Propionic Acid-Producing Strains Previously Designated as *Corynebacterium xerosis*, *C. minutissimum*, *C. striatum*, and CDC Group I₂ and Group F₂ Coryneforms Belong to the Species *Corynebacterium amycolatum*

G. WAUTERS,^{1*} A. DRIESSEN,¹ E. AGERON,² M. JANSSENS,¹ AND P. A. D. GRIMONT²

Microbiology Unit, University of Louvain, Brussels, Belgium,¹ and Unité des Entérobactéries, INSERM U389, Institut Pasteur, Paris, France²

Propionic acid-producing *Corynebacterium* strains that lacked mycolic acids and were formerly identified as *Corynebacterium minutissimum*, *Corynebacterium xerosis*, *Corynebacterium striatum*, and CDC group I₂ and F₂ strains were studied to determine their relatedness to *Corynebacterium amycolatum*. A total of 60 strains were used for phenotypic characterization studies, and 26 of these strains were used for genetic studies. DNA-DNA hybridization experiments performed at 65°C revealed that the levels of relatedness between the propionic acid-producing strains and the type strain of *C. amycolatum* were more than 70% and that the ΔT_m values ranged from 0 to 5°C (ΔT_m is the difference between the denaturation temperature of a homoduplex and the denaturation temperature of a heteroduplex); these values are consistent with inclusion of these strains in the species *C. amycolatum*. Currently used conventional tests, such as urease, nitrate reduction, and sugar fermentation tests, were not suitable for accurate identification of *C. amycolatum*. Phenotypic differentiation of this species from related taxa should be based on the following characteristics in addition to propionic acid production: lipid requirement, Tween esterase activity, tyrosine clearing, alkaline phosphatase activity, α -glucosidase activity, and β -glucuronidase activity.

Corynebacterium amycolatum has been described as a mycolic acid-less *Corynebacterium* species that exhibits variability in its biochemical properties, especially urease activity and fermentation of sucrose, maltose, and trehalose (6). The various biochemical profiles of *C. amycolatum* overlap those of *Corynebacterium xerosis*, *Corynebacterium striatum*, and *Corynebacterium minutissimum*, as well as those of coryneform group I₂ and F₂ strains described by Hollis and Weaver (12). It has been suggested that *C. amycolatum* and strains lacking mycolic acids and identified as members of *C. minutissimum*, *C. striatum*, and CDC groups I₂ and F₂ are related on the basis of the results of chemotaxonomic studies (1).

It has been proposed that the volatile fatty acids produced from glucose can be used to improve the identification of corynebacteria (16). Some strains that have the characteristics of *C. minutissimum* produce propionic acid, while other strains, including the type strain of *C. minutissimum*, do not (9). Similarly, most strains currently identified as *C. xerosis* strains and some strains identified as *C. striatum* strains produce propionic acid, but the type strains of these species do not produce propionic acid. Other strains that produce propionic acid are members of *Corynebacterium diphtheriae*, *Corynebacterium kutscheri*, CDC groups I₂ and F₂, and the recently described species *Corynebacterium glucuronolyticum* (10) and *Corynebacterium seminale* (17). The type strain of *C. amycolatum* also produces propionic acid.

The purpose of this study was to examine the relationship between *C. amycolatum* and mycolateless propionic acid-producing (PAP) coryneform bacteria that have a cellular fatty acid pattern consistent with the pattern found in the genus *Corynebacterium*. Although PAP strains identified as *C. minutissimum*, *C. striatum*, *C. xerosis*, and CDC group I₂ and F₂

strains that were isolated from human sources differ phenotypically in some characteristics, as shown by the results of conventional tests, all of these organisms lack mycolates and have many common cultural, biochemical, and chemotaxonomic characteristics. DNA relatedness studies revealed that all of these organisms are members of *C. amycolatum*.

MATERIALS AND METHODS

Bacterial strains. A total of 60 mycolateless PAP strains obtained from various clinical sources were tested to determine their phenotypic characteristics. The organisms were presumptively identified by using previously described methods (5, 12). The strains which we used and their origins are shown in Table 1.

Reference strains of the most relevant *Corynebacterium* species found in humans, including the type strain of *C. amycolatum*, were compared with the study strains.

Growth conditions. A requirement for lipid was assessed by comparing growth on tryptic soy agar containing 1% Tween 80 and growth on tryptic soy agar lacking Tween 80.

Biochemical properties. Catalase, oxidase, carbohydrate fermentation, pyrazinamidase, indole production, urea hydrolysis, nitrate reduction, gelatin hydrolysis, and esculin hydrolysis tests were performed by conventional methods (15). Tween esterase was detected by the method of Sierra (18). The tyrosine clearing test was performed on nutrient agar containing 0.5% (wt/vol) L-tyrosine. Alkaline phosphatase, pyrrolidonyl arylamidase, α -glucosidase, β -glucosidase, α -galactosidase, β -galactosidase, and β -glucuronidase tests were performed by using diagnostic tablets (Rosco Diatabs, Taastrup, Denmark). An identification analysis was performed in parallel by using a commercial API Coryne kit (API System bioMérieux, Marcy l'Etoile, France). API 50CH panels (API System bioMérieux) were used to test acid production from a large number of carbohydrates.

Production of propionic acid was detected after 48 h of growth in glucose broth by gas-liquid chromatography, using a model IGC 121 DFL chromatograph (Intersmat, Brussels, Belgium), and a packed glass column; the solid phase was SP 1000 obtained from Chrompack International, Middelburg, The Netherlands.

Cell wall fatty acids were extracted by the method of Lambert and Moss (13) from cells grown for 48 h on brain heart blood agar. The fatty acid analysis was performed at 45 to 210°C by using the capillary column of a Delsi DI 200 chromatograph (Intersmat) equipped with split-splitless injection and a flame ionization detector. The results were expressed as relative percentages by using an Enica 31 integrator obtained from Delsi Nermag Instruments (Intersmat).

Mycolic acids were detected by thin-layer chromatography as described by Minnikin et al. (14).

DNA-DNA hybridization. The cells used for DNA extraction were grown in

*Corresponding author. Mailing address: Microbiology Unit, U.C.L./ 5490, avenue Hippocrate, 54, B-1200 Brussels, Belgium. Phone: 32 2 7645490. Fax: 32 2 7649440.

TABLE 1. PAP strains used in this study

Strain	API code	Former taxon	Origin
22	2 100 325	<i>C. minutissimum</i>	Blood
30	2 100 325	<i>C. minutissimum</i>	Blood
116	2 100 104	<i>C. minutissimum</i>	Ear
197	2 100 324	<i>C. minutissimum</i>	Urethra
199	2 100 324	<i>C. minutissimum</i>	Wound
200	2 100 324	<i>C. minutissimum</i>	Unknown
208	2 100 324	<i>C. minutissimum</i>	Wound
215	2 100 324	<i>C. minutissimum</i>	Blood
223	2 100 324	<i>C. minutissimum</i>	Ear
244	2 100 324	<i>C. minutissimum</i>	Vaginal swab
281	2 100 324	<i>C. minutissimum</i>	Urine
283	2 100 324	<i>C. minutissimum</i>	Skin
292	2 100 324	<i>C. minutissimum</i>	Urethra
295	2 100 324	<i>C. minutissimum</i>	Wound
299	2 100 324	<i>C. minutissimum</i>	Wound
303	2 100 324	<i>C. minutissimum</i>	Throat
308	2 100 324	<i>C. minutissimum</i>	Throat
310	2 100 324	<i>C. minutissimum</i>	Ear
353	2 100 325	<i>C. minutissimum</i>	Peritoneal fluid
378	2 100 324	<i>C. minutissimum</i>	Ear
379	2 100 324	<i>C. minutissimum</i>	Ear
181	3 100 324	CDC group I ₂	Pus
227	3 100 324	CDC group I ₂	Unknown
240	3 100 324	CDC group I ₂	Blood
245	3 100 324	CDC group I ₂	Skin graft
273	3 100 324	CDC group I ₂	Skin
275	3 110 324	CDC group I ₂	Skin
291	3 100 325	CDC group I ₂	Pus
297	3 100 324	CDC group I ₂	Genital swab
298	3 100 324	CDC group I ₂	Genital swab
306	3 100 324	CDC group I ₂	Throat
311	3 100 324	CDC group I ₂	Throat
326	3 100 324	CDC group I ₂	Nose
400	3 100 324	CDC group I ₂	Urine
409	3 110 304	CDC group I ₂	Blood
213	3 100 325	<i>C. xerosis</i>	Pus, eye
224	3 100 325	<i>C. xerosis</i>	Urine
231	3 100 325	<i>C. xerosis</i>	Bile
234	3 100 325	<i>C. xerosis</i>	Urine
281	3 100 325	<i>C. xerosis</i>	Pus
289	3 100 325	<i>C. xerosis</i>	Blood
290	3 100 325	<i>C. xerosis</i>	Blood
293	3 100 325	<i>C. xerosis</i>	Blood
321	3 100 325	<i>C. xerosis</i>	Wound
330	3 100 325	<i>C. xerosis</i>	Unknown
219	3 100 305	<i>C. striatum</i>	Blood
229	3 100 105	<i>C. striatum</i>	Urine
232	3 100 305	<i>C. striatum</i>	Bursite
233	3 100 325	<i>C. striatum</i>	Wound
284	3 100 105	<i>C. striatum</i>	Skin
294	3 100 305	<i>C. striatum</i>	Wound
319	3 100 305	<i>C. striatum</i>	Blood
337	3 100 305	<i>C. striatum</i>	Skin
382	3 100 305	<i>C. striatum</i>	Catheter
272	2 101 324	CDC group F ₂	Skin
333	2 101 124	CDC group F ₂	Ear
28	2 100 305	NI ^a	Peritoneal fluid
84	2 100 305	NI ^a	Pus
296	2 100 305	NI ^a	Ear
309	2 100 105	NI ^a	Ear

^a NI, not identified (strain that has the biochemical profile of *C. minutissimum* but is maltose negative).

brain heart infusion (Difco) supplemented with 0.1% (vol/vol) Tween 80 at 37°C with shaking for 2 days. The cells were washed twice in 50 mM Tris buffer and then resuspended in 15 ml of a buffer containing 50 mM Tris and 100 mM sucrose. Lysozyme (60 mg) was added, and the mixture was incubated at 37°C overnight. Then 1 ml of a 2% (wt/vol) sodium dodecyl sulfate solution and 100

μl of a proteinase K solution (10 mg/ml) were added, and the mixture was incubated at 60°C for 2 h. DNA was purified as described elsewhere (4). The exact procedure used for in vitro labeling of DNA with tritium-labeled nucleotides and for hybridization experiments (S1 nuclease-trichloroacetic acid procedure) have been described previously (11). The temperature at which 50% of a reassociated DNA became hydrolyzable by S1 nuclease (T_m) was determined as described by Crosa et al. (8). ΔT_m (the difference between the T_m of a homoduplex and the T_m of a heteroduplex) is an estimate of the level of divergence between two DNAs (3).

Antibiotic susceptibility. Antibiotic-susceptibility was determined by the disk diffusion method.

RESULTS

Morphological and cultural characteristics. The PAP strains that had the biochemical profiles of *C. minutissimum*, *C. xerosis*, *C. striatum*, and CDC groups I₂ and F₂ and the type strain of *C. amycolatum* had similar cell and colony morphologies. Gram staining revealed short, somewhat granular, gram-positive rods in typical V-shaped and palisade arrangements. The colony sizes were quite irregular, ranging from 0.3 to 1.5 mm after 24 h of incubation on blood agar. The colonies were grayish and had a characteristic dry, waxy appearance, whereas the colonies of true *C. minutissimum* and *C. striatum* strains glistened. The type strain of *C. xerosis* also produced dry colonies, but the colonies were yellowish. None of the strains had a requirement for lipid.

Biochemical properties. The strains were catalase positive and oxidase negative. Glucose was fermented with the production of acetic acid and a large amount of propionic acid by the strains which we studied, as well as *C. amycolatum*. As determined with API 50CH panels, all of the strains produced acid from D-glucose, D-fructose, D-mannose, glycerol, and D-ribose. The results were variable for galactose, maltose, sucrose, and trehalose, and acid was not produced from L-arabinose, ribitol, galactitol, cellobiose, D-fucose, mannitol, L-sorbose, D-glucitol, D-xylose, L-rhamnose, *myo*-inositol, *N*-acetyl-D-glucosamine, α-methyl-D-glucoside, amygdalin, salicin, lactose, turanose, and melibiose. Esculin was not hydrolyzed.

All of the strains were negative for indole production, gelatin hydrolysis, Tween 80 esterase activity, tyrosine clearing, pyrrolidonyl arylamidase activity, α-galactosidase activity, β-galactosidase activity, β-glucosidase activity, and β-glucuronidase activity. The α-glucosidase test was negative for all of the strains except strains 275 and 409, for which the test was strongly positive as determined by both the API Coryne method and the Rosco Diatabs method. The tests for pyrazinamidase activity, starch hydrolysis, and alkaline phosphatase activity were positive. Variable results were obtained in the tests for urease activity and nitrate reduction. The results of sucrose and maltose fermentation, nitrate reduction, and urease tests allowed us to presumptively place the strains in the following species or groups: 21 strains in *C. minutissimum*, 10 strains in *C. xerosis*, 9 strains in *C. striatum*, 14 strains in CDC group I₂, and 2 strains in CDC group F₂. Four strains could not be placed in any known species since they exhibited the profile of *C. minutissimum* but were maltose negative. The type strain of *C. amycolatum* had biochemical characteristics that were consistent with previous descriptions. This strain fit the profile of CDC group F₂, but, as noted previously, strains of *C. amycolatum* may have different biochemical profiles (1, 6).

The presumptive identities determined by the API Coryne system and by conventional tests were the same for all but six strains. Strain 116 was identified as a member of *Corynebacterium jeikeium* and strains 28, 84, and 296 were identified as CDC group G strains by the API Coryne system. However, these identities were not consistent with the lack of a requirement for lipid or serum by these four strains. Strains 291 and

TABLE 2. Cellular fatty acid composition of 50 mycolateless PAP *Corynebacterium* strains

Fatty acid	%	
	Mean	Range
C _{14:0}	1.27	<1-4
C _{15:0}	2.8	<1-12
C _{16:0}	29.94	15-62
C _{16:1}	2.29	<1-9
C _{17:0}	8.11	<1-16
C _{17:1}	6.65	<1-22
C _{18:0}	9.08	3-21
C _{18:1} ^{9c}	28.72	11-47
C _{18:2}	9.34	1-22
C _{18:0} + C _{18:2}	18.42	5-31

233 were both identified as *C. xerosis* strains by the API Coryne system.

Cellular fatty acid compositions. The fatty acid patterns of the strains determined by gas-liquid chromatography revealed that these organisms contained straight-chain saturated and unsaturated fatty acids, which is consistent with the description of the genus *Corynebacterium* (2). No tuberculostearic acid was present. The quantitative fatty acid compositions of 50 PAP strains were determined, and the results are shown in Table 2.

Mycolic acids. The 60 strains which we studied lacked mycolic acids, in contrast to the type strains of *C. minutissimum*, *C. striatum*, *C. xerosis*, and *Corynebacterium pseudodiphtheriticum*, which were used as positive controls.

DNA-DNA hybridization. The relative levels of reassociation between unlabeled DNAs from *Corynebacterium* species and labeled DNAs from strains 22 and 319 and *C. amycolatum* CIP 103452^T (T = type strain) are shown in Table 3. The levels of relatedness between strains 22 and 319 and the type strains of *C. minutissimum* and *C. striatum* ranged from 1 to 17%. The levels of relatedness among strains 22, 319, and CIP 103452^T ranged from 72 to 87%, with ΔT_m values between 2.0 and 4.0°C. Thus, these three strains represent a single DNA relatedness group (20). The 4 strains designated PAP *C. minutissimum*, the 5 strains designated PAP *C. xerosis*, the 3 strains designated PAP *C. striatum*, the 10 strains designated PAP CDC group I₂, and the 2 strains designated PAP CDC group F₂ exhibited levels of relatedness to one, two, or three of the reference strains (strains 22, 319, and CIP 103452^T) of more than 70%, with ΔT_m values of less than 5°C. The reassociation values were less than 70% with only the following six pairs of strains: strains 275 and 22, 197 and 319, 181 and 319, 272 and 319, 213 and 103452, and 293 and 103452. These strains were associated with higher levels of reassociation (>70%) with labeled DNAs of the other reference strains and with ΔT_m values of less than 5°C. Thus, the six low values which we obtained (levels of reassociation, 58 to 69%) should be considered experimental artifacts.

Susceptibility of strains to antibiotics. A total of 19 of 54 strains were resistant to ampicillin, whereas the true *C. striatum* and *C. minutissimum* strains (15 and 16 strains, respectively) were susceptible to this antibiotic. Some of the strains which we studied were resistant to several antibiotics.

DISCUSSION

As shown by the DNA-DNA hybridization results, the 60 PAP mycolateless *Corynebacterium* strains that fit the biochemical profiles of *C. minutissimum*, *C. xerosis*, *C. striatum*, CDC group I₂, and CDC group F₂ exhibit high levels of DNA

TABLE 3. Levels of DNA-DNA hybridization of *Corynebacterium* strains at 65°C

Source of unlabeled DNA	% Relatedness with labeled DNA from:		
	Strain 22	Strain 319	Strain CIP 103452 ^T
PAP <i>C. minutissimum</i> strains			
22	100 (0.0) ^a	73 (2.5)	87 (4.0)
197	95	69	92 (3.0)
215	91 (0.0)	73	83 (5.0)
292	88	73	81 (3.0)
PAP <i>C. xerosis</i> strains			
213	87	70 (3.0)	68 (3.0)
231	87 (2.0)	93	77
281	92	75	92 (1.0)
290	80	91	100 (3.0)
293	85	88	67 (3.6)
PAP <i>C. striatum</i> strains			
219	83 (1.5)	91	84 (4.0)
232	95	96	100 (3.0)
319	78	100 (0.0)	86 (2.0)
PAP CDC group I ₂ strains			
181	74 (1.0)	58 (2.4)	ND ^b
240	81	68	86 (4.0)
245	93	68 (4.7)	77 (4.0)
275	62 (1)	70 (2.5)	86 (1.0)
291	74 (0.0)	83 (0.0)	96 (3.0)
297	83	76 (2.5)	94 (4.0)
298	82	70 (0.0)	89 (4.0)
306	80 (1.6)	73 (3.2)	87 (5.0)
311	88	72	68 (4.0)
326	95	75	73 (5.0)
PAP CDC group F ₂ strains			
272	81	62	83 (3.0)
333	72 (3.0)	ND	81 (0.0)
Unidentified PAP strains			
28	97	75	94 (3.0)
84	97	70	95 (4.0)
<i>C. amycolatum</i> CIP 103452 ^T	72 (3.6)	75	100 (0.0)
<i>C. striatum</i> ATCC 6940 ^T	17	1	
<i>C. minutissimum</i> ATCC 23348 ^T	6	8	
<i>C. minutissimum</i> NCTC 10284	5	3	
<i>C. xerosis</i> CIP 100653 ^T	17	12	
<i>C. diphtheriae</i> ATCC 27010 ^T	7	4	
<i>C. kitchneri</i> ATCC 15677 ^T	2	3	
<i>C. renale</i> NCTC 7448 ^T	5	1	
<i>C. pseudodiphtheriticum</i> ATCC 7091 ^T	5	2	
<i>C. urealyticum</i> ATCC 43042 ^T	12	11	
<i>C. urealyticum</i> CIP 103425	18	ND	
<i>C. jeikeium</i> 21	11	6	
CDC group G ₂ strain 265	1	1	

^a The values in parentheses are ΔT_m values (in degrees Celsius).

^b ND, not done.

complementarity with each other and with the type strain of *C. amycolatum*, and the ΔT_m values range from 0 to 5°C. Therefore, all of these strains should be included in the species *C. amycolatum*. The ΔT_m values between 3 and 5°C suggest that there is some genomic heterogeneity within the *C. amycolatum* genomospecies. Previously, Barreau et al. (1) demonstrated, on the basis of the results of whole-cell protein electrophoresis, whole-cell sugar analysis, and cell wall component studies, that *C. amycolatum*, "*Corynebacterium asperum*," some strains of *C. minutissimum*, *C. striatum*, CDC group I₂, and CDC group F₂ were closely related. These authors did not include strains identified as *C. xerosis* in their mycolic acid-less isolates, while in our study, all of the strains identified as *C. xerosis* by con-

TABLE 4. Differential characteristics of *C. amycolatum* and other nonlipophilic fermenting corynebacteria isolated from humans

Species	No. of strains	Propionic acid production	Urease activity	Pyrazinamidase activity	Nitrate reduction	Tween 80 esterase activity	Tyrosine clearing	Alkaline phosphatase activity	β -Glucuronidase activity	α -Glucosidase activity	Acid production from:			
											Glucose	Sucrose	Maltose	Galactose
<i>C. amycolatum</i>	60	+	v	+	v	—	—	+	—	v	+	v	v	v
<i>C. diphtheriae</i>	13	+	—	—	v	v	—	—	—	+	+	—	+	v
<i>C. xerosis</i> ^b		—	—	+	+	—	—	+	—	+	+	+	+	+
<i>C. minutissimum</i>	16	—	—	+	—	+	+	+	—	—	+	v	+	—
<i>C. striatum</i>	15	—	—	+	+	+	+	+	—	—	+	v	—	+
<i>C. glucuronolyticum</i> ^d		+	v	+	v	ND	ND	v	+	v	+	+	v	ND

^a +, positive reaction; —, negative reaction; v, variable reaction; ND, not determined.

^b Data for the type strain.

^c Very weak positive reaction obtained with Rosco tablets.

^d Data from reference 10.

ventional methods produced propionic acid and were devoid of mycolates. Indeed, we did not find any *C. xerosis* strain that had the characteristics of type strain CIP 100653. The genetic heterogeneity of *C. xerosis* strains was discussed in a recent study by Coyle et al. (7).

In a previous study Van Bosterhaut et al. described the isolation of three PAP *C. minutissimum* strains from highly relevant clinical samples (19); these strains (strains 22, 28, and 215) were included in our study. These organisms lack mycolic acids and belong to the species *C. amycolatum*, as shown by DNA-DNA hybridization data. It is obvious that the strains that exhibit the PAP *C. minutissimum* profile are not genetically related to the type strain of *C. minutissimum*; similarly, the PAP *C. xerosis* strains are not related to the type strain of *C. xerosis*, and the PAP *C. striatum* strains are not related to the type strain of *C. striatum*.

Current conventional identification tests, which are based mainly on sugar fermentation, urease activity, and nitrate reduction data, do not result in accurate identification of *C. amycolatum*. Moreover, this species is not included in the API Coryne database. Therefore, it could be foreseen that *C. amycolatum* strains could be misidentified as *C. minutissimum*, *C. xerosis*, *C. striatum*, CDC group F₂, and CDC group I₂ strains.

If propionic acid production and chemotaxonomic characteristics, such as the absence of mycolic acids and the presence of specific cell wall fatty acids, are not used, *C. amycolatum* can be distinguished from other fermenting corynebacteria by the following phenotypic characteristics: from *C. diphtheriae* by positive pyrazinamidase and alkaline phosphatase tests; from *C. minutissimum* and *C. striatum* by the absence of Tween esterase activity and tyrosine decomposition; from *C. xerosis* by the lack of α -glucosidase activity in the majority of the strains; from *C. glucuronolyticum* and *C. seminale* by the absence of β -glucuronidase activity; from *C. jeikeium* and other lipophilic species by the lack of a lipid requirement; and from most of these species by a lack of Tween esterase activity. Propionibacteria also produce propionic acid, but their cellular fatty acids are mainly branched fatty acids. Moreover, although some strains grow aerobically, their growth is enhanced by anaerobic conditions. Characteristics which differentiate *C. amycolatum* and the main fermenting, nonlipophilic *Corynebacterium* species of human origin are shown in Table 4.

Our results confirm that the mycolic acid-less corynebacteria are related to *C. amycolatum*, as suggested by Barreau et al. (1). Our results also demonstrate the usefulness of detection of propionic acid production by corynebacteria as proposed by Estrangin et al. (9). Indeed, *C. amycolatum* includes the vast majority of the nonlipophilic PAP corynebacteria of human origin other than *C. diphtheriae*.

ACKNOWLEDGMENT

We are grateful to V. Avesani for help with the determinations of fatty acid profiles.

REFERENCES

1. Barreau, C., F. Bimet, M. Kiredjian, N. Rouillon, and C. Bizet. 1993. Comparative chemotaxonomic studies of mycolic acid-free coryneform bacteria of human origin. *J. Clin. Microbiol.* **31**:2085–2090.
2. Bernard, K. A., M. Bellefeuille, and E. P. Ewan. 1991. Cellular fatty acid composition as an adjunct to the identification of asporogenous, aerobic, gram-positive rods. *J. Clin. Microbiol.* **29**:83–89.
3. Brenner, D. J. 1978. Characterization and clinical identification of *Enterobacteriaceae* by DNA hybridization. *Prog. Clin. Pathol.* **7**:71–117.
4. Brenner, D. J., A. C. McWhorter, J. K. Leete Knudson, and A. G. Steigerwalt. 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J. Clin. Microbiol.* **15**:1133–1140.
5. Clarridge, J. E., and C. A. Spiegel. 1995. *Corynebacterium* and miscellaneous irregular gram-positive rods, *Erysipelothrix*, and *Gardnerella*, p. 357–378. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. G. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
6. Collins, M. D., R. A. Burton, and D. Jones. 1988. *Corynebacterium amycolatum* sp. nov., a new mycolic acid-less *Corynebacterium* species from human skin. *FEMS Microbiol. Lett.* **49**:349–352.
7. Coyle, M. B., R. B. Leonard, D. J. Nowowiejski, A. Malknazi, and D. J. Finn. 1993. Evidence of multiple taxa within commercially available reference strains of *Corynebacterium xerosis*. *J. Clin. Microbiol.* **31**:1788–1793.
8. Crosa, J. H., D. J. Brenner, and S. Falkow. 1973. Use of a single-strand-specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J. Bacteriol.* **115**:904–911.
9. Estrangin, E., B. Thiers, and Y. Peloux. 1987. Apport des microméthodes et de l'analyse en chromatographie en phase gazeuse des acides carboxyliques issus de la fermentation du glucose dans l'identification des corynébactéries. *Ann. Biol. Clin.* **45**:285–289.
10. Funke, G., K. A. Bernard, C. Bucher, G. E. Pfyffer, and M. D. Collins. 1995. *Corynebacterium glucuronolyticum* sp. nov. isolated from male patients with genitourinary infections. *Med. Microbiol. Lett.* **4**:204–215.
11. Grimont, P. A. D., M. Y. Popoff, F. Grimont, C. Coynault, and M. Melmin. 1980. Reproducibility and correlation study of three deoxyribonucleic acid hybridization procedures. *Curr. Microbiol.* **4**:325–330.
12. Hollis, D. G., and R. E. Weaver. 1986. Guide for identification of coryneform

- bacteria. Centers for Disease Control, Atlanta.
13. **Lambert, M. A., and C. W. Moss.** 1989. Cellular fatty acid compositions and isoprenoid quinone contents of 23 *Legionella* species. *J. Clin. Microbiol.* **27**:465–473.
 14. **Minnikin, D. E., I. G. Hutchinson, A. B. Galdicott, and M. Goodfellow.** 1980. Thin layer chromatography of methanolysates of mycolic acid containing bacteria. *J. Chromatogr.* **188**:221–223.
 15. **Nash, P., and M. M. Krenz.** 1991. Culture media, p. 1226–1288. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
 16. **Reddy, C. A., and M. Kao.** 1978. Value of acid metabolic products in identification of certain corynebacteria. *J. Clin. Microbiol.* **7**:428–433.
 17. **Riegel, P., R. Romy, D. de Briel, G. Prévost, F. Jehl, F. Bimet, R. Christen, and H. Monteil.** 1995. *Corynebacterium seminale* sp. nov., a new species associated with genital infections in male patients. *J. Clin. Microbiol.* **33**: 2244–2249.
 18. **Sierra, G.** 1957. A simple method for detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **23**:15–22.
 19. **Van Bosterhaut, B., R. Cuvelier, E. Serruys, E. Pouthier, and G. Wauters.** 1992. Three cases of opportunistic infection caused by propionic acid producing *Corynebacterium minutissimum*. *Eur. J. Clin. Microbiol. Infect. Dis.* **11**:628–631.
 20. **Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper.** 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* **37**:463–464.