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Comparative Chemotaxonomic Studies of Mycolic Acid-Free Coryneform Bacteria of Human Origin

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Forty-two clinical isolates were classified as *Corynebacterium minutissimum*, *Corynebacterium striatum*, and *Corynebacterium* CDC group I by the API Coryne system. The chemotaxonomic characteristics of the isolates were determined by thin-layer chromatographic analysis. Twenty-six isolates were found to have a type IV cell wall (*meso*-di-aminopimelic acid arabinose, galactose) but did not contain mycolic acids. These 26 isolates shared chemotaxonomic characteristics with those of mycolic acid-free reference strains (including the *Corynebacterium amycolatum* NCFB 2768 type strain, "*Corynebacterium asperum*," and coryneform CDC groups I2 and F2). The total protein profiles of the isolates determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were similar to each other and to that of the *C. amycolatum* type strain. The profiles of the reference strains "*Corynebacterium asperum*" (CIP 100836, CIP 80.54, CIP 79.37, CIP 52.13), coryneform bacteria CDC groups I2 and F2 (CDC F5771, F5890, G723, G1970), and *C. amycolatum* were closely related. Thus, the mycolic acid-negative strains with a chemotype IV wall may belong to a single taxon. DNA hybridization studies could confirm this hypothesis. The present study shows the importance of chemotaxonomic analysis for verifying strain identifications and completing results from biochemical tests, particularly for coryneform bacteria.

It was recently shown (6, 9) that some bacterial strains in the genus *Corynebacterium* do not contain mycolic acids. These molecules are of particular importance in the definition of authentic members of the genus *Corynebacterium* (7, 19). Therefore, it can be argued that any bacterium devoid of mycolic acids cannot be classified in this genus. However, we have frequently collected clinical isolates that possess all of the phenotypic characteristics of coryneform bacteria but that do not contain mycolic acids. All of these strains were identified as *Corynebacterium* spp. by standard culture and biochemical tests. Many of these strains have been identified by a new commercial kit (11, 12) as *C. minutissimum*, *C. striatum*, or related organisms of CDC group I.

To study further those strains that are devoid of mycolic acids, we compared them with other reference strains in a detailed chemotaxonomic analysis.

In the present study, cell wall components such as polar lipids, mycolic acids, peptidoglycan, and sugar polymers were characterized by thin-layer chromatography. Total protein patterns were studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

Clinical strains. A total of 42 clinical isolates were obtained from the Laboratoire des Identifications Bactériennes de l'Institut Pasteur. These isolates were initially selected on the basis of coryneform morphology and good growth on brain heart infusion (BHI) agar (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). They were identified by using the API Coryne system (API-BioMérieux, Inc., La Balme les Grottes, France).

Inocula were prepared and inoculations were performed according to the supplier's instructions. The results were interpreted as described by the supplier for the API analytical profile index.

Reference strains. *Corynebacterium* species were obtained from the Collection de l'Institut Pasteur (CIP), Paris, France; the American Type Culture Collection (ATCC); or the National Collection of Food Bacteria (NCFB) or were kindly provided by E. Weaver, Centers for Disease Control and Prevention (CDC), Atlanta, Ga. The following strains were studied: "*C. asperum*" (quotation marks indicate that it is not an approved bacterial name) CIP 79.37, CIP 80.54, CIP 100836, and CIP 52.13; *C. minutissimum* CIP 100652T (ATCC 23348, type strain) *C. striatum* CIP 81.15T (ATCC 6940, type strain) *C. amycolatum* CIP 103462T (NCFB 2768, type strain), CDC group I1 strain E8873; CDC group I2 strains F5771, G675, and F5890; and CDC group F2 strains G723 and G1970.

Growth conditions. For chemotaxonomic analysis, bacteria were cultivated for 24 to 36 h at 30°C, with shaking, in 300 ml of BHI broth. After the purity of the culture was verified, cells were killed by adding 2% (vol/vol) formalin (E. Merck AG, Darmstadt, Federal Republic of Germany), washed twice, resuspended in distilled water, and freeze-dried.

Amino acid analysis of peptidoglycan. Cell wall material was prepared by a modification of the method of Keddie and Cure (15). Five milligrams of freeze-dried bacteria was suspended in 2 ml of a 5% (wt/vol) KOH solution and heated at 98°C. The turbidity was recorded with a Bausch & Lomb model 21 spectrophotometer at 390 nm until a steady optical density was attained. Then, 10 ml of distilled water was added. The preparation was subsequently centrifuged at 10,000 × *g* for 10 min (model Centrikon H-401; Kontron & Hermle KG, Gosheim, Switzerland), washed, and hydrolyzed by adding 2 ml of 6 M hydrochloric acid and heating at 100°C for 18 h. The hydrolysate was filtered through a 0.22-μm-pore-size cellulose membrane (Millex; Millipore Corp., Bedford, Mass.) and dried at 80°C under vacuum, and the residue was dissolved in 30 μl of 10% 1-propanol. A 2-μl

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TABLE 1. Reference strains identified by the API Coryne system and cell wall components

Species name as received[a]	Accession no.[b]	Biochemical reaction[c]					Analytical profile	API[d]	MYC[e]
		URE	NIT	RIB	MAL	SUC			
C. amycolatum	CIP 103452	+	−	+	+	−	2101324	GrF	−
C. striatum	CIP 81.15	−	+	−	−	+	3100105	STR	+
C. minutissimum	CIP 100652	−	−	−	+	+	2100125	MIN	+
"*C. asperum*"	CIP 79.37	+	+	+	+	−	3101324	GrF	−
"*C. asperum*"	CIP 80.54	+	−	+	+	−	2101324	GrF	−
"*C. asperum*"	CIP 100836	−	−	+	+	−	2100324	MIN	−
"*C. asperum*"	CIP 52.13	−	−	+	±	−	2100324[f]	MIN	−
CDC group I1	E8873	−	+	−	−	−	3100104[g]	GrI	+
CDC group I1	F435	−	+	−	−	−	3100104[g]	GrI	+
CDC group I1	F8257	−	+	−	−	−	3100100[g]	GrI	+
CDC group I2	F5771	−	+	+	+	−	3100324[h]	GrI	−
CDC group I2	F5890	−	+	+	+	−	3100324[h]	GrI	−
CDC group I2	G675	−	+	+	+	−	3100324[h]	GrI	−
CDC group F2	G723	+	+	+	+	−	3101324[i]	GrF	−
CDC group F2	G1970	+	−	+	+	−	2101324[i]	GrF	−

[a] All strains were chemotype IV (*meso*-di-aminopimelic acid, arabino-galactane).
[b] CIP strains were from the Collection de l'Institut Pasteur. All other strains were from CDC.
[c] URE, urease; NIT, nitrate reduction; RIB, ribose; MAL, maltose; SUC, sucrose.
[d] API, species identified by the API profile index. GrF, CDC group F; STR, *C. striatum*; MIN, *C. minutissimum*; GrI, CDC group I.
[e] MYC, mycolic acids.
[f] Delayed reaction for maltose.
[g] Tween positive = *C. striatum*; Tween negative = CDC group I (according to API). Sucrose negative = CDC group I; sucrose positive = *C. striatum* (according to CDC).
[h] Maltose positive = CDC group I2 (according to CDC).
[i] Sucrose negative = CDC group F2 (according to CDC).

aliquot was spotted onto high-performance thin-layer chromatography cellulose plates (10 by 10 cm; Art. 5787; Merck). The plates were developed by two-dimensional chromatography as described by Brenner et al. (4); the first dimension was 1-propanol and 50% acetic acid (20:10); the second dimension was methanol, pyridine, 10% HCl (20:2.5:5.5).

The amino acids were revealed by spraying the plates with freshly prepared 0.2% (wt/vol) ninhydrin in ethanol-collidine (95:5); this was followed by heating at 90°C.

Sugar analysis of whole-cell extracts. Five milligrams of freeze-dried bacteria was hydrolyzed in 1 ml of 1 M sulfuric acid at 100°C for 2 h. The hydrolysate was mixed with 220 mg of barium hydroxide and neutralized with a saturated barium hydroxide solution. The resulting precipitate was centrifuged at 8,000 × *g* for 20 min, and the supernatant was collected, filtered onto a cellulose membrane (Millex) as described above, and dried under an airstream at 60°C. The extract was then taken up with 50 μl of 10% 1-propanol. Five microliters was applied onto high-performance thin-layer chromatography cellulose plates (10 by 20 cm; Art. 5786; Merck) to form a 12-mm band. A mixture of pure sugars at 0.5% (wt/vol) in 10% (vol/vol) 1-propanol was used as a standard. The sugars were separated by three developments with ethyl acetate-pyridine-water (100:35:25) as described by Schaal (24) and were revealed by spraying the plates with aniline phthalate reagent (1.5 g of phthalic acid, 1 ml of aniline, 100 ml of water-saturated butanol) and then heating at 90°C.

Polar lipid analysis. Polar lipids were extracted by the method of Blight and Dyer (3); 100 mg of freeze-dried bacteria was shaken for 4 h with 3.8 ml of chloroform-methanol-water (20:10:8) and then centrifuged at 10,000 × *g* for 20 min, and the extraction was repeated. The two supernatants were pooled, 2 ml of chloroform and 2 ml of water were added, and the mixture was shaken gently. The lipid phase was separated by centrifugation at 10,000 × *g* for 10 min, filtered through a 1PS-Separator Phase Filter (Whatman, Maidson United Kingdom), and finally evaporated under a nitrogen stream. The dried lipids were redissolved in 200 μl of chloroform-methanol (1:1). Five microliters was applied onto Kieselgel G60 plates (10 by 20 cm; Art. 5626; Merck) to form a 12-mm band. As described by Mangold (20), the components were separated by a single development with chloroform-methanol-acetic acid-water (60:25:0.8:4) and were revealed by spraying with molybdenum blue reagent for phosphate esters (10), α-naphthol reagent for glycolipids (13), ninhydrin reagent (see amino acid analysis above) for free amino groups, and vanillin-sulfuric acid for total visualization (5).

Mycolic acids. Mycolic acids were detected as described by Minnikin et al. (21, 22). Briefly, 50 mg of freeze-dried bacteria was hydrolyzed in 1 ml of methanol-toluene-sulfuric acid (30:15:1) at 70°C for 16 h. After cooling, 1 ml of hexane was added and the mixture was shaken and allowed to separate. The upper phase was collected and evaporated under a stream of nitrogen. Residues were dissolved in 100 μl of diethyl ether. Five-microliter samples of each extract were spotted onto Kieselgel G60 plates. The components were separated by using three developments with hexane-ether (85:15) and were revealed by spraying with 10% (wt/vol) phosphomolybdic acid in ethanol and then heating at 110°C.

PAGE. (i) Preparation of protein extracts. Three culture tubes containing BHI agar medium were prepared for each strain. Bacteria were grown for 24 h at 30°C under aerobic conditions, harvested directly from the agar, and suspended in 500 μl of 0.015 M Tris hydrochloride buffer (pH 8.6) containing 2% (wt/vol) 1,4-dithiothreitol (Sigma). About 0.8 g of 0.13-mm-diameter glass beads (Verre et Technique, Arceuil, France) was added, and the bacterial cells were disrupted by strong shaking for 2 min with a Vibrogen apparatus (Buhler Vibrogen, Touzart et Matignon, Paris, France) (23). The liquid was collected with a micropipette and was centrifuged for 1 min at 10,000 × *g* in a conical tube.

TABLE 2. Clinical isolates identified by API coryne and their cell wall components

Accession no.[a]	Clinical source	Biochemical reaction[b]					Analytical profile	API[c]	MYC[d]	CHEMO[e]
		URE	NIT	RIB	MAL	SUC				
LIB 169.87	Catheter	−	−	+	−	+	2100325	MIN	−	MAF.Gr
LIB 356.87	Pus	−	−	+	−	+	2100325	MIN	−	MAF.Gr
LIB 433.87	Blood	−	−	±	+	−	6100324[f]	MIN	+	MIN
LIB 436.87	Pus	−	+	+	−	+	3100305	STR	+	STR
LIB 478.87	Skin	−	+	−	−	+	3100105	STR	+	STR
LIB 488.87	Fistula	−	−	−	+	+	2100125	MIN	+	MIN
LIB 057.88	Catheter	−	−	+	+	+	2100325	MIN	+	MIN
LIB 360.88	Urine	−	−	+	+	+	2100325	MIN	−	MAF.Gr
LIB 388.88	Blood	−	−	+	±	+	6100325[g]	MIN	+	MIN
LIB 559.88	Catheter	−	−	+	−	+	2100305[h]	STR	−	MAF.Gr
LIB 588.88	Catheter	−	−	+	−	+	2100305[i]	STR	−	MAF.Gr
LIB 619.88	Pus	−	−	+	+	−	2100324[j]	MIN	−	MAF.Gr
LIB 637.88	Pus	−	−	+	−	+	2100325	MIN	−	MAF.Gr
LIB 647.88	Pus	−	−	+	+	+	2100325	MIN	+	MIN
LIB 740.88	Blood	−	−	+	+	+	2100325	MIN	−	MAF.Gr
LIB 767.88	Synovial fluid	−	+	−	−	+	3100105	STR	+	STR
LIB 814.88	Catheter	−	−	+	−	+	2100305[h]	STR	−	MAF.Gr
LIB 082.89	Eye	−	−	+	+	+	2100325	MIN	−	MAF.Gr
LIB 269.89	Blood	−	−	+	+	+	2100325	MIN	−	MAF.Gr
LIB 322.89	Urine	−	−	±	+	+	6100325[g]	MIN	+	MIN
LIB 350.89	Catheter	−	−	+	+	+	2100325	MIN	−	MAF.Gr
LIB 361.89	Catheter	−	+	−	−	+	3100105	STR	+	STR
LIB 379.89	Pus	−	−	+	+	+	2100325	MIN	−	MAF.Gr
LIB 493.89	Blood	−	−	+	+	+	6100325[g]	MIN	+	MIN
LIB 551.89	Pus	−	+	+	±	−	3100324	GrI	−	MAF.Gr
LIB 552.89	Pus	−	−	−	+	+	2100125	MIN	+	MIN
LIB 565.89	Mouth	−	−	+	−	+	2100325	MIN	−	MAF.Gr
LIB 580.89	Vagina	−	+	+	±	−	3100324	GrI	−	MAF.Gr
LIB 584.89	Urine	−	−	+	+	+	2100325	MIN	−	MAF.Gr
LIB 609.89	Urine	−	−	±	±	+	2100325	MIN	+	MIN
LIB 617.89	Pus	−	+	+	−	+	3100305	STR	+	STR
LIB 698.89	Skin	−	−	+	−	+	2100305[h]	STR	−	MAF.Gr
LIB 856.89	Urine	−	−	−	+	+	2140125[j]	MIN	−	MAF.Gr
LIB 336.90	Pus	−	−	+	+	−	2100324[f]	MIN	+	MIN
LIB 416.90	Abscess	−	−	+	+	−	2100324[f]	MIN	−	MAF.Gr
LIB 557.90	Abscess	−	−	−	+	+	2100125	MIN	+	MIN
LIB 565.90	Pus	−	−	+	+	−	2100324[f]	MIN	−	MAF.Gr
LIB 598.90	Dialysate	−	−	+	+	+	2100325	MIN	−	MAF.Gr
LIB 808.90	Vagina	−	−	+	+	−	2100324[f]	MIN	−	MAF.Gr
LIB 080.91	Blood	−	−	+	+	−	2100325	MIN	−	MAF.Gr
LIB 094.91	Sputum	−	−	+	±	+	2100325[f]	MIN	−	MAF.Gr
LIB 131.91	Catheter	−	−	+	+	−	2100324	MIN	−	MAF.Gr

[a] All strains were chemotype IV (mDAP, arabinogalactan). LIB, Laboratoire des Identifications Bactériennes, Institut Pasteur.
[b] URE, urease; NIT, nitrate reduction; RIB, ribose; MAL, maltose; SUC, sucrose.
[c] API, species identified by the API profile index. MIN, *C. minutissimum*; STR, *C. striatum*; GrI, CDC group I.
[d] MYC, mycolic acids.
[e] CHEMO, identified according to chemotaxonomic data. MAF.Gr, MAF group. See footnote *c* for definitions of the other abbreviations in this column.
[f] *C. minutissimum* or *C. jeikeium* (differentiated by cultural properties).
[g] *C. minutissimum* or *C. xerosis* (*C. xerosis* is nitrate reduction positive according to CDC.)
[h] *C. striatum* or CDC group G2 (differentiated by cultural properties).
[i] *C. minutissimum* or *C. jeikeium* (differentiated by cultural properties).
[j] *C. minutissimum* scoring esculin positive.

The supernatants were kept on ice throughout this procedure. The Bio-Rad protein assay (Bio-Rad, Paris, France) was used to determine protein concentrations. Extracts were stored frozen at −20°C until use.

(ii) **Electrophoresis.** Samples were heated at 100°C for 5 min with an equal volume of denaturing buffer (0.35 M Tris buffer [pH 6.8] containing 2% SDS, 10% glycerol, and 1.6% 1,4-dithiothreitol). Discontinuous gels were prepared as described by Laemmli (17), with 12% (wt/vol) polyacrylamide used for the separating gel. Wells were loaded with 25 to 30 μg of protein. A Protean II cell apparatus (Bio-Rad) was used for electrophoresis (at a constant voltage of 155 V for the stacking gel and 205 V for the separating gel) until the marker dye (bromophenol blue) had migrated 10 cm. Gels were then stained with Coomassie blue.

Protein patterns were analyzed with a DESAGA CD60 densitometer (DESAGA, Heidelberg, Federal Republic of Germany), and clusters were obtained by computer analysis of the raw data. Briefly, densitometric values were obtained from each protein profile, and the resulting matrix was analyzed by the clustering algorithm described previously (2).

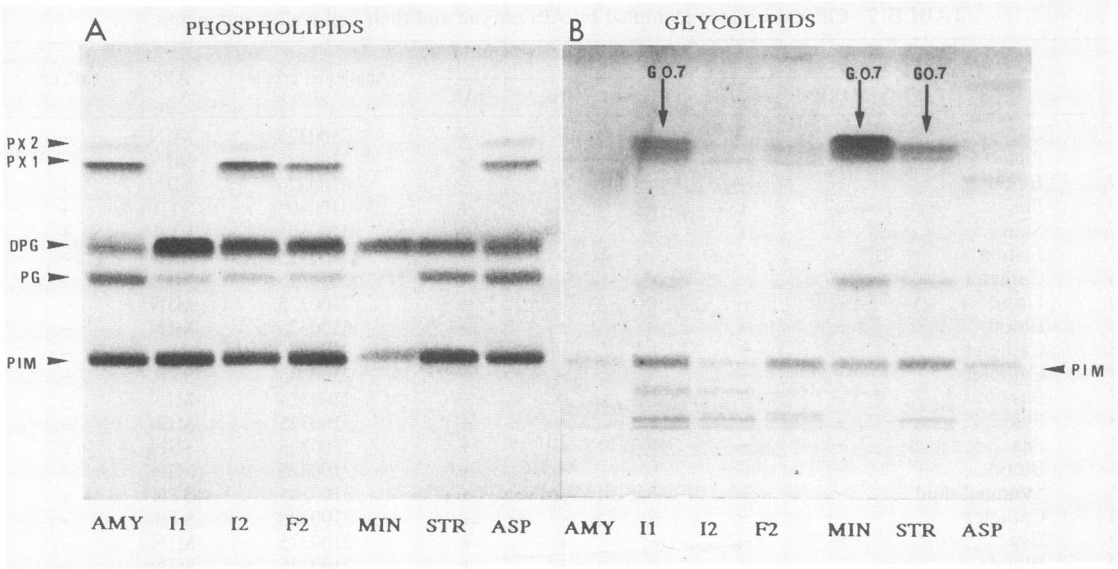

FIG. 1. Analysis of polar lipids by thin-layer chromatography. (A) Specific staining of phosphate esters. (B) Specific staining of glycolipids. PX2 and PX1, unknown phospholipids. PX2 was often weakly labeled (see fig. 2). PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PIM, phosphatidylinositol mannosides. Arrows show the glycolipid with an R_f of 0.7. AMY, *C. amycolatum*; I2, I1, and F2, CDC groups I2, I1, and F2; MIN, *C. minutissimum*; STR, *C. striatum*; ASP, "*C. asperum*."

RESULTS AND DISCUSSION

Phenotypic characteristics. All 42 clinical isolates examined in the present study shared the same cultural and morphological features. There were facultatively anaerobic, gram-positive coryneform bacteria which were not acid fast. They grew well on the usual nutrient agars (BHI or Columbia) between 35 and 37°C and produced raised, opaque, creamyish colonies when they were grown on BHI agar. All the isolates were urease negative.

With the API Coryne system, 31 of 42 of the clinical isolates were classified as *C. minutissimum*, 9 of 42 were classified as *C. striatum*, and 2 of 42 were classified as CDC group I. These results were obtained by following the manufacturer's instructions exactly. Some of the numeric profiles were open to more than one interpretation. The questionable reactions and the reasons for the definitive choices are given in Tables 1 and 2. It should be noted that CDC groups I2 and F2 are not covered by the API Coryne system.

Chemotaxonomic data. Screening for wall chemotype IV (i.e., *meso*-di-aminopimelic acid, arabinose, and mycolic acids) showed 26 clinical isolates and 10 reference strains to be mycolic acid-free (MAF). The other reference strains and 16 clinical isolates contained conventional cell wall constituents (7) (Table 1). These latter isolates were identified correctly by the API system (11 *C. minutissimun* and 5 *C. striatum*); the identification of each of these strains was confirmed by comparing their total protein patterns with those of the *C. minutissimum* and *C. striatum* type strains.

Polar lipids identical to phosphatidylglycerol, diphosphatidylglycerol (DPG), and phosphatidylinositol mannosides were common to all clinical isolates and reference strains. However, all bacteria that belonged to the MAF group were easily differentiated by (i) one or two unknown phospholipids that migrated faster than DPG and (ii) the absence of a major glycolipid with an R_f of 0.7 detected only in bacteria with mycolic acids (Fig. 1). The polar lipid patterns did not allow differentiation between MAF reference strains, but supported the fact that clinical isolates were members of the MAF group (Fig. 2).

These findings from polar lipid analysis confirm the importance of these molecules as chemotaxonomic markers, as emphasized previously (1, 18).

With the API Coryne system, 20 of the 26 MAF clinical isolates were identified as *C. minutissimum*, 4 were identified as *C. striatum*, and 2 were identified as CDC group I. The MAF reference strains, however, were classified in CDC group I or F (Table 1).

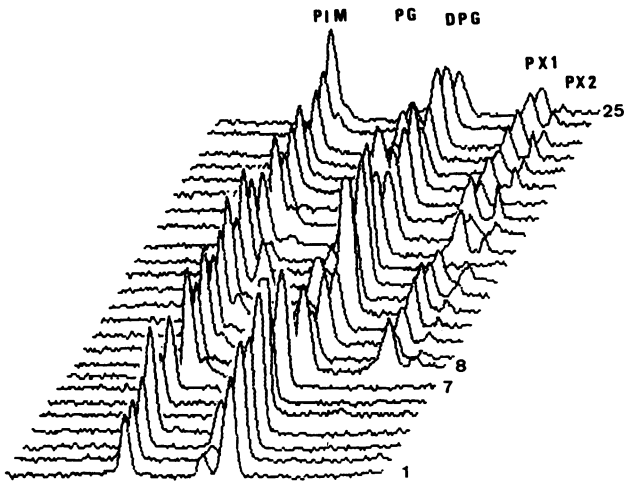

FIG. 2. Scanning of thin-layer chromatograms stained for phospholipids. Chromatograms 1 to 7, clinical strains containing mycolic acids; chromatograms 8 to 25, clinical strains containing no mycolic acids. From left to right, the peaks are phosphatidylinositol mannosides (PIM), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and two unknown phospholipid (PX1 and PX2).

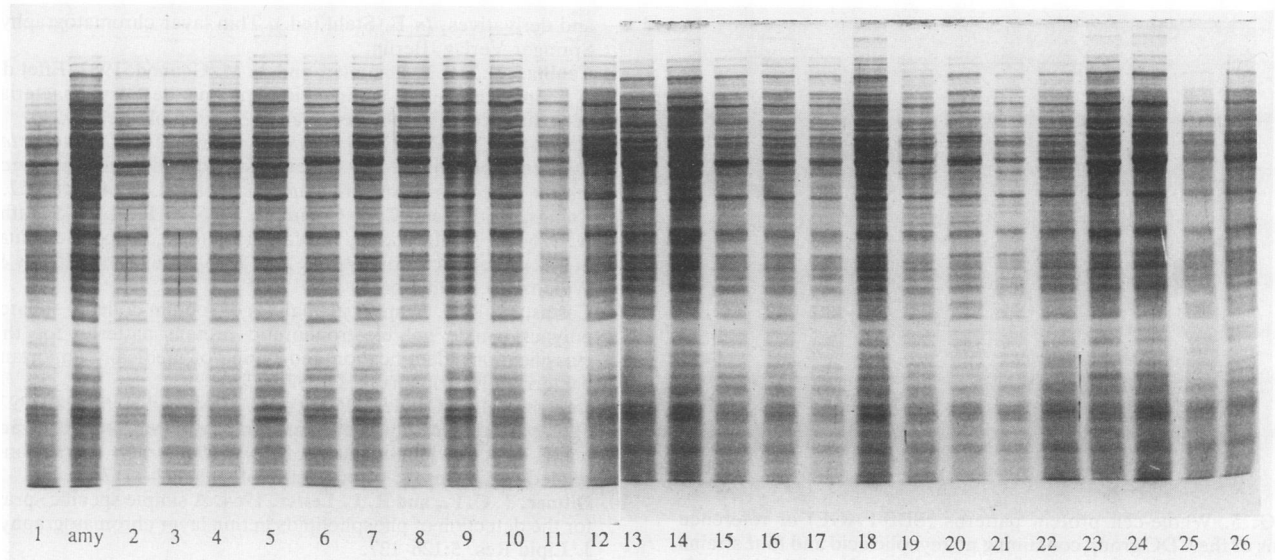

FIG. 3. Whole-cell protein patterns (SDS-PAGE) of 26 MAF clinical strains and the type strain *C. amycolatum*. Lanes: amy, *C. amycolatum*; 1 to 26, clinical strains 169.87, 356.87, 360.88, 559.88, 588.88, 619.88, 637.88, 740.88, 814.88, 082.89, 269.89, 350.89, 379.89, 551.89, 565.89, 580.89, 584.89, 698.89, 336.90, 416.90, 565.90, 598.90, 808.90, 080.91, 094.91, and 131.91, respectively (see Table 2).

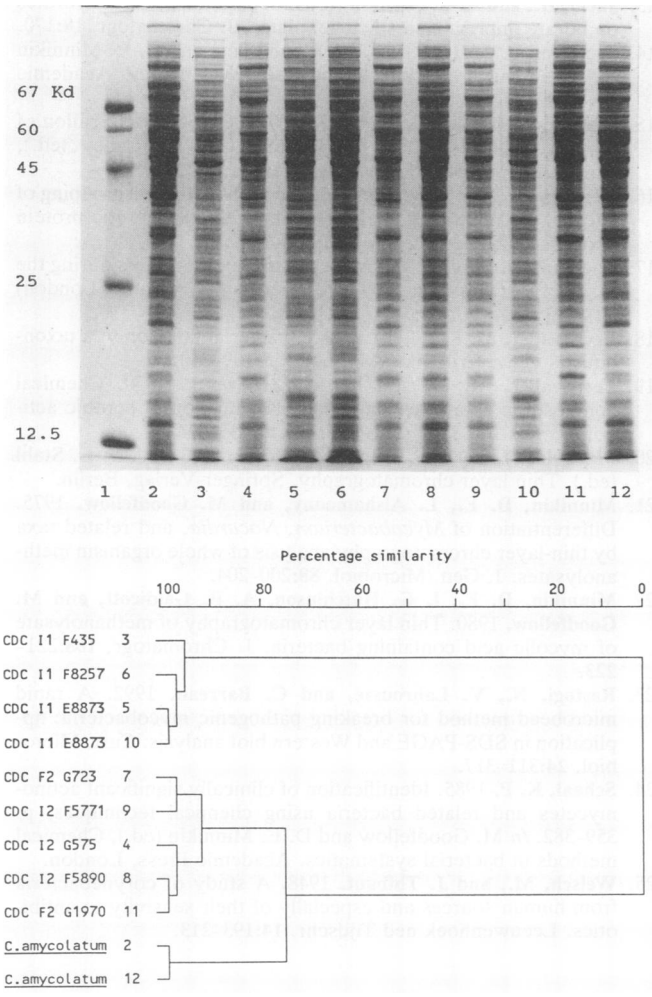

FIG. 4. Numerical analysis of whole-cell protein patterns (SDS-PAGE) of reference strains containing mycolic acids compared with those of reference strains containing no mycolic acid. The dendrogram is of similarities obtained by using chi-square coefficients calculated between absorbance profiles.

All the MAF clinical isolates had very similar patterns of total proteins by SDS-PAGE (Fig. 3). This SDS-PAGE profile was similar to that of *C. amycolatum*.

SDS-PAGE protein profile analysis is considered to be one of the most accurate methods for bacterial identification. Many investigators consider this approach to be simpler than and as reliable as hybridization analysis (8, 14, 16). Nevertheless, only DNA hybridization can determine whether the MAF isolates indeed all belong to the same taxon, because this is the reference technique for determination of a species.

Cluster analysis of SDS-PAGE profiles showed that coryneforms devoid of mycolic acids and those which contained mycolates belonged to two unrelated clusters (Fig. 4). *C. amycolatum* fell in the same cluster as the MAF strains. Its protein profile was almost identical to those of "*C. asperum*" and F2 or I2 CDC group isolates (Fig. 5). Further preliminary classification analysis suggested that there are probably subgroups within the MAF group.

The four strains of "*C. asperum*" shared the same chemotaxonomic properties. This species was described by Welsch and Thibaut in 1948 (25) and has always been conserved by CIP. Diphtheroid bacteria named "*C. asperum*" have been found in the human upper respiratory tract. On the basis of morphology, fermentative ability, and susceptibility to antibiotics, it was suggested that these bacteria form a distinct group. However, they have different sugar fermentative abilities, for example, to maltose. The phenotypes of these strain are similar to those of the clinical isolates examined in the present study.

Both *C. amycolatum* and "*C. asperum*" were identified as CDC group F or *C. minutissimum* by the API Coryne system (Table 2).

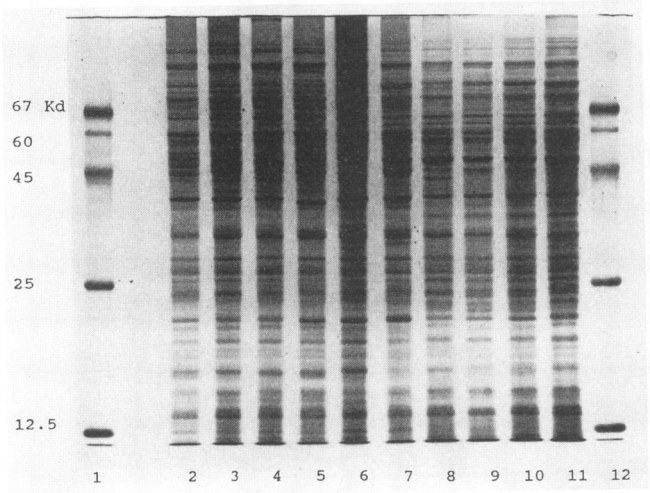

FIG. 5. Whole-cell protein patterns (SDS-PAGE) of reference strains of the CDC group containing no mycolic acid and four strains of "*C. asperum*." Lanes 1 and 12, molecular mass standard; lane 2, CDC group I2 strain F5890; lane 3, CDC group I2 strain F5771; lane 4, CDC group F2 strain G675; lane 5, CDC group F2 strain G723; lane 6, CDC group F2 strain G1970; lane 7, *C. amycolatum* 103452; lane 8, "*C. asperum*" 100836; lane 9, "*C. asperum*" 80.54; lane 10, "*C. asperum*" 79.37; lane 11, "*C. asperum*" 52.13.

In conclusion, our findings confirm and complete some recently published observations (9) that mycolic acids are not present in isolates described as coryneform bacteria.

Although the present study included a relatively small number of clinical isolates, it seems probable that many bacteria isolated from humans and identified as *C. minutissimum* or *C. striatum* by routine identification tests could belong to a group closely related to *C. amycolatum*, "*C. asperum*", or coryneform CDC group I2 or F2.

A simple test for mycolic acid could thus be sufficient to validate API Coryne test results indicating *C. minutissimum* or *C. striatum*. Such a test involves thin-layer chromatography but requires only standard equipment and could at least be performed in reference laboratories.

The results of the present study also demonstrate the necessity of detailed chemotaxonomic studies in the field of "diphtheroid" bacteria and suggest that further DNA-DNA hybridization studies are required to confirm the relationship between "*C. asperum*," *C. amycolatum*, and CDC groups F2 and I2.

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