Journal of Clinical Microbiology, Nov. 1993, p. 2907–2912 Vol. 31, No. 11
0095-1137/93/112907-06$02.00/0
Copyright © 1993, American Society for Microbiology

Characteristics of CDC Group 1 and Group 1-Like Coryneform Bacteria Isolated from Clinical Specimens

GUIDO FUNKE,[1]* GLADYS MARTINETTI LUCCHINI,[1] GABY E. PFYFFER,[1] MARCELLO MARCHIANI,[2] AND ALEXANDER VON GRAEVENITZ[1]

Institute of Medical Microbiology, University of Zürich, Gloriastrasse 32, CH-8028 Zürich,[1] and Microbiology Laboratory, Institute of Botany, University of Neuchâtel, CH-2007 Neuchâtel,[2] Switzerland

Received 1 April 1993/Returned for modification 24 May 1993/Accepted 16 August 1993

Fifteen strains of CDC group 1 coryneform and biochemically similar bacteria were isolated from clinical specimens. Of the 15 strains isolated, 11 were derived from abscesses and purulent lesions, mostly from the upper part of the body, and 3 were grown from blood cultures. Nine strains were associated with mixed anaerobic but no other aerobic flora. Seven strains exhibited the classical biochemical profile of CDC coryneform group 1; however, eight strains were unable to reduce nitrate and were called "group 1-like." Other reactions to differentiate CDC group 1 and group 1-like coryneform rods include alpha-hemolysis on human blood agar, fermentation of adonitol, and the presence of alkaline phosphatase. Fifteen strains showed marked CAMP reactions on different erythrocyte agars. Gas-liquid chromatography of volatile and nonvolatile fatty acids as well as cellular fatty acid patterns and the composition of cell wall components suggest that CDC group 1 and group 1-like coryneform bacteria do not belong to the genus *Corynebacterium* but possibly to the genus *Actinomyces* or *Arcanobacterium*. DNA-DNA hybridization studies revealed that group 1 and group 1-like strains represent different species.

Gram-positive coryneform rods (GPCRs) are frequently encountered in the clinical laboratory but are often regarded as contaminants or as nonpathogenic. In recent years, several reports emphasizing the involvement of GPCRs as pathogens in a variety of clinical conditions have been published (8, 19).

Starting in 1990, we attempted to identify all potentially significant GPCRs isolated in our clinical laboratory in order to establish their role in disease. Using the system for GPCR identification of Hollis and Weaver (15), we found that 15 strains showed biochemical profiles identical or similar (group 1-like) to those of CDC group 1 coryneform bacteria (23). So far, only 21 strains of CDC group 1 coryneform bacteria have been reported (14). We describe here the clinical, biochemical, structural, and molecular characteristics of 15 additional CDC group 1 and group 1-like coryneform bacteria isolated from clinical specimens.

MATERIALS AND METHODS

Strains, media, and growth conditions. Clinical material was cultured aerobically at 37°C with 5% CO_2 on Columbia agar with 5% sheep blood, the same medium with colistin and nalidixic acid, Columbia chocolate agar, and MacConkey agar without CO_2 (all media were from Becton Dickinson Microbiology Systems [BBL], Cockeysville, Md., unless specified otherwise). For anaerobic cultures, brucella agar with 5% sheep blood, kanamycin-vancomycin agar with laked blood, and phenylethyl blood agar plates were used (26). Aerobically growing rods were identified by standard procedures (18). Obligate anaerobes were reported as mixed anaerobic flora if examination of anaerobic plates revealed more than three morphologically different strains.

CDC group 1 coryneform strains 1 to 3 were received from R. E. Weaver, Special Bacteriology Reference Laboratory, Centers for Disease Control and Prevention (CDC), Atlanta, Ga., as reference strains. Strains 4 and 5, initially diagnosed as "atypical CDC coryneform group A-4" (7), were later also shown to be members of CDC coryneform group 1 (23) and were therefore used as reference strains as well. Strains 6 to 20 were isolated in our clinical laboratory between July 1990 and March 1993.

For hybridization studies, the following strains were obtained from the Swiss National Collection of Type Cultures, Lausanne, Switzerland: *Corynebacterium minutissimum* ATCC 23348, *Corynebacterium xerosis* ATCC 373, *Corynebacterium striatum* ATCC 6940, *Arcanobacterium haemolyticum* ATCC 9345, *Actinomyces pyogenes* ATCC 19411, *Actinomyces israelii* ATCC 10048, and *Actinomyces viscosus* LA (Lausanne) 762.

Biochemical profiles. Traditional media used for biochemical characterization were prepared as described by Nash and Krenz (21). Nitrate reduction was tested in nitrate broth (Difco, Detroit, Mich.). Cystine Trypticase agar medium contained 1% carbohydrates and was supplemented with 5% rabbit serum. For adonitol fermentation, we used a basal medium containing 0.5% Bacto beef extract (Difco), 1% Trypticase peptone, 0.5% NaCl, 0.0006% bromothymol blue, and 0.5% adonitol. The commercial API Coryne (RAPID Coryne) and API ZYM (API Biomérieux SA, Marcy l'Etoile, France) systems were used following the manufacturer's instructions. The CAMP reaction (4) was performed on Columbia and Mueller-Hinton agars with 5% sheep blood, human blood, or ox blood with *Staphylococcus aureus* ATCC 25923. Incubation was carried out at 37°C without CO_2 for tests dependent on changes in pH; otherwise, incubation was carried out at 37°C in an atmosphere with 5% CO_2.

Susceptibility to antimicrobial agents. Antimicrobial susceptibility patterns were generated for six antibiotics commonly used in the treatment of infections caused by GPCRs (penicillin G, erythromycin, clindamycin, tetracycline, gen-

* Corresponding author.

TABLE 1. Origins of CDC group 1 and group 1-like coryneform bacteria isolated from clinical specimens

Strain	Patient sex, age[a]	Diagnosis or source	Direct Gram stain[b]	Quantity in primary cultures[c]		
				Coryneforms	Anaerobes[d]	Others
Group 1						
1	NK	Ear	NK	—	—	—
2	NK	Shunt fluid	NK	—	—	—
3	NK	Eye	NK	—	—	—
4	NK	Endophthalmitis	NK	—	—	—
5	NK	Endophthalmitis	NK	—	—	—
6	F, 23	Suppurative hidradenitis	3 WBC, 0 MO	3	0	2[e]
7	F, 30	Infected mammary hematoma	5 WBC, 5 GPC	5	5	0
8	F, 53	Mammary abscess	3 WBC, 0 MO	3	4	0
9	F, 49	Ischiofemoral abscess	5 WBC, 5 GPC	3	5	0
10	M, 36	Axillary furuncle	3 WBC, 0 MO	3	4	0
11	M, 50	Mammary gland abscess	3 WBC, 0 MO	3	3	0
12	F, 62	Blood culture, septic shock	ND	1	0	0
Group 1-like						
13	M, 15	Blood culture, Ewing's sarcoma	ND	1	0	0
14	M, 63	Mammary gland abscess	3 WBC, 0 MO	5	3	0
15	M, 40	Iliac crest abscess	3 WBC, 5 GPCR	5	0	0
16	M, 87	Malum perforans pedis	5 WBC, 5 GPCR	5	0	0
17	F, 42	Infected presternal atheroma	3 WBC, 0 MO	5	4	0
18	M, 44	Infected neck atheroma	3 WBC, 0 MO	2	4	0
19	M, 57	Infected atheroma of upper arm	3 WBC, 3 GPC	3	3	0
20	M, 76	Blood culture, hip fracture	ND	1	0	0

[a] NK, not known; F, female; M, male.
[b] 3, <10 leukocytes (WBC) or microorganisms per oil immersion field (×1,000); 5, ≥10 leukocytes or microorganisms per oil immersion field; MO, microorganisms; GPC, gram-positive cocci; ND, not done; GPCR, gram-positive coryneform rods.
[c] 1, detected in blood culture; 2, detected after enrichment in fluid thioglycolate only; 3, approximately 10^3 CFU/ml was detected; 4, approximately 10^4 CFU/ml was detected; 5, ≥10^5 CFU/ml was detected; —, reference strain.
[d] All cultures with anaerobic bacteria contained at least three different anaerobic species.
[e] Coagulase-negative staphylococci were detected.

tamicin, and vancomycin). MICs were tested by the agar dilution procedure (Mueller-Hinton agar supplemented with 5% sheep blood) according to the guidelines of the National Committee for Clinical Laboratory Standards (22).

Gas-liquid chromatography. Volatile and nonvolatile fatty acids from the fermentation of glucose were determined by gas-liquid chromatography (13) on a Sigma 300 capillary chromatograph (Perkin-Elmer, Norwalk, Conn.) of grown brain heart infusion broth supplemented with 1% glucose. Ratios of volatile and nonvolatile fatty acids from fermentation were calculated from chromatogram curves by using a 3390A integrator (Hewlett-Packard, Palo Alto, Calif.). Cellular fatty acid patterns were analyzed by using the Microbial Identification System (Microbial ID, Inc. Newark, Del.) from cultures grown for 48 h at 37°C with 5% CO_2 on Trypticase soy agar with 5% sheep blood (27).

Analysis of cell wall components. For detection of *meso*-diaminopimelic acid and mycolic acids in whole-cell hydrolysates, the methods outlined by Schaal (24) were applied. Cell wall components were separated by thin-layer chromatography and were then visualized.

DNA analysis. DNA was isolated and purified by hydroxyapatite chromatography (3). Determination of the G+C content was performed by the thermal denaturation method described by Mandel and Marmur (20).

For hybridization studies, total genomic DNA was isolated by following a standard miniprep method described by Ausubel et al. (1), with the modification that the cells were incubated with proteinase K and sodium dodecyl sulfate (SDS) for 4 h. For slot hybridizations, 0.5 μg of chromosomal DNA per slot was bound to a Nylon 66 membrane (BiodyneB; Pall Biosupport, East Hills, N.Y.) and was hybridized with 50 ng of genomic DNA of the probe strain labelled with ^{32}P by using a random primer labelling kit (Boehringer, Mannheim, Germany). Prehybridization and hybridization were carried out in 5× SSC (1× SSC is 0.15 M NaCl plus 15 mM Tri sodium citrate)–5× Denhardt's solution–50 mM sodium phosphate buffer (pH 6.5)–0.5% SDS–250 μg of denaturated salmon sperm DNA per ml–50% formamide. On the basis of an average G+C content of 55% for the strains examined, the hybridization temperature was 60°C (conditions corresponding to 10 to 16°C below the melting temperature [T_m]). Membranes were washed three times for 5 min each time in a solution containing 2× SSC and 0.1% SDS at room temperature and 2 times for 15 min each time in 0.1× SSC–0.1% SDS at 50°C. Hybrids were detected by autoradiography with NiF RX film (Fuji Photo Film Co., Ltd., Tokyo, Japan).

RESULTS

Table 1 summarizes the clinical data. Eleven of the 15 strains were grown from abscesses and purulent lesions, mostly from the upper part of the body. Direct Gram stains of the clinical samples revealed the presence of GPCR in only two samples. In only 1 of the 15 clinical specimens could other aerobically growing bacteria be isolated in addition to the CDC group 1 and group 1-like coryneform bacteria, while 9 of the 15 strains were associated with mixed anaerobic flora. Three strains were grown in pure culture from blood in both aerobic and anaerobic bottles. (For the remainder of this report, the two groups of bacteria are referred to as group 1 and group 1-like, respectively.) Strain 13 came from a patient with underlying neoplasia treated

TABLE 2. Biochemical properties of CDC group 1 and group 1-like coryneform bacteria

Reaction	% Positive reactions	
	Group 1 (n = 12)	Group 1-like (n = 8)
Alpha-hemolysis on:		
Sheep blood agar	50	0
Human blood agar	100	0
Catalase	100	100
Nitrate reduction	100	0
Urea hydrolysis	0	0
Esculin hydrolysis	0	0
Triple sugar iron slant acid	100	100
Triple sugar iron butt acid	100	100
Fermentation (after 24 h) of:		
Glucose	100	100
Maltose	100	100
Sucrose	100	100
Mannitol	100	100
Xylose	100	88
Adonitol	0	100
Assayed for the following enzyme[a]:		
Alkaline phosphatase	0	63 (w)
Esterase (C_4)	100 (m)	100 (m)
Esterase lipase (C_8)	100 (w)	100 (w)
Lipase (C_{14})	0	0
Leucine arylamidase	100 (s)	100 (s)
Valine arylamidase	0	0
Cystine arylamidase	8 (w)	0
Trypsin	0	0
Chymotrypsin	0	0
Acid phosphatase	0	0
Phosphoamidase	0	0
α-Galactosidase	100 (m)	100 (m)
β-Galactosidase	100 (s)	100 (s)
β-Glucuronidase	0	0
α-Glucosidase	100 (s)	100 (s)
β-Glucosidase	0	0
n-Acetyl-β-glucosaminidase	0	0
α-Mannosidase	100 (m)	75 (w)
α-Fucosidase	0	0

[a] As determined by the API ZYM system; (w), hydrolyzed substrate, approximately 5 nmol; (m), hydrolyzed substrate, approximately 20 nmol; (s), hydrolyzed substrate, >40 nmol.

with immunosuppressive drugs, whereas strains 12 and 20 were derived from initially immunocompetent patients. Strain 12 was cultured in three blood culture sets from a patient who developed septic shock following fever of unknown etiology. All the other patients were immunocompetent. No predisposition for infection with group 1 or group 1-like strains was observed regarding sex or age. Two of the 15 strains were isolated from patients hospitalized at the Zürich University Hospital; the remaining ones were cultured from specimens from patients treated in 10 different secondary-care hospitals in the Zürich area.

All 20 isolates tested could be grown under strictly anaerobic conditions. Six strains of group 1 exhibited alpha-hemolysis on sheep blood agar (Table 2), and all group 1 strains produced alpha-hemolysis on human blood agar. In

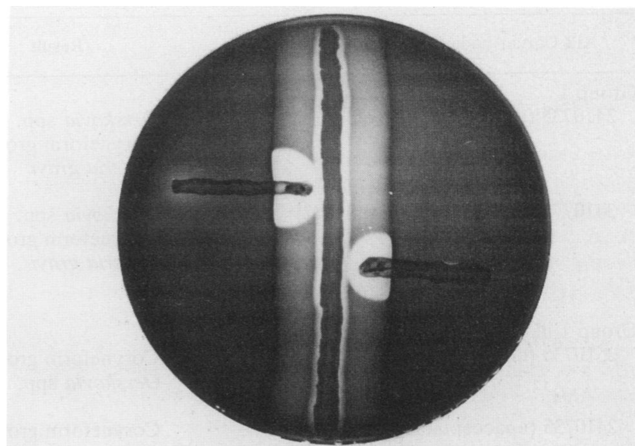

FIG. 1. CAMP reactions of CDC group 1 and group 1-like coryneform bacteria. Sheep blood agar plate after 20 h of incubation in 37°C with 5% CO_2. Left, CDC group 1 coryneform bacteria; right, CDC group 1-like coryneform bacteria.

comparison, group 1-like strains did not show any kind of hemolysis on any of the blood agars tested. Another major difference between group 1 and group 1-like strains was the inability of group 1-like strains to reduce nitrate. Using a different basal medium instead of cystine Trypticase agar, we observed that group 1-like but not group 1 strains were able to ferment adonitol. Alkaline phosphatase was present only in group 1-like strains (63%) but not in any of the group 1 strains (Table 2). In both group 1 and group 1-like strains, leucine arylamidase, β-galactosidase, and α-glucosidase were found to be the most active enzymes tested.

All 20 strains examined showed a strong CAMP reaction (Fig. 1) on Columbia as well as on Mueller-Hinton agar bases by using sheep and ox blood. Only 1 of the 20 strains examined was not able to induce a CAMP phenomenon on human blood agar.

Table 3 depicts the numerical patterns and the corresponding identifications that were obtained with the API Coryne system. All identifications were deemed either doubtful or unacceptable.

Group 1 and group 1-like strains were generally susceptible to antibiotics administered in the treatment of infections caused by GPCRs. Only one strain of group 1-like bacteria was resistant to tetracycline (MIC, >64 mg/liter). MICs (in milligrams per liter) for both group 1 and group 1-like strains were as follows: penicillin G, <0.03; erythromycin, <0.03; clindamycin, <0.03; tetracyline, 0.125 to 1; gentamicin, 1 to 2; and vancomycin, 0.125 to 0.25.

Quantitative analysis of volatile and nonvolatile fatty acids showed that succinic acid was the major end product of fermentation of both group 1 and group 1-like strains. The succinic acid/lactic acid ratio was found to be 3.7 ± 1.1 (standard deviation [SD]) for group 1 (n = 12) and 4.0 ± 0.7 (SD) for group 1-like (n = 8) strains. The succinic acid/acetic acid ratio was 1.1 ± 0.4 (SD) for group 1 and 1.5 ± 0.4 (SD) for group 1-like strains.

Cellular fatty acid profiles are shown in Table 4. The cell walls of both group 1 and group 1-like strains contained 16:0, 18:1 *cis*-9, and 18:0 fatty acid methyl esters (FAMEs) as their major constituents.

Cell walls of group 1 (strains 1 to 9) and group 1-like (strains 13 to 19) bacteria contained neither *meso*-diaminopimelic acid nor mycolic acids.

TABLE 3. API Coryne patterns

API Coryne code (no. of strains)	Result	No. of mismatched reactions	% id[a]	*T* index[b]
Group 1				
3410735 (unacceptable profile) (10)	*Oerskovia* spp.	6		
	Coryneform group A	5		
	Listeria grayi	4		
3410775 (unacceptable profile) (2)	*Oerskovia* spp.	6		
	Coryneform group A	4		
	Listeria grayi	3		
Group 1-like				
2510735 (doubtful profile) (5)	Coryneform group A	3	89.9	0.26
	Oerskovia spp.	6	10.1	0.08
2410735 (unacceptable profile) (2)	Coryneform group A	4		
	Oerskovia spp.	7		
2410335 (unacceptable profile) (1)	Coryneform group A	5		
	Listeria grayi	3		

[a] Percentage of identification, an estimate of how closely the profile corresponds to the taxon relative to all of the other taxa in the data base.
[b] The *T* index is an estimate of how closely the profile corresponds to the most typical set of reactions for each taxon.

The G+C content of group 1 strains ($n = 2$) was found to be 55 to 58 mol%, and it was found to be 55 mol% for group 1-like strains ($n = 2$).

Figure 2 shows the results of hybridizations with group 1 (strain 2) and group 1-like (strain 13) bacteria as probes, respectively. When a group 1 strain was used as a probe, all group 1 strains showed marked hybridization signals, whereas for group 1-like strains, only weak cross-hybridization could be detected. No hybridization signals were obtained for *Corynebacterium* spp., *Actinomyces* spp., or *A. haemolyticum*. Using a group 1-like strain as a probe, other group 1-like strains showed strong hybridization signals, whereas group 1 strains hybridized much more weakly.

DISCUSSION

From a collection of GPCRs encountered in our clinical laboratory during a 33-month period, we examined 15 strains plus 5 reference strains of CDC group 1 coryneform and biochemically similar rods using biochemical, chemotaxonomic, and molecular methods. These strains could be separated into two different groups which were referred to as CDC group 1 coryneform and CDC group 1-like coryneform bacteria. (Between April and June 1993 we isolated nine additional strains of group 1 bacteria and five additional strains of group 1-like bacteria from clinical specimens).

TABLE 4. Cellular fatty acid profiles of CDC group 1 and group 1-like coryneform bacteria

Component	% of cellular fatty acids (mean ± SD [range])	
	Group 1 ($n = 12$)	Group 1-like ($n = 8$)
14:0 FAME	3 ± 1 (1–4)	3 ± 1 (2–4)
16:1 *cis*-7 FAME	3 ± 2 (0–5)	2 ± 1 (1–3)
16:0 FAME	38 ± 6 (31–45)	35 ± 2 (32–39)
18:1 *cis*-9 FAME	30 ± 4 (26–35)	30 ± 3 (26–34)
18:1 *trans*-11 FAME	1 ± 1 (0–3)	2 ± 2 (0–4)
Feature 6[a]	3 ± 1 (2–5)	5 ± 1 (3–7)
18:0 FAME	16 ± 3 (14–21)	18 ± 2 (16–21)
Feature 7[b]	2 ± 3 (0–8)	3 ± 3 (2–10)

[a] 18:2 *cis*-9 FAME, 18:2 *cis*-12 FAME, 18:0 anti-iso FAME.
[b] 18:1 *cis*-11 FAME, 18:1 *trans*-6 FAME, 18:1 *trans*-9 FAME.

Seven strains of group 1 bacteria and eight strains of group 1-like bacteria were isolated from similar sites of patients with similar diagnoses treated in different hospitals. We were not able to obtain concurrent or subsequent samples for culture from these patients since all suppurative lesions were either drained or removed surgically.

Sixty percent of the strains were associated with mixed anaerobic flora. All group 1 and group 1-like strains were found to be facultatively anaerobic GPCRs. Of the 21 strains of group 1 bacteria that have been described so far, only 4 were derived from abscesses (14). Unfortunately, no information regarding the flora associated with these strains is available.

The only clinical report of a group 1 isolate came from Ellis et al. (10), who described a patient with acute natural valve endocarditis caused by a group 1 strain. However, we question their diagnosis of a group 1 isolate because the identification scheme that they used did not include group 1 bacteria. Further evidence is provided by the authors themselves by citing two case reports of group I endocarditis. Therefore, group 1 may have been reported incorrectly instead of group I. The strain is no longer available (23a).

For differentiation of group 1 and group 1-like bacteria, the ability to reduce nitrate is the key reaction. Additionally, alpha-hemolysis on human blood agar and fermentation of adonitol provide a clear-cut distinction. Na'was et al. (23) observed a weak fermentation of adonitol in 8 of 13 group 1 strains. The lack of observable adonitol fermentation by our group 1 strains was probably due to the buffering ability of the medium used.

Further differential criteria between group 1 and group 1-like rods can be obtained by using the API ZYM system since both groups exhibit distinctive profiles. The API ZYM system has not been extensively applied for the identification of GPCRs. Enzymatic profiles of *Actinomyces* spp. suggest that group 1 and group 1-like rods are more closely related to

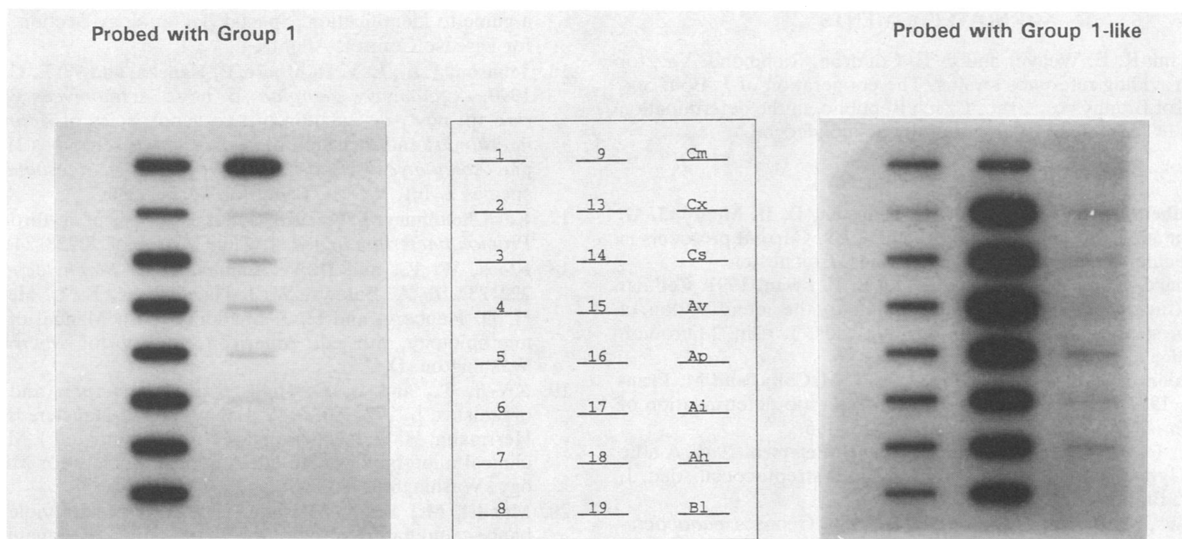

FIG. 2. DNA-DNA hybridization studies of CDC group 1 and group 1-like coryneform bacteria. 1 to 9, CDC group 1 coryneform strains; 13 to 19, CDC group 1-like coryneform strains; Cm, *C. minutissimum*; Cx, *C. xerosis*; Cs, *C. striatum*; Av, *A. viscosus*; Ap, *A. pyogenes*; Ai, *A. israelii*; Ah, *A. haemolyticum*; Bl, blank.

this genus (e.g., by the presence of α-galactosidase or β-galactosidase) than to *Corynebacterium* spp. (12).

All but 1 of the 20 strains tested on two different media showed a marked CAMP reaction usually not seen in GPCRs except in *Corynebacterium renale*, *Propionibacterium acnes*, and *Rhodococcus equi* (17, 28). Therefore, we propose that the CAMP reaction be used as a confirmatory test for the diagnosis of group 1 or group 1-like strains.

The absence of group 1 strains in the data base of commercial identification sets for coryneform bacteria, e.g., RAPID Coryne (API Coryne) (11), may contribute to the fact that group 1 strains have not been diagnosed more often. By using the minimal sets of tests recommended for identification of GPCRs by Hollis and Weaver (15) and Coyle and Lipsky (8) as well as the tests presented here, clinical laboratories should, however, be able to recognize such strains.

Group 1 and group 1-like strains cannot be separated by their cellular fatty acid patterns. Na'was et al. (23) detected fatty acids in 13 group 1 strains that were qualitatively similar but quantitatively different from ours (higher percentages of 14:0, 16:1 *cis*-7, and 16:0 FAMEs but lower ones for 18:1 *cis*-9 and 18:0 FAMEs). Since we used the same detection system, these differences may be due to their technique of growing strains at 35°C on heart infusion agar supplemented with 5% defibrinated rabbit blood, whereas we cultured our strains at 37°C in 5% CO_2 on Trypticase soy agar supplemented with sheep blood. Our findings are, however, in good agreement with the data of Bernard et al. (2), who investigated five strains of group 1 bacteria. The fatty acid profiles of group 1 and group 1-like strains are similar to those of most of the other named *Corynebacterium* spp. (2, 27), but the presence of about 3% 14:0 FAME suggests a closer relationship of group 1 and group 1-like bacteria to *Actinomyces* spp. or *A. haemolyticum* (2, 16).

Further evidence for a possible relationship of group 1 and group 1-like strains to *Actinomyces* spp. or *A. haemolyticum* is provided by the analysis of volatile and nonvolatile fatty acids produced from glucose. Coudron et al. (7) reported (without quantitative analysis) that group 1 strains produce succinic acid as a major end product. We observed that group 1 and group 1-like strains produce about four times more succinic acid than lactic acid. Such large amounts of succinic acid are, to our knowledge, only produced by *Actinomyces* spp. (13).

We were not able to detect *meso*-diaminopimelic acid or mycolic acids as cell wall components in 16 strains of group 1 or group 1-like bacteria tested. De Briel et al. (9) reported a lack of corynomycolic acids in one group 1 strain. The feature excludes group 1 and group 1-like strains from the genus *Corynebacterium* (6). Cell walls of *Actinomyces* spp. or *A. haemolyticum* also do not contain *meso*-diaminopimelic acid or mycolic acids (5, 25).

The G+C contents of group 1 and group 1-like bacteria are in the range of those for *Corynebacterium* spp. (51 to 65%) (6), slightly out of the range of those for *Actinomyces* spp. (57 to 69%) (25), and out of the range of those for *A. haemolyticum* (48 to 52%) (5).

For hybridization studies, *C. minutissimum*, *C. xerosis*, and *C. striatum* were taken as classical representatives of *Corynebacterium* spp. exhibiting biochemical characteristics similar to those of group 1 and group 1-like strains (19) but differing from them in their inability to ferment mannitol and xylose. *A. viscosus* was selected since its basic biochemical profile, with the exception of mannitol fermentation (15), is similar to those of group 1 and group 1-like strains. Finally, *A. pyogenes* and *A. israelii* were selected as classical representatives of the genus *Actinomyces*, and *A. haemolyticum* was selected because it is the only species within that genus. These hybridization studies revealed that group 1 and group 1-like strains are unrelated to the corynebacteria listed above. Both species also have only little DNA homologies with *Actinomyces* spp. or *A. haemolyticum*. Since we observed only weak cross-hybridization between group 1 and group 1-like strains, we concluded that group 1 and group 1-like strains represent different species. However, only sequence analysis of the 16S rRNA genes will clarify the phylogenetic relationship of group 1 and group 1-like strains to the genus *Actinomyces* and, perhaps, other bacterial genera.

ACKNOWLEDGMENTS

We thank R. E. Weaver and P. E. Coudron, Richmond, Va., for kindly providing reference strains. The cooperation of J. Boháček, Institute of Biophysics, Brno, Czech Republic, in the determination of the G+C contents is also gratefully acknowledged.

REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. John Wiley & Sons, Chichester.
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. **Cashion, P., M. A. Holder-Franklin, J. McCully, and M. Franklin.** 1977. A rapid method for the base ratio determination of bacterial DNA. Anal. Biochem. **87:**461–466.
4. **Christie, R., N. E. Atkins, and E. Munch-Petersen.** 1944. A note on a lytic phenomenon shown by group B streptococci. Aust. J. Exp. Biol. Med. Sci. **22:**197–200.
5. **Collins, M. D., and C. S. Cummins.** 1986. Genus *Arcanobacterium*, p. 1287–1288. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
6. **Collins, M. D., and C. S. Cummins.** 1986. Genus *Corynebacterium*, p. 1266–1276. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
7. **Coudron, P. E., R. C. Harris, M. G. Vaughan, and H. P. Dalton.** 1985. Two similar but atypical strains of coryneform group A-4 isolated from patients with endophthalmitis. J. Clin. Microbiol. **22:**475–477.
8. **Coyle, M. B., and B. A. Lipsky.** 1990. Coryneform bacteria in infectious diseases: clinical and laboratory aspects. Clin. Microbiol. Rev. **3:**227–246.
9. **De Briel, D., F. Couderc, P. Riegel, F. Jehl, and R. Minck.** 1992. High-performance liquid chromatography of corynomycolic acids as a tool in identification of *Corynebacterium* species and related organisms. J. Clin. Microbiol. **30:**1407–1417.
10. **Ellis, M. E., C. Mullangi, A. Hokail, S. M. Hussain Qadri, and M. Akhtar.** 1991. Acute natural valve endocarditis due to Corynebacterium group 1 in a normal competent host. Eur. Heart J. **12:**842–843.
11. **Gavin, S. E., R. B. Leonard, A. M. Briselden, and M. B. Coyle.** 1992. Evaluation of the Rapid CORYNE identification system for *Corynebacterium* species and other coryneforms. J. Clin. Microbiol. **30:**1692–1695.
12. **Gruner, E., A. von Graevenitz, and M. Altwegg.** 1992. The API ZYM system: a tabulated review from 1977 to date. J. Microbiol. Methods **16:**101–118.
13. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg.
14. **Hollis, D. G.** 1992. Presented at the 92nd General Meeting of the American Society for Microbiology.
15. **Hollis, D. G., and R. E. Weaver.** 1981. Gram-positive organisms: a guide to identification. Special Bacteriology Section, Centers for Disease Control, Atlanta.
16. **Johnson, J. L., L. V. H. Moore, B. Kaneko, and W. E. C. Moore.** 1990. *Actinomyces georgiae* sp. nov., *Actinomyces gerencseriae* sp. nov., designation of two genospecies of *Actinomyces naeslundii*, and inclusion of *A. naeslundii* serotypes II and III and *Actinomyces viscosus* serotype II in *A. naeslundii* genospecies 2. Int. J. Syst. Bacteriol. **40:**273–286.
17. **Kar Choudhury, T. K.** 1978. Synergistic lysis of erythrocytes by *Propionibacterium acnes*. J. Clin. Microbiol. **8:**238–241.
18. **Kloos, W. E., and D. W. Lambe.** 1991. *Staphylococcus*, p. 222–237. *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.
19. **Krech, T., and D. G. Hollis.** *Corynebacterium* and related organisms, p. 277–286. *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.
20. **Mandel, M., and J. Marmur.** 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. Methods Enzymol. **12(part B):**195–206.
21. **Nash, P., and M. M. Krenz.** 1991. Culture media, p. 1226–1288. *In* A. Balows, W. J. Hausler, Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
22. **National Committee for Clinical Laboratory Standards.** 1991. MIC interpretive standards of three categories of susceptibility for organisms other than *Haemophilus* and *Neisseria gonorrhoeae*, 4th ed. Approved standard. NCCLS document M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
23. **Na'was, T. E., D. G. Hollis, C. W. Moss, and R. E. Weaver.** 1987. Comparison of biochemical, morphologic, and chemical characteristics of Centers for Disease Control fermentative coryneform groups 1, 2 and A-4. J. Clin. Microbiol. **25:**1354–1358.

23a. **Qadri, S. M. H.** Personal communication.

24. **Schaal, K. P.** 1985. Identification of clinically significant actinomycetes and related bacteria using chemical techniques, p. 359–381. *In* M. Goodfellow and D. E. Minnikin (ed.), Chemical methods in bacterial systematics, Academic Press, London.
25. **Schaal, K. P.** 1986. Genus *Actinomyces*, p. 1383–1418. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
26. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1985. Wadsworth anaerobic bacteriology manual, 4th ed. Star Publishing Company, Belmont, Calif.
27. **von Graevenitz, A., G. Osterhout, and J. Dick.** 1991. Grouping of some clinically relevant gram-positive rods by automated fatty acid analysis. APMIS **99:**147–154.
28. **Zaki, M. M.** 1965. Relation between staphylococcal beta-lysin and different corynebacteria. Vet. Rec. **77:**941.