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Isolation of Gram-Positive Rods That Resemble but Are Clearly Distinct from *Actinomyces pyogenes* from Mixed Wound Infections

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Beginning in 1990, gram-positive rods resembling *Actinomyces pyogenes* were found with increasing frequency in mixed cultures from various infectious processes, most of them from patients with otitis, empyema, pilonidal cysts, perianal abscesses, and decubitus ulcers. Ribotyping and hybridization showed that these gram-positive rods could be divided into five groups not related to known *Actinomyces* species. Biochemical markers for reliable differentiation into these groups, however, could not be found. Therefore, naming new species is not warranted unless parameters are discovered that allow identification without DNA hybridization. These gram-positive rods have been isolated only in mixed cultures with anaerobes, *Staphylococcus aureus*, *Streptococcus "milleri,"* enterococci, and gram-negative rods. Their exact role in these possibly synergistic infections needs further investigation.

Gram-positive rods are found with increasing frequency as pathogens. In recent years, bacteria such as nontoxigenic *Corynebacterium diphtheriae*, *C. jeikeium*, *C. pseudodiphtheriticum*, *Arcanobacterium haemolyticum*, *Rhodococcus equi*, and bacteria of Centers for Disease Control group D2 (recently named *C. urealyticum* [37]) have been recognized as important pathogens in patients with endocarditis, intravascular devices, prostheses, or encrusted cystitis and in immunosuppressed patients (9, 29, 35, 44).

As a consequence, gram-positive rods are now being identified to the species level more often in clinical microbiological laboratories. During 1990, five strains morphologically and biochemically resembling *Actinomyces* (formerly *Corynebacterium*) *pyogenes* (38), a species capable of growing well aerobically, were isolated from specimens submitted to the Department of Medical Microbiology of the University of Zürich. Identical strains were found 30 times during 1991 and 36 times in the first 6 months of 1992, always in mixed cultures with other bacteria. This report contains data from a detailed phenotypic and genotypic analysis of 12 strains isolated consecutively from specimens taken from deep-wound infections between August and October 1991.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Clinical specimens were cultured aerobically at 37°C with 5% CO_2 on Columbia agar with 5% sheep blood, Columbia agar with 5% sheep blood, colistin and nalidixic acid, and Columbia chocolate agar; MacConkey agar was incubated aerobically without CO_2. Anaerobic cultures were set up on brucella agar with 5% sheep blood, kanamycin-vancomycin agar with laked blood (42), and phenylethyl blood agar (42) (all media were from Becton Dickinson Microbiology Systems, Cockeysville, Md., unless otherwise specified). Bacteria growing aerobically were identified by standard methods (14, 15, 20, 27); obligate anaerobes were identified by RapID ANA II (Innovative Diagnostic Systems, Atlanta, Ga.), API 20A, or ATB 32A (both from API, La-Balme-les-Grottes, France) combined with gas-liquid chromatography of volatile and nonvolatile fatty acids from prereduced, anaerobically sterilized (PRAS) chopped-meat broth containing carbohydrates (23); if anaerobes could not be identified by these means, they were only identified to the genus level, e.g., *Bacteroides* sp.

A. pyogenes-like (APL) gram-positive rods were designated APL1 to APL12. Five *A. pyogenes* isolates of animal origin fitting the description of Reddy et al. (38) (AP13 to AP17) were obtained from J. Nicolet, University of Bern, Bern, Switzerland. Another typical *A. pyogenes* strain (AP18) was isolated from multiple blood cultures from a patient with septicemia and submitted as a reference culture for identification from a laboratory in Fribourg, Switzerland; a further typical strain (AP20) was isolated later during the study from a case of whitlow. The type strain of *A. pyogenes* (NCTC 5224 = ATCC 19411), AP19, was obtained through the Swiss National Collection of Type Cultures in Lausanne.

Strains representing the following 11 *Actinomyces* species (numbered 21 to 31) were included in ribotyping and hybridization experiments (obtained either through the American Type Culture Collection or the Swiss National Collection of Type Cultures; type strains are designated by the letter T): *A. israelii* ATCC 10048, *A. odontolyticus* ATCC 17982, *A. naeslundii* ATCC 12104^T, *A. viscosus* LA (Lausanne) 762, *A. meyeri* ATCC 33972, *A. bovis* ATCC 13683^T, *A. gerencseriae* ATCC 23860^T, *A. georgiae* ATCC 49285^T, *A. hordeovulneris* ATCC 35275^T, *A. denticolens* ATCC 43322^T, and *A. howellii* ATCC 43323^T. The *Arcanobacterium haemolyticum* strain used in hybridization experiments was type strain ATCC 9345.

The strains were maintained in skim milk at −70°C. Two of the atypical isolates resembling *A. pyogenes* (APL5 and APL9) were unfortunately lost after basic biochemical examination but before they could be investigated genotypically and by extended PRAS biochemistry.

Biochemical and enzymatic characterization. Strains were

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identified in accordance with the criteria outlined by Coyle and Lipsky (9) and Hollis and Weaver (24). Media used for biochemical reactions were prepared as described by Nash and Krenz (36); basal media were obtained from Becton Dickinson. Cystine Trypticase agar (CTA) media contained 1% of the carbohydrates and were supplemented with 5% rabbit serum. Gelatin degradation was tested by adding film strips (Diagnostics Pasteur, Marnes-la-Coquette, France) to a dense suspension (McFarland no. 5) of the bacteria in saline. Degradation of casein, tyrosine, and xanthine was determined as described by Land et al. (32). Nitrate reduction was tested in nitrate broth, and esculin hydrolysis was tested on mannitol-esculin agar slants (both from Difco, Detroit, Mich.). Starch hydrolysis was evaluated by checking for lack of blueness around growth after addition of aqueous iodine solution to colonies grown on Mueller-Hinton agar. Lecithinase and lipase reactions were done on modified McClung-Toabe agar (36). Catalase activity was determined by adding 30% hydrogen peroxide solution to Trypticase soy broth cultures. The CAMP reaction was performed on Columbia sheep blood agar with *Staphylococcus aureus* ATCC 25923. DNase test agar with methyl green (Difco) was used with addition of 0.1 ml of sterile rabbit serum to 4 ml of medium.

Incubation was done at 37°C without CO_2 for tests dependent on changes in pH but otherwise at 37°C with 5% CO_2; anaerobic incubations were done at 37°C in an anaerobic chamber (Jouan, Nantes, France) containing a mixture of 10% hydrogen, 10% CO_2, and 80% nitrogen or in anaerobe jars (Oxoid, Basingstoke, England).

Additionally, a battery of reactions in PRAS media was tested as described by Johnson et al. (25) and Holdeman et al. (23); PRAS media were obtained from Carr-Scarborough Microbiologicals (Stone Mountain, Ga.). In preliminary experiments, cultures were tested for maximum growth in peptone-yeast extract-glucose broth medium with and without Tween 80 (0.02%, vol/vol) and inoculated (i) anaerobically under CO_2, (ii) aerobically and then restoppered (to introduce a small amount of oxygen), and (iii) aerobically with tubes covered with sterile aluminium foil (25). Eventually, strains were inoculated and incubated either anaerobically or microaerobically, depending on the optimal conditions for acid formation for a particular strain. Tween 80 was added to all of the media.

The commercial identification systems API CORYNE (16, 18), API ZYM (22) (both from API), and RapID ANA II (Innovative Diagnostic Systems) were used in accordance with the instructions of the manufacturers.

Gas-liquid chromatography. Volatile and nonvolatile fatty acids from fermentation were determined by gas-liquid chromatography of PRAS chopped-meat broth with carbohydrates, in which bacteria were grown, gassed with CO_2 (23). Cellular fatty acids were analyzed by the Microbial Identification System (MIS) of Hewlett-Packard, currently marketed by Microbial ID, Inc. (Newark, Del.), from cultures grown for 24 h at 37°C with 5% CO_2 on Trypticase soy agar (Becton Dickinson) with 5% sheep blood (39, 43). The software used was version 3.5 of both the clinical and the general Trypticase soy blood agar libraries. The broth procedure described for use with the anaerobe MIS library (version 3.5) was also used (19, 25). For the broth procedure, 10 ml of PRAS peptone-yeast-glucose medium with Tween 80 (PYG-T; Carr-Scarborough) was inoculated with 0.2 ml of an actively growing culture in PRAS chopped-meat broth (23); after overnight incubation at 37°C, another subculture to several tubes of PYG-T medium was made to obtain sufficient cell mass. After centrifugation, the pellet was washed with 3 ml of 0.7% aqueous $MgSO_4$ and centrifuged again. The cells were lysed and saponified, and the fatty acids were methylated, extracted, washed, and analyzed by the MIS as described by Ghanem et al. (19).

Streptococcal antigens. Agglutination for streptococcal antigens found in *A. pyogenes* (30) was done by the Phadebact *Streptococcus* Test (Karo Bio Diagnostics AB, Huddinge, Sweden) and the Prolex Streptococcal Latex Kit (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada).

Susceptibility to antimicrobial agents. MICs were determined by the E-test procedure (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar with 5% sheep blood (26). The plates were swabbed with an inoculum corresponding to the turbidity of a McFarland 0.5 standard, and incubation was done at 37°C without CO_2.

DNA analysis. Total genomic DNA was isolated by the standard miniprep procedure described by Ausubel et al. (1), except that cells were incubated in proteinase K and sodium dodecyl sulfate (SDS) for 4 to 6 h.

Ribotyping was performed essentially as described by Martinetti and Altwegg (34), with restriction enzymes *Cla*I, *Sma*I, and *Pvu*II (Boehringer, Mannheim, Federal Republic of Germany).

For slot hybridizations (1), 1.8 μg of chromosomal DNA was bound per slot to a supported Nylon 66 membrane (BiodyneB; Pall Biosupport, East Hills, N.Y.) and hybridized with 50 ng of probe strain genomic DNA labelled with ^{32}P by using a random-primer labelling kit (Boehringer). Prehybridization and hybridization were done in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt's solution–50 mM sodium phosphate buffer (pH 6.5)–0.5% SDS–250 mg of denatured herring sperm DNA per ml–50% formamide. On the basis of an average G+C-content of 63% (range, 57 to 69%) for actinomycetes (40), hybridization temperatures were 45°C (optimal conditions corresponding to 25 to 30°C below the T_m) and 60°C (stringent conditions corresponding to 10 to 15°C below the 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 twice for 15 min each time in 0.1× SSC–0.1% SDS at 50°C. Hybrids were detected by autoradiography using NiF RX film (Fuji Photo Film Co., Ltd., Tokyo, Japan).

For quantitative analysis, pieces of membrane carrying the DNA of one slot were cut out and each piece was introduced into a reaction tube containing 10 ml of toluol as scintillation liquid. Counts per minute were assessed during 10 min on a liquid scintillation analyzer (Packard).

RESULTS

Clinical data. Clinical data and the bacteria isolated from the specimens are summarized in Table 1. Gram-positive rods resembling *A. pyogenes* were always isolated in mixed culture with anaerobes, *S. aureus*, *S. "milleri,"* enterococci, or gram-negative rods. Pure cultures were never found. This was also the case for all of the other 59 specimens from which they had been isolated between January 1990 and June 1992. The clinical spectrum of these infections was similar to that of the 12 cases shown in Table 1. Overall, the 71 strains were isolated from the following: 22 pilonidal cysts, 19 perianal abscesses, seven intra-abdominal infections, four wound infections of the female genital tract, three chronic otitis ear discharge fluid samples, three superficial abdominal wound infections, three cases of suppurative hydradenitis,

TABLE 1. Origins of and accompanying bacteria isolated from specimens containing APL gram-positive rods

Isolate	Sex[a], age (yr)	Diagnosis	Aerobically growing bacteria	Obligately anaerobic bacteria
APL1	F, 30	Perianal abscess	APL, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Citrobacter amalonaticus*, *Enterococcus faecalis*	*Bacteroides fragilis*, *Bacteroides* sp., *Fusobacterium nucleatum*, *Peptostreptococcus* sp.
APL2	F, 68	Subhepatic abscess after laparoscopic cholecystectomy	APL, *Escherichia coli*	*Bacteroides fragilis*, *Peptostreptococcus* sp., *Veillonella* sp., *Lactobacillus* sp.
APL3	M, 29	Pilonidal cyst	APL	*Peptostreptococcus anaerobius*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus* sp.
APL4	M, 26	Pilonidal cyst	APL, *Streptococcus "milleri,"* coagulase-negative staphylococci	*Bacteroides fragilis*
APL5 (lost)	M, 29	Pilonidal cyst	APL	*Peptostreptococcus anaerobius*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus* sp.
APL6	F, 4	Chronic otitis media	APL, *Pseudomonas aeruginosa*	*Peptostreptococcus asaccharolyticus*, *Peptostreptococcus* sp., *Prevotella melaninogenica*, *Bacteroides* sp.
APL7	M, 32	Chronic otitis media	APL, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus faecalis*	*Peptostreptococcus asaccharolyticus*
APL8	M, 32	Perianal abscess	APL	*Bacteroides fragilis*, *Prevotella melaninogenica*, *Bacteroides* sp., *Lactobacillus* sp.
APL9 (lost)	M, 25	Pilonidal cyst	APL, *Streptococcus "milleri"*	*Peptostreptococcus* sp.
APL10	M, 54	Perianal abscess	APL, coagulase-negative staphylococci	None
APL11	M, 66	Drainage after lung resection	APL, *Staphylococcus aureus*	None
APL12	M, 59	Pleural empyema	APL	*Bacteroides* sp., *Fusobacterium nucleatum*, *Propionibacterium acnes*, *Lactobacillus* sp., *Veillonella* sp.

[a] F, female; M, male.

two mammillary abscesses, two cases of pleural empyema, one bronchial secretion sample, one inguinal abscess, one gluteal abscess, one decubital ulcer, one case of whitlow, and one patient with osteomyelitis.

DNA analysis. Ribotyping (Fig. 1) and slot hybridization analysis (see Fig. 3) clearly indicated that the 10 strains resembling *A. pyogenes* were very diverse and could be arranged in five different groups with one to three strains per group, as summarized in Table 2. Hybridization group I comprised strains APL1, APL3, and APL7; group II included APL2, APL4, and APL8; group III included APL6; group IV included APL10 and APL12; and group V included APL11. Cross-hybridization between groups was minimal (see Fig. 3). Strains belonging to the same group showed identical, or at least very similar, rDNA patterns. Strains APL10 and APL12 were identical with each of the three enzymes used, and the same was true for strains APL2, APL4, and APL8. Strain APL3 was different from strains APL1 and APL7; however, the difference was restricted to a single band with each enzyme. Strains APL6 and APL11 had unique rDNA patterns and made up single-member hybridization groups. Slot hybridization of all 10 APL strains with *Arcanobacterium haemolyticum* showed no hybridization.

In contrast to the APL strains, true *A. pyogenes* strains (Fig. 1, lanes 14 to 20) represented a relatively homogeneous group. Hybridization results were very similar under both stringent and optimal hybridization conditions (see Fig. 3). This homogeneity was confirmed by rDNA patterns (Fig. 1), which were very similar for these strains but definitively different from all of the other strains analyzed. There were minor differences in the rDNA patterns of strains AP15 and AP17 (Fig. 1, lanes 16 and 18), although biochemically these two strains did not differ from the other true *A. pyogenes* strains.

The representative strains of the 11 known *Actinomyces* species showed different ribotypes (Fig. 2). APL strains did not hybridize significantly with these other *Actinomyces* strains (Fig. 3), with the exception of APL11, which had 56% homology with *A. odontolyticus*; all of the other hybridization homologies visible in Fig. 3 were below 30%. For APL11, *A. odontolyticus* could not be excluded with certainty by comparing its biochemical reactions with those previously published (25).

Cultural and biochemical properties. The biochemical properties of the bacterial isolates tested are shown in Table 3. The gram-positive rods resembling *A. pyogenes* showed three colony types (Table 3). All three types showed no hemolysis when incubated anaerobically. Except for the crumbly colonies of APL11, all of the colonies were smooth. Colony sizes were equal under aerobic and anaerobic incubations. In contrast, true *A. pyogenes* strains grew larger white colonies 0.8 to 1 mm in diameter which turned

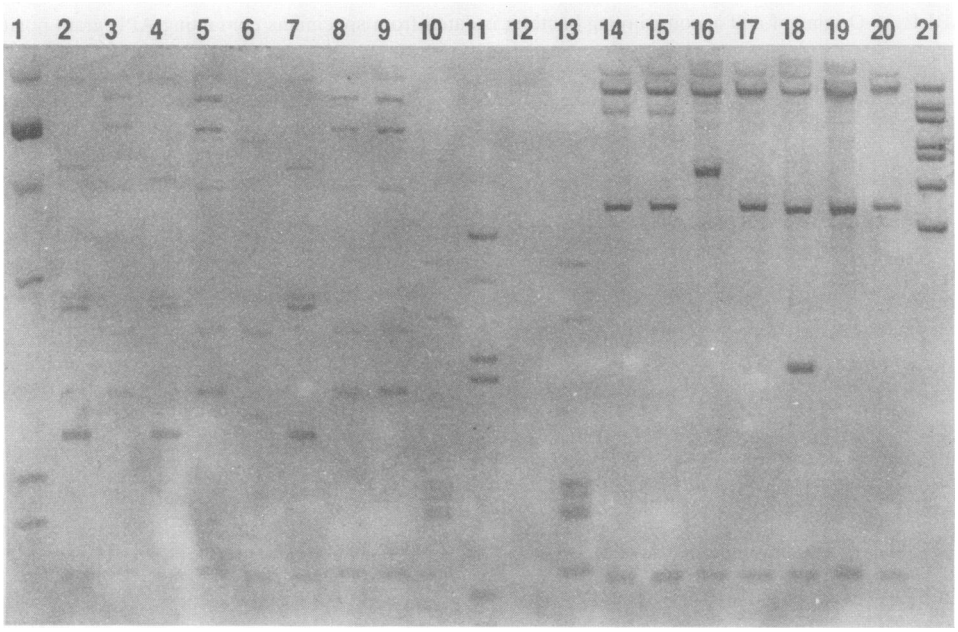

FIG. 1. rDNA patterns of genomic DNAs of APL and *A. pyogenes* strains digested with *Sma*I. Lanes: 1, molecular size marker lambda DNA digested with *Hin*dIII (fragment sizes, from the top to the bottom, are 23.1, 9.4, 6.6, 4.3, 2.3, and 2.0 kb; 2, APL1; 3, APL2; 4, APL3; 5, APL4; 6 to 8, APL6 to APL8; 9, APL8; 10 to 11, APL10 to APL11; 12, APL11; 13, APL12; 14 to 19, AP13 to AP18; 20, type strain *A. pyogenes* ATCC 19411; 21, molecular size marker *Serratia fonticola* (standard) DNA digested with *Hin*dIII (fragment sizes, from the top to the bottom, are 14.6, 11.8, 10.6, 8.5, 7.9, 6.6, and 5.4 kb).

greyish-green after several days; beta-hemolysis was stronger, and larger colonies were formed aerobically than anaerobically. Growth of APL1 to APL12 in broth cultures was moderate, and that of AP13 to AP20 was good; all broth cultures were turbid, with slight formation of a smooth sediment. Microscopically, all of the strains, including the true *A. pyogenes* strains, formed straight and slightly curved rods, with some branching, of about 0.5 to 0.8 μm in diameter and a length of 2 to 3 μm; some of the cells showed slight clubbing. Most cells stained gram positively, with some irregularities giving a beaded appearance. All of the strains produced a weak CAMP reaction with *S. aureus* ATCC 25923 by showing only a narrow band of increased hemolysis on sheep blood agar along the *Actinomyces* streak; this CAMP phenomenon was more marked with the true *A. pyogenes* strains. With the exception of *A. israelii*, all 10 strains of known *Actinomyces* spp. investigated showed this narrow band of increased hemolysis, too.

TABLE 2. Comparison of hybridization groups and rRNA gene patterns

Strain	Hybridization group	Dot blot hybridization result obtained with labelled strain:					rRNA gene pattern		
		APL3	APL6	APL8	APL10	APL11	*Cla*I	*Sma*I	*Pvu*II
APL1	I	+					A	A	A
APL2	II			+			B	B	B
APL3	I	+					C[a]	C[a]	C[a]
APL4	II			+			B	B	B
APL6	III		+				D	D	D
APL7	I	+					A	A	A
APL8	II			+			B	B	B
APL10	IV				+		E	E	E
APL11	V					+	F	F	F
APL12	IV				+		E	E	E

[a] With ribotype C, only one band was different from ribotype A for each of the restriction enzymes.

All of the strains produced lactic and succinic acids in chopped-meat broth with carbohydrates under CO_2; in thioglycolate broth, they produced only lactic acid, owing to the lack of CO_2 (23, 41).

Problems were encountered with esculin hydrolysis and starch hydrolysis: strains APL1, APL3, APL11, and APL12 were clearly positive in PRAS esculin medium but required a very large inoculum and prolonged incubation on mannitol-esculin agar slants. Starch degradation was clearly positive for all of the strains when done on Mueller-Hinton agar, but only few strains were positive in PRAS starch medium, even after 3 weeks of incubation.

In the API ZYM strip, APL gram-positive rods showed fewer positive enzymatic reactions than did true *A. pyogenes* strains. These reduced enzymatic activities also resulted in lack of identification by the API CORYNE system. True *A. pyogenes* strains showed the typical enzymatic patterns described by Lämmler and Blobel (30) and were identified excellently by the API CORYNE system (all ID values, 99.9%; all T values, 0.97). In the RapID ANA II, identifications of APL bacteria yielded either *A. odontolyticus* or *A. meyeri* without correlation to the groups determined by ribotype and hybridization; true *A. pyogenes* strains were also identified as *A. odontolyticus* or *A. meyeri* by RapID ANA II.

Gas-liquid chromatography of cellular fatty acids. The plate method used by von Graevenitz et al. (43) proved to be unsatisfactory, owing to insufficient growth of APL strains on Trypticase soy agar. Many attempts yielded only an insufficient total peak area. True *A. pyogenes* strains also remained unidentified by this plate method. The Virginia Polytechnic Institute broth method (19, 25) using PRAS

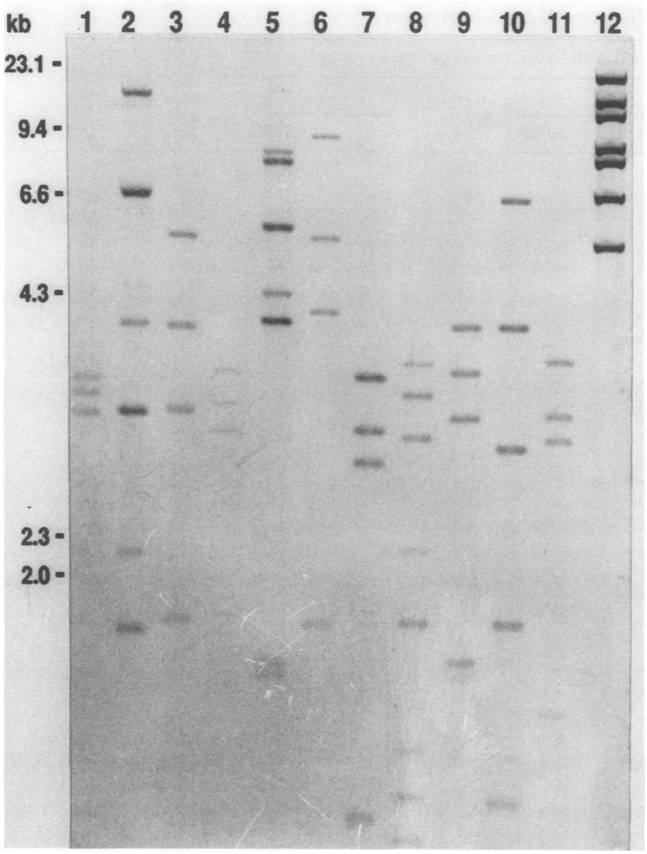

FIG. 2. rDNA patterns of genomic DNAs of *Actinomyces* species digested with *Sma*I. Lanes: 1, *A. israelii* ATCC 10048; 2, *A. odontolyticus* ATCC 17982; 3, *A. naeslundii* ATCC 12104^T; 4, *A. viscosus* LA (Lausanne) 762; 5, *A. meyeri* ATCC 33972; 6, *A. bovis* ATCC 13683^T; 7, *A. gerencseriae* ATCC 23860^T; 8, *A. georgiae* ATCC 49285^T; 9, *A. hordeovulneris* ATCC 35275^T; 10, *A. denticolens* ATCC 43322^T; 11, *A. howellii* ATCC 43323^T; 12, molecular size marker *S. fonticola* (standard) DNA digested with *Hin*dIII. The sizes of the *S. fonticola* molecular size marker fragments are given in Fig. 1. Positions of fragments of molecular size marker lambda DNA digested with *Hin*dIII are indicated at the left.

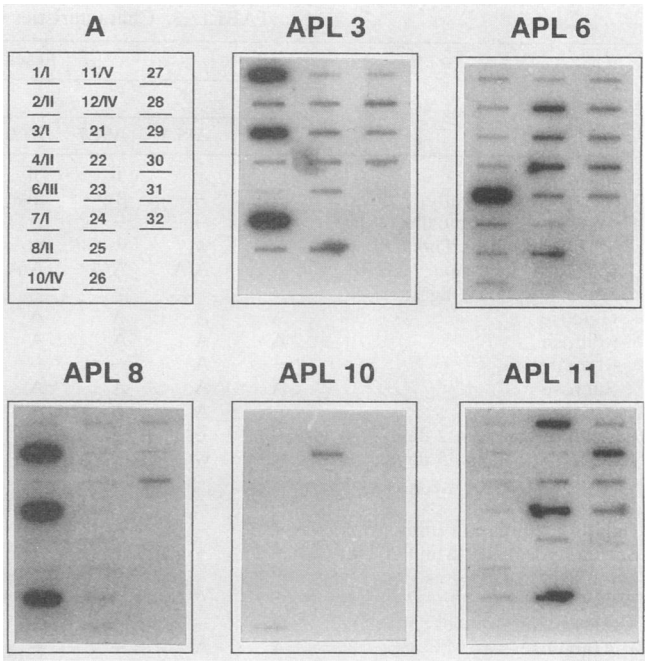

FIG. 3. Slot blot of genomic DNAs isolated from APL strains, *A. pyogenes* strains, and strains of other *Actinomyces* species hybridized at 45°C. (A) Schematic illustration of the positions of the genomic DNAs of the strains. This schema remains constant in all slot blots. Slots numbered 1 to 12 represent APL strains; the hybridization groups are designated with roman numerals. Slots: 21, *A. israelii* ATCC 10048; 22, *A. odontolyticus* ATCC 17982; 23, *A. naeslundii* ATCC 12104^T; 24, *A. viscosus* LA (Lausanne) 762; 25, *A. meyeri* ATCC 33972; 26, *A. bovis* ATCC 13683^T; 27, *A. gerencseriae* ATCC 23860^T; 28, *A. georgiae* ATCC 49285^T; 29, *A. hordeovulneris* ATCC 35275^T; 30, *A. denticolens* ATCC 43322^T; 31, *A. howellii* ATCC 43323^T. Slot 32 is a negative control containing 2× SSC without DNA. The strain designations above the slot blot panels correspond to the labelled probes used for hybridization.

PYG-T medium resulted in acceptable total interpretable peak areas of 51,000 to 93,000. As shown in Table 4, identifications were, with one exception (APL2 was identified as a *Lactobacillus* sp.) within the genus *Actinomyces* but at similarity levels of <0.500. The true *A. pyogenes* strains AP13 to AP20 were identified as either *A. georgiae* (similarity index, 0.214 to 0.375), *A. meyeri* (0.270), *A. naeslundii* (0.243 to 0.317), *Actinomyces* sp. strain DO1 (0.176 to 0.402), or bifidobacteria (0.113 to 0.335) and occasionally as streptococci (0.078 to 0.138). It must be mentioned that *A. pyogenes* is not included in the MIS data base for anaerobes. APL strains had higher contents than did true *A. pyogenes* strains of 16:1 *cis*-9 fatty acid methyl ester (FAME), 18:1 *cis*-9 FAME, and the fatty acids combined in feature 10 (18:1C11/T9/T6 FAME or un-17.834 equivalent chain length [ECL]) and lower amounts of 16:0 FAME and 18:0 FAME.

In summary, gram-positive rods resembling *A. pyogenes* were distinguished from true *A. pyogenes* strains primarily by formation of smaller colonies; weaker beta-hemolysis and CAMP reactions; negative casein, DNA, and gelatin degradation; negative agglutination with *Streptococcus* group B and G antibodies in both of the agglutination kits used; negative pyrrolidonyl arylamidase; and overall fewer enzymatic reactivities. The cellular fatty acid content was higher in unsaturated 16:1 *cis*-9 FAME and 18:1 *cis*-9 FAME and lower in saturated 16:0 FAME and 18:0 FAME.

DISCUSSION

Beginning in 1990, facultatively anaerobic gram-positive rods resembling *A. pyogenes* were found with increasing frequency in mixed cultures from various infectious processes. These bacteria seem to be widely distributed, as 7 of 71 isolates were reference cultures submitted for identification by other laboratories up to 60 km away from Zürich. These bacteria could not be identified by the commonly used commercial system API CORYNE. When the traditional biochemical schemes of Coyle and Lipsky (9) and Hollis and Weaver (24) were used, the bacteria looked very much like *A. pyogenes*, negative gelatin hydrolysis being the only atypical reaction. RapID ANA II and gas-liquid chromatography by the MIS failed on both APL bacteria and true *A. pyogenes* strains by identifying them as other *Actinomyces* spp., although *A. pyogenes* is included in the RapID ANA II data base; it is, however, not included in the MIS anaerobe library.

TABLE 3. Characteristics of APL bacteria and *A. pyogenes*[a]

Test	Result obtained with hybridization group:										Result obtained with AP13–AP20
	I			II			III (APL6)	IV		V (APL11)	
	APL1	APL3	APL7	APL2	APL4	APL8		APL10	APL12		
Colony type[a]	i	i	ii	ii	ii	ii	ii	ii	i	iii	iv
Hemolysis aerobically, 2 days	–[b]	–	βw	βw	βw	βw	βw	βw	–	βw	β
Hemolysis anaerobically, 2 days	–	–	–	–	–	–	–	–	–	–	βw to β
CAMP reaction, 2 days	w	w	w	w	w	w	w	w	w	w	+
Triple sugar iron agar	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
Cystine Trypticase agar:											
Glucose	A	A	A	A	A	A	A	A	A	A	A
Maltose	A	A	A	A	A	A	A	A	A	A	A
Mannitol	–	A	–	–	–	–	–	–	–	–	7A, 1–
Sucrose	A	A	A	A	A	A	A	A	A	A	A
Xylose	A	A	A	A	A	A	A	A	A	A	A
Esculin hydrolysis, 2 days	–	–	–	–	–	–	–	–	–	–	–
Esculin hydrolysis, 5 days	w	w	–	–	–	–	–	–	w	w	–
Casein degradation	–	–	–	–	–	–	–	–	–	–	+
DNase, 5 days	–	–	–	–	–	–	–	–	–	–	+
Gelatin degradation (film strips)	–	–	–	–	–	–	–	–	–	–	+
Starch hydrolysis (Mueller-Hinton agar)	+	+	+	+	+	+	+	+	+	+	+
PRAS:											
Amygdalin	–	–	–	–	–	–	–	–	–	–	–
Arabinose	A	A	–	–	–	–	–	–	A	–	–
Cellobiose	A	A	–	–	–	–	–	–	A	–	–
Erythritol	–	–	–	w	w	–	A	A	w	–	–
Esculin acid	–	w	–	–	–	–	–	–	w	–	–
Esculin hydrolysis	+	+	–	–	–	–	–	–	+	+	–
Fructose	A	A	A	A	A	–	A	A	A	A	–
Galactose	A	A	–	–	–	A	–	–	A	–	A
Glucose	A	A	A	A	A	A	A	A	A	A	A
Glycogen	w	w	A	–	–	–	–	–	–	–	A
Inositol	A	A	–	–	–	A	–	A	A	–	6A, 2–
Lactose	A	A	–	–	–	–	–	–	A	–	A
Maltose	A	A	A	A	A	A	A	A	A	w	A
Mannitol	w	w	–	–	–	–	–	–	w	–	1w, 7–
Mannose	A	A	A	w	A	A	A	–	A	–	4A, 4–
Melezitose	A	A	–	–	–	–	–	–	A	–	2A, 6–
Melibiose	A	A	–	–	A	–	–	–	A	–	–
Raffinose	A	A	–	–	–	–	–	–	A	–	–
Rhamnose	–	–	–	–	–	–	–	–	–	–	–
Ribose	A	A	A	A	A	A	A	A	w	–	A
Salicin	A	A	–	–	–	–	–	–	A	–	–
Sorbitol	–	–	–	–	–	–	–	–	–	–	6A, 2–
Sucrose	w	A	A	A	A	A	A	A	w	A	A
Trehalose	–	A	–	–	–	–	–	–	w	–	A
Xylose	A	A	A	A	A	w	A	A	A	w	A
Gelatin hydrolysis, 3 wk	–	–	–	–	–	–	–	–	–	–	+
Milk, curd formation	+	+	–	–	–	–	–	–	+	–	7+, 1–
Starch degradation, 3 wk	+	–	+	–	–	–	–	+	–	–	1+, 7–
API ZYM											
Alkaline phosphatase[c]	–	–	–	–	–	w	–	–	–	–	+
Esterase (C4)	w	w	w	w	–	–	w	w	w	w	w
Esterase lipase (C8)	w	w	w	w	w	–	w	w	w	w	w
Leucine arylamidase	+	+	+	–	+	+	+	+	+	+	+
Valine arylamidase	–	+	–	–	–	–	–	–	–	–	–
Trypsin	–	–	–	–	–	–	–	–	w	w	w to +
Acid phosphatase	–	+	–	–	–	+	–	–	–	–	w to +
α-Galactosidase	–	+	–	–	–	–	–	–	–	–	–
β-Galactosidase	–	+	–	–	–	w	–	–	–	–	+
β-Glucuronidase	–	–	–	–	–	–	–	–	–	–	w to +
α-Glucosidase	w	+	–	–	w	+	w	w	+	+	+
β-Glucosidase	–	+	–	–	–	–	–	–	–	–	–
β-Glucosamidase	–	+	–	–	–	+	–	–	–	–	+
α-Fucosidase	–	+	–	–	–	–	–	w	–	–	–
API CORYNE											
Pyrazinamidase	+	+	+	+	–	–	+	+	+	+	–
Pyrrolidonyl arylamidase	–	–	–	–	–	–	–	–	–	–	+

Continued on following page

TABLE 3—*Continued*

Test	Result obtained with hybridization group:										Result obtained with AP13–AP20
	I			II			III (APL6)	IV		V (APL11)	
	APL1	APL3	APL7	APL2	APL4	APL8		APL10	APL12		
Agglutination with Strep B antibody	–	–	–	–	–	–	–	–	–	–	w
Agglutination with Strep G antibody	–	–	–	–	–	–	–	–	–	–	+
MIC range (mg/liter) of:											
Clindamycin				≤0.016–0.047[d]							0.023–0.047
Erythromycin				<0.016[d]							<0.016
Gentamicin				0.19–2[d]							0.38–1
Penicillin G				≤0.016–0.032[d]							≤0.016–0.016
Tetracycline				0.094–0.25[d]							0.125–0.25
Vancomycin				0.19–0.75[d]							0.19–0.75

[a] All strains were negative for catalase, urease, nitrate reduction, and indole formation; tyrosine, xanthine, lipase, lecithinase, milk digestion (all after 3 weeks of incubation); API ZYM lipase, cystine arylamidase, chymotrypsin, phosphohydrolase, and α-mannosidase.

[b] i, whitish, slightly dull, circular, low convex colonies about 0.3 to 0.5 mm in diameter after 48 h of incubation aerobically with 5% CO_2 on sheep blood agar with no hemolysis; ii, similar to i but more greyish and more transparent with weak beta-hemolysis; iii, slightly yellow and opaque with weak beta-hemolysis; iv, white colonies 0.8 to 1 mm in diameter with pronounced beta-hemolysis which turn greyish green after several days of incubation. +, positive reaction; A, acid formation (in PRAS media, pH reduction of ≥0.8); w, weakly positive reaction (in PRAS media, pH reduction of 0.5 to 0.7); –, negative reaction (in PRAS media, pH reduction of ≤0.4).

[c] API ZYM reactions: w, color intensity 1 to 2; +, color intensity 3 to 5 according to manufacturer's color chart.

[d] Result obtained for all strains in all five hybridization groups.

Most isolates were from pilonidal cysts, perianal abscesses, intraabdominal infections, and, further, from patients with chronic otitis, empyema, and various other abscesses. Although the bacteriology of these infections has been investigated thoroughly (3, 13), *A. pyogenes* and related bacteria have rarely been mentioned, whereas the role of *P. aeruginosa*, *S. aureus*, *S. "milleri,"* and the obligate anaerobes has been well described.

Ribotyping and hybridization showed that these gram-positive rods could be divided into five groups not related to known *Actinomyces* spp., with the possible exception of one of those groups showing 56% hybridization with *A. odontolyticus*. The morphologically and biochemically similar species *Arcanobacterium haemolyticum* could be excluded by lack of hybridization with the APL strains, as well as absence of an antagonistic hemolytic effect with *S. aureus*

TABLE 4. Fatty acids of APL bacteria and *A. pyogenes*[a]

Component	% of total fatty acids in hybridization group:										% of total fatty acids in AP13 to AP20	
	I			II			III (APL6)	IV		V (APL11)		
	APL1	APL3	APL7	APL2	APL4	APL8		APL10	APL12		Range	$\bar{x}$ ± SD
10:0 FAME	4.3	5.3	5.9	5.1	4.8	3.2	4.7	5.7	3.8	1.9	2.7–3.5	3.11 ± 0.31
12:0 FAME	2.5	2.9	1.5	1.2	1.3	1.0	1.3	1.5	2.0	4.0	0.9–2.0	1.61 ± 0.41
14:1 *cis*-9 FAME	1.6	1.8							1.4			
14:0 FAME	6.1	7.4	3.0	2.5	3.0	2.4	2.6	2.8	5.5	19.6	5.0–10.2	7.94 ± 2.50
16:1 *cis*-7 FAME									0.7	1.2	0.9–3.6	2.33 ± 0.89
16:1 *cis*-9 FAME	10.9	12.1	8.0	6.9	6.4	6.4	6.3	6.7	10.4	4.1	2.3–2.9	2.64 ± 0.21
16:0 FAME	10.9	13.4	11.0	9.2	10.8	12.3	12.7	11.1	10.3	18.3	28.0–37.7	32.56 ± 3.38
Feature 8[b]	1.5	1.4							1.6			
18:2 *cis*-9,12 FAME	1.9	1.8							2.0	1.3		
18:1 *cis*-9 FAME	43.8	38.8	56.8	60.8	59.5	60.1	59.3	58.4	44.1	42.2	30.4–40.7	35.98 ± 3.70
Feature 10[b]	10.8	11.8	8.2	7.3	7.6	8.5	7.8	7.7	11.1	5.9	3.1–5.2	4.05 ± 0.71
18:1 *cis*-11 DMA[c]	1.6								1.6			
un-18.199 18:0a DMA			2.4	2.7	2.5	2.0	2.1	2.3			0.9–1.1	1.0 ± 0.08
Feature 12[b]	1.6			1.3	1.4	1.1		1.2	1.6			
18:0 FAME	2.6	2.1	3.3	3.0	2.7		3.1	1	2.2	1.5	5.9–15.0	8.96 ± 3.13

[a] Identification given by the MIS with similarity indices: APL1, 0.313 for *A. odontolyticus* serotype II, 0.183 for *Fusobacterium* sp. strain A, 0.162 for *A. meyeri*; APL2, 0.417 for *Lactobacillus crispatus*, 0.355 for *L. acidophilus*, 0.328 for *Gemella morbillorum* CFA G3; APL3, 0.277 for *A. odontolyticus* serotype II, 0.145 for *Peptostreptococcus asaccharolyticus*, 0.132 for *Fusobacterium* sp. strain A; APL4, 0.413 for *A. odontolyticus* serotype II, 0.380 for *G. morbillorum*, 0.327 for *A. meyeri*; APL6, 0.480 for *A. odontolyticus* serotype II, 0.380 for *A. meyeri*, 0.332 for *A. odontolyticus* serotype I; APL7, 0.382 for *A. meyeri*, 0.355 for *A. odontolyticus* serotype II, 0.324 for *L. acidophilus*; APL8, 0.488 for *A. odontolyticus* serotype II, 0.443 for *Actinomyces* sp. strain DO1, 0.334 for *A. meyeri*; APL10, 0.392 for *A. odontolyticus* serotype II, 0.351 for *A. meyeri*, 0.317 for *L. acidophilus*; APL11, 0.483 for *A. odontolyticus* serotype I, 0.398 for *Bifidobacterium* sp. strain DO5, 0.231 for *Clostridium malenominatum*; APL12, 0.333 for *A. odontolyticus* serotype II, 0.160 for *Fusobacterium* sp. strain A, 0.139 for *Actinomyces* sp. strain DO1.

[b] Feature 8, 17:1 *cis*-9 FAME or 17:2 FAME (16.803 ECL). Feature 10, 18:1C11/T9/T6 FAME or un-17.834 ECL. Feature 12, un-18.622 ECL or 19:0 i FAME.

[c] DMA, dimethylacetyl.

ATCC 25923, the positive xylose reaction, and the mostly negative acid phosphatase and beta-glucosamidase results of APL strains (5, 9, 31).

True *A. pyogenes* strains are a relatively homogeneous group. There was some minor variation in the rDNA patterns of two strains, as has to be expected since ribotyping has allowed subtyping within most of the species analyzed so far (21, 34).

The minimal cross-hybridization between the groups of APL bacteria shows that they are new species rather than subspecies of *A. pyogenes*. Naming new species is not warranted unless there are biochemical parameters that allow their identification without DNA hybridization. Biochemical markers for reliable differentiation into these groups, however, could not be found. Further investigation of these strains will require analysis of cell walls (40), DNA base composition, and isoprenoid quinone type (8).

In recent years, numerous new *Actinomyces* species have been described from human (*A. georgiae* and *A. gerencseriae*) (25) and animal (*A. denticolens*, *A. hordeovulneris*, *A. howellii*, and *A. slackii*) (6, 10–12) sources. As stated by Johnson et al. (25), it continues to be difficult to differentiate between *Actinomyces* species by the usual biochemical tests, owing to great variations within a species defined by DNA homology and serology. A recent study using the RapID ANA II and API ZYM systems has shown that there are probably more organisms similar to *Actinomyces* spp. which require further study (5).

Owing to the mixed nature of the infections, the pathogenic role of gram-positive rods resembling *A. pyogenes* is difficult to define. *A. pyogenes* itself is a well-known animal pathogen and has been described as a causative agent of bovine mastitis and other pyogenic infections in various domestic animals (17, 31, 33). Reports of human infections are rare: an epidemic of leg ulcers in school children has been reported from Thailand (28). There are single-case reports of empyema and bacteremia (2, 7); our isolate AP18 also originated from a bacteremic patient living on a farm. Gahrn-Hansen and Frederiksen recently reported 11 human cases of *A. pyogenes* infections, mainly various abscesses, collected since 1968 (17).

Our report is meant to draw the attention of bacteriologists to the fact that gram-positive rods resembling *A. pyogenes* are isolated with increasing frequency from clinical specimens. Part of this increase is probably due to higher awareness of infections by gram-positive rods. The exact pathogenic potential of APL bacteria has to be evaluated further. It may well be that the different DNA groups do not have the same virulence potential. At least it seems that APL bacteria are implicated in synergistic infections with staphylococci, streptococci, gram-negative bacteria, and especially anaerobes.

One problem of correct recognition of many *Actinomyces* spp. is posed by their ability also to grow aerobically to some extent and the fact that this property is not taken into account by most of the traditional identification schemes used in clinical laboratories (9, 29) or used in conjunction with determinations of cellular fatty acids (4). As many *Actinomyces* spp. can grow fairly well aerobically (40), they should be included in tables and commercial systems used for identification of aerobically isolated gram-positive rods.

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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, England.
2. **Barnham, M.** 1988. *Actinomyces pyogenes* bacteremia in a patient with carcinoma of the colon. J. Infect. **17**:231–234.
3. **Bartlett, J. G.** 1990. Anaerobic bacteria: general concepts, p. 1828–1842. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill & Livingstone, New York.
4. **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.
5. **Brander, M. A., and H. R. Jousimies-Somer.** 1992. Evaluation of the RapID ANA II and API ZYM systems for identification of *Actinomyces* species from clinical specimens. J. Clin. Microbiol. **30**:3112–3116.
6. **Buchanan, A. M., J. L. Scott, M. A. Gerencser, B. L. Beaman, S. Jang, and E. L. Biberstein.** 1984. *Actinomyces hordeovulneris* sp. nov., an agent of canine actinomycosis. Int. J. Syst. Bacteriol. **34**:439–443.
7. **Chlosta, E. M., G. K. Richards, E. Wagner, and J. F. Holland.** 1970. An opportunistic infection with *Corynebacterium pyogenes* producing empyema. Am. J. Clin. Pathol. **53**:167–170.
8. **Collins, M. D., D. Jones, R. M. Kroppenstedt, and K. H. Schleifer.** 1982. Chemical studies as a guide to the classification of *Corynebacterium pyogenes* and "*Corynebacterium haemolyticum*." J. Gen. Microbiol. **128**:335–341.
9. **Coyle, M. B., and B. A. Lipsky.** 1990. Coryneform bacteria in infectious diseases: clinical and laboratory aspects. Clin. Microbiol. Rev. **3**:227–246.
10. **Dent, V. E., and R. A. D. Williams.** 1984. *Actinomyces howellii*, a new species from the dental plaque of dairy cattle. Int. J. Syst. Bacteriol. **34**:316–320.
11. **Dent, V. E., and R. A. D. Williams.** 1984. *Actinomyces denticolens* Dent & Williams sp. nov.: a new species from the dental plaque of cattle. J. Appl. Bacteriol. **56**:183–192.
12. **Dent, V. E., and R. A. D. Williams.** 1986. *Actinomyces slackii* sp. nov. from dental plaque of dairy cattle. Int. J. Syst. Bacteriol. **36**:392–395.
13. **Eykyn, S. J., and I. Phillips.** 1989. Miscellaneous anaerobic infections, p. 567–589. *In* S. M. Finegold and L. W. George (ed.), Anaerobic infections in humans. Academic Press, San Diego.
14. **Facklam, R. R., and J. A. Washington II.** 1991. *Streptococcus* and related catalase-negative gram-positive cocci, p. 238–257. *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.
15. **Farmer, J. J., III, and M. T. Kelly.** 1991. *Enterobacteriaceae*, p. 360–383. *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. **Freney, J., M. T. Duperron, C. Courtier, W. Hansen, F. Allard, J. M. Boeufgras, D. Monget, and J. Fleurette.** 1991. Evaluation of API Coryne in comparison with conventional methods for identifying coryneform bacteria. J. Clin. Microbiol. **29**:38–41.
17. **Gahrn-Hansen, B., and W. Frederiksen.** 1992. Human infections with *Actinomyces pyogenes* (*Corynebacterium pyogenes*). Diagn. Microbiol. Infect. Dis. **15**:349–354.
18. **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.
19. **Ghanem, F. M., A. C. Ridpath, W. E. C. Moore, and L. V. H. Moore.** 1991. Identification of *Clostridium botulinum*, *Clostridium argentinense*, and related organisms by cellular fatty acid analysis. J. Clin. Microbiol. **29**:1114–1124.
20. **Gilardi, G. L.** 1991. *Pseudomonas* and related genera, p. 429–441. *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.

21. **Grimont, F., and P. A. D. Grimont.** 1986. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann. Inst. Pasteur **137B**:165–175.
22. **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.
23. **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.
24. **Hollis, D. G., and R. E. Weaver.** 1981. Gram-positive organisms: a guide to identification. Special Bacteriology Section, Centers for Disease Control, Atlanta.
25. **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. **30**:273–286.
26. **Jorgensen, J. H., A. W. Howell, and L. A. Maher.** 1991. Quantitative antimicrobial susceptibility testing of *Haemophilus influenzae* and *Streptococcus pneumoniae* by using the E-test. J. Clin. Microbiol. **29**:109–114.
27. **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.
28. **Kotrajaras, R., and H. Tagami.** 1987. *Corynebacterium pyogenes*. Its pathogenic mechanism in epidemic leg ulcers in Thailand. Int. J. Dermatol. **26**:45–50.
29. **Krech, T., and D. G. Hollis.** 1991. 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.
30. **Lämmler, C., and H. Blobel.** 1986. Tentative identification of *Actinomyces pyogenes* with antisera against group G streptococci. Zentralbl. Bakteriol. Hyg. A **262**:357–360.
31. **Lämmler, C., and H. Blobel.** 1988. Comparative studies on *Actinomyces pyogenes* and *Arcanobacterium haemolyticum*. Med. Microbiol. Immunol. **177**:109–114.
32. **Land, G. L., M. R. McGinnis, J. Staneck, and A. Gatson.** 1991. Aerobic pathogenic *Actinomycetales*, p. 340–359. *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.
33. **Madsen, M., G. H. Sorensen, B. Aalbaek, J. W. Hansen, and H. Bjorn.** 1992. Summer mastitis in heifers—studies on the seasonal occurrence of *Actinomyces pyogenes*, *Peptostreptococcus indolicus* and *Bacteroidaceae* in clinically healthy cattle in Denmark. Vet. Microbiol. **30**:243–255.
34. **Martinetti, G., and M. Altwegg.** 1990. rRNA gene restriction patterns and plasmid analysis as a tool for typing *Salmonella enteritidis*. Res. Microbiol. **141**:1151–1162.
35. **Morris, A., and I. Guild.** 1991. Endocarditis due to *Corynebacterium pseudodiphtheriticum*. Rev. Infect. Dis. **13**:887–892.
36. **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.
37. **Pitcher, D., A. Soto, F. Soriano, and P. Valeroguillen.** 1992. Classification of coryneform bacteria associated with human urinary tract infection (group D2) as *Corynebacterium urealyticum* sp. nov. Int. J. Syst. Bacteriol. **42**:178–181.
38. **Reddy, C. A., C. P. Cornell, and A. M. Fraga.** 1982. Transfer of *Corynebacterium pyogenes* (Glage) Eberson to the genus *Actinomyces* as *Actinomyces pyogenes* (Glage) comb. nov. Int. J. Syst. Bacteriol. **32**:419–429.
39. **Sasser, M., and M. D. Wichman.** 1991. Identification of microorganisms through use of gas chromatography and high-performance liquid chromatography, p. 111–118. *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.
40. **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.
41. **Schaal, K. P. (University of Bonn, Bonn, Germany).** Personal communication.
42. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1985. Wadsworth anaerobic bacteriology manual, 4th ed. Star Publishing Co., Belmont, Calif.
43. **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.
44. **Zuber, P. L. F., E. Gruner, M. Altwegg, and A. von Graevenitz.** 1992. Invasive infection with non-toxigenic *Corynebacterium diphtheriae* among drug users. Lancet **339**:1359.