their opinion, to consider each a distinct species. Based on these research findings, it would seem that the current classification of subspecies within *Cl. michiganense* is far from ideal.

The classification within the genus *Clavibacter* appears to be incompletely resolved, and incorrect at this time. Based on the results reported here and elsewhere (Yamada & Komagata, 1972; Collins & Jones, 1980;

Döpfer *et al.*, 1982; Riley *et al.*, 1988), and in concurrence with others (Collins & Bradbury, 1986; Riley *et al.*, 1988), *Cl. michiganense* subspecies *michiganense*, *insidiosum*, *sepedonicum*, *nebraskense* and *tessellarius* warrant full species status in the genus *Clavibacter*.

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