Taxonomic Study of *Corynebacterium* Group ANF-1 Strains: Proposal of *Corynebacterium afermentans* sp. nov. Containing the Subspecies *C. afermentans* subsp. *afermentans* subsp. nov. and *C. afermentans* subsp. *lipophilum* subsp. nov.

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We have determined the cell wall composition, guanine-plus-cytosine (G+C) contents of the DNA, rRNA gene restriction patterns, and the levels of DNA-DNA relatedness of 11 strains identified biochemically as Centers for Disease Control (CDC) *Corynebacterium* group absolute nonfermenter 1 (*Corynebacterium* group ANF-1). For seven of these strains, growth is abundant on 5% sheep blood agar, which differentiates them from the four other strains, whose growth requires a lipid supplement such as Tween 80. Two of the lipid-requiring strains produced mucoid colonies on 1% Tween 80-supplemented sheep blood agar. All strains possess cell wall component type IV, short-chain mycolic acids, and G+C contents of DNA of 66 to 68 mol% as determined by reverse-phase high-performance liquid chromatography. DNA-relatedness experiments by an S1 nuclease procedure showed that nine of these strains, including two of the lipid-requiring strains, constitute a new genomic species less than 40% related to *Corynebacterium* species and other coryneform groups. The lipid-requiring strain T18502 exhibited 98% DNA relatedness with another lipid-requiring strain, T88593 (difference in thermal denaturation midpoint [ΔT_m] = 2°C) and 71 to 77% similarity with the nonlipophilic strains (ΔT_m range of from 2 to 5°C). Conversely, the DNA relatedness between strain LCDC 88199 and the six other nonlipophilic strains ranged from 86 to 100% (ΔT_m range of from 1 to 3°C) and was only 73 and 76% with the lipophilic strains T18502 and T88593, respectively (ΔT_m , 3 and 4°C). These results indicated that these two cultural types of bacteria constitute two subspecies within the new genomic species. These subspecies can be identified within the genus *Corynebacterium* by their phenotypic characteristics and rRNA gene restriction patterns by *Pvu*II and *Eco*RI endonuclease digestion. The two mucoid strains were not related to other *Corynebacterium* group ANF-1 strains or *Corynebacterium* species reference strains. Further studies should allow the determination of the taxonomic status of these mucoid strains. Therefore, we propose a new species, *Corynebacterium afermentans* sp. nov., which contains two subspecies: *C. afermentans* subsp. *afermentans* subsp. nov. (type strain, LCDC 88199 = CIP 103499) for nonlipophilic *Corynebacterium* group ANF-1 strains and *C. afermentans* subsp. *lipophilum* subsp. nov. (type strain, T18502 = CIP 103500) for two lipid-requiring *Corynebacterium* group ANF-1 strains.

Corynebacterium group absolute nonfermenter 1 (ANF-1) is a bacterial taxon first described in 1981 at the Centers for Disease Control (CDC) (Atlanta, Ga.) in a guide to the identification of medically important gram-positive organisms (12). This unnamed taxon group consists of pleomorphic gram-positive rods which do not produce acid from any sugars and do not possess urease activity. *Corynebacterium* group ANF-1 differs from *Corynebacterium* group ANF-3 in its inability to reduce nitrate to nitrite.

Although their natural habitat is not well known, *Corynebacterium* group ANF-1 strains have been recovered from human clinical specimens, particularly from blood samples and ears (12), but no clinical infections were reported for this taxon group in a review of the clinical aspects of coryneform bacteria (5).

The taxonomic status of *Corynebacterium* group ANF-1 is not clearly established. The presence of straight-chain or unsaturated cellular fatty acids (2) and the presence of short-chain mycolic acids in some strains (7) were reported to be common features for this and other *Corynebacterium* species, but no complete chemotaxonomic study and, to

date, no genomic studies have been performed. Therefore, the purpose of this study was to characterize *Corynebacterium* group ANF-1 strains by cell wall composition, G+C contents of DNA, ribotyping, and DNA-DNA hybridization.

We studied 11 strains which have been identified according to the biochemical classification of the CDC as belonging to *Corynebacterium* group ANF-1. We compared biochemical properties and determined the genetic relationship of these *Corynebacterium* group ANF-1 strains with medically relevant *Corynebacterium* species and related organisms. On the basis of DNA-DNA hybridization and ribotyping results, we propose that two types of *Corynebacterium* group ANF-1 should be recognized as subspecies of a new species in the genus *Corynebacterium* for which we propose the name *Corynebacterium afermentans*.

MATERIALS AND METHODS

Bacterial strains. The 11 strains identified as *Corynebacterium* group ANF-1 and the reference strains or clinical isolates of *Corynebacterium* species and related organisms studied are listed in Table 1.

Corynebacterium group ANF-1 LCDC 88199 was a gift from K. A. Bernard, Laboratory Centre for Disease Control

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TABLE 1. G+C contents of and levels of DNA relatedness between strains of *C. afermentans* and other *Corynebacterium* species or related organisms

Source(s) of DNA ^a	G+C content (mol%) ^b	% Relative binding at 65°C with the following labeled strain ^c :	
		LCDC 88199 ^T	T18502 ^T
<i>C. afermentans</i> subsp. <i>afermentans</i>			
LCDC 88199 ^T	66	100 (0.0)	71 (4.0)
T53478	66	96 (1.0)	73 (4.0)
T94156	66	100 (1.0)	73 (3.5)
T18953	66	86 (2.0)	76 (3.5)
T8354	66	98 (1.0)	77 (5.0)
T88284	66	100 (2.0)	76 (2.0)
T51462	67	94 (3.0)	76 (3.0)
<i>C. afermentans</i> subsp. <i>lipophilum</i>			
T18502 ^T	68	73 (4.0)	100 (0.0)
T88593	ND ^d	76 (3.0)	98 (2.0)
<i>C. bovis</i> ATCC 7715 ^T	74	11	
<i>C. cystitidis</i> CIP 69.40	61	3	
<i>C. diphtheriae</i> NCTC 380	55	3	
<i>C. glutamicum</i> IAM 12435 ^T	55	3	
<i>C. jeikeium</i> CIP 82.51	63	6	
<i>C. minutissimum</i> ATCC 23348 ^T	61	16	
<i>C. minutissimum</i> ATCC 23346	62	8	
<i>C. mycetoides</i> CIP 55.51 ^T	68	24	40
<i>C. pilosum</i> ATCC 29592 ^T	62	10	
<i>C. pseudodiphtheriticum</i> CIP 102940	55	6	
<i>C. pseudotuberculosis</i> CIP 52.97	54	18	
<i>C. renale</i> CIP 69.37	61	5	
<i>C. striatum</i> ATCC 6940 ^T	61	10	
<i>C. urealyticum</i> ATCC 43042 ^T	65	1	12
<i>C. xerosis</i> ATCC 373 ^T	71	5	
<i>Corynebacterium</i> group ANF-1 like			
T30014	66	30 (8.0)	37 (9.5)
T71705	ND	39	33
<i>Corynebacterium</i> group ANF-3 B77159	57	12	
<i>Corynebacterium</i> group F-1 G5911	63	21	
<i>Corynebacterium</i> group G-1 F8156	62	13	
<i>Corynebacterium</i> group G-2 G795	62	12	
<i>Corynebacterium</i> group I-1 F435	62	1	

^a *Corynebacterium* groups F-1, G-1, G-2, and I-1 strains were a generous gift from R. E. Weaver, CDC. *Corynebacterium* group ANF-3 B77159 was isolated in our laboratory.

^b G+C contents are data from this study and reference 16.

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

^d ND, not determined.

(Ottawa, Ontario, Canada). The other 10 *Corynebacterium* group ANF-1 clinical strains had been isolated from blood cultures (strains T53478, T94156, T18953, T8354, T51462, T18502, T88593, T30014, T88284, and T71705). These clinical isolates were from patients hospitalized in Strasbourg (France). The following strains of recognized *Corynebacte-*

rium species were obtained from the Collection of the Institut Pasteur (CIP), the American Type Culture Collection (ATCC), the National Collection of Type Cultures (NCTC), and the Institute of Applied Microbiology (IAM): *C. bovis* NCTC 3324 (ATCC 7715, type strain), *C. cystitidis* CIP 69.40, *C. diphtheriae* CIP A102 (Park-Williams 8), *C. glutamicum* IAM 12435 (ATCC 13022, type strain), *C. jeikeium* CIP 82.51, *C. minutissimum* NCTC 10288 (ATCC 23348, type strain) and ATCC 23346, *C. mycetoides* CIP 55.51 (ATCC 21134, type strain), *C. pilosum* ATCC 29592 (type strain), *C. pseudodiphtheriticum* CIP 102940, *C. pseudotuberculosis* bv. *equi* CIP 52.97, *C. renale* CIP 69.37, *C. striatum* CIP 81.15 (ATCC 6940, type strain), *C. urealyticum* ATCC 43042 (type strain), and *C. xerosis* ATCC 373 (type strain). The reference strains which are not the type strains of the named species were verified to possess the same biochemical characteristics as and G+C% contents of DNA similar to those of those reported for the type strains (4, 14). The strains of *Corynebacterium* groups F-1 (G5911), G-1 (F8156), G-2 (G795), and I-1 (F435) were a generous gift from R. E. Weaver, CDC. The *Corynebacterium* group ANF-3 strain B77159 was isolated in our laboratory from a human respiratory tract specimen and identified according to the method of Hollis and Weaver (12).

Growth conditions and biochemical tests. Bacteria were grown aerobically at 37°C on 5% sheep blood agar (Trypticase soy agar, Biomérieux, Marcy l'Etoile, France) and on 5% sheep blood agar supplemented with 1% (vol/vol) Tween 80 (polyoxyethylene sorbitan mono-oleate) (Merck, Darmstadt, Germany).

The procedures used for the determination of biochemical characteristics have been described elsewhere (16). The isolates were identified according to the method of Hollis and Weaver (12). Fermentation tests were verified with API Coryne systems (Biomérieux). Enzymatic activities were examined by the API Zym system according to the manufacturer's instructions (Biomérieux). Assimilation tests were performed by the API 20 NE system. For acetate, propionate, and lactate assimilation tests, a mineral base (15) was supplemented with 2 g of these components per liter. Antimicrobial susceptibilities were determined by the Bauer diffusion method with 5% sheep blood agar and an inoculum of approximately 10^5 CFU/ml (1).

Cell wall analysis. Samples for amino acid and sugar determinations were prepared and separated by thin-layer chromatography, and the components were identified as described previously (20). Reverse-phase high-performance liquid chromatography was performed for the determination of mycolic acids. Saponification, derivatization conditions, and comparison of mycolic acid patterns were carried out by following a previously published procedure (7).

DNA base composition. Cultures were grown for 48 h on Mueller-Hinton agar (Oxoid, Basingstoke, England) supplemented with 1% (vol/vol) Tween 80. Cells were harvested and resuspended in 20 ml of 0.2 M sucrose–0.05 M Tris-HCl (pH 8.0). Incubation at 37°C for 2 h in the presence of 2.5 mg of lysozyme per ml was followed by the addition of 10 mg of sodium dodecyl sulfate (SDS) at 55°C for 2 h. DNA was then extracted and purified by the method of Brenner et al. (3). After being purified on cesium chloride gradients, the DNA for G+C content determination was hydrolyzed with DNase I and nuclease P1 (8) and the moles percent G+C was determined by reverse-phase high-performance liquid chromatography of the deoxynucleotides as described previously (16).

DNA-DNA hybridization. DNAs from *Corynebacterium*

group ANF-1 strains LCDC 88199 and T18502 were labeled by nick translation with [^3H]dCTP (Amersham International, Amersham, United Kingdom) with a commercial kit (Boehringer GmbH, Mannheim, Germany).

DNA-DNA hybridization was carried out at 65°C for 16 h in 0.42 M NaCl by the S1 nuclease-trichloroacetic method (11). The denaturation temperature (T_m) at which 50% of the reassociated DNA becomes hydrolyzable by S1 nuclease was determined in 0.2 M NaCl by the method of Crosa et al. (6). ΔT_m is the difference between the T_m of a homoduplex (in a homologous reaction) and the T_m of heteroduplexes (in heterologous reactions).

Ribotyping. Ribotyping was performed as described by Grimont and Grimont (9). DNA was digested for 4 h at 37°C with *Eco*RI or *Pvu*II restriction endonuclease. DNA fragments were separated overnight by agarose gel electrophoresis (2 V/cm) in 0.5× TEB (10× TEB is 0.89 M Tris, 0.89 M boric acid, and 25 mM EDTA Na₂ [pH 8.3]) and then transferred onto an Immobilon P membrane (Millipore) with 0.5 M NaOH by the method of Southern (19). Prehybridization was performed in 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM sodium phosphate, and 1 mM EDTA [pH 7.7]) (17), 5× Denhardt's solution (17), and 0.1% (wt/vol) SDS at 50°C for 2 h. *Escherichia coli* 16S and 23S rRNAs, previously dephosphorylated with calf intestine alkaline phosphatase (Boehringer), were 5' labeled with [γ - ^{32}P]ATP by T4 polynucleotide kinase. Hybridization between *Corynebacterium* DNA and *E. coli* rRNA was performed overnight at 60°C in the prehybridization solution supplemented with 2.5×10^6 dpm (50 ng) of 5'- ^{32}P -labeled rRNA per 20 ml and per membrane. After hybridization, the filters were washed twice in 0.1× SSPE-0.1% (wt/vol) SDS at 37°C for 15 min. Hybridized bands were visualized by autoradiography at -70°C with intensifying screens for a maximum of 24 h.

RESULTS

Phenotypic characterization. The 11 strains were identified as belonging to *Corynebacterium* group ANF-1 by Hollis and Weaver's identification scheme (12). These strains do not produce acid from any sugars or nitrite from nitrate, and they do not possess urease activity. Four of these strains (T18502, T88593, T30014, and T71705) showed a lipid requirement, such as Tween 80, which has not been described for this taxon group. Tween 80 is the most commonly used component in studies characterizing lipophilic or lipid-dependent strains. The term "lipid requiring" is appropriate for the four strains of *Corynebacterium* group ANF-1 with which no visible growth occurs in brain heart infusion broth in 48 h and with which only very small colonies appear after several days on sheep blood agar alone. In contrast, on 1% Tween 80-supplemented sheep blood agar, large colonies are observed in 48 h. Furthermore, we observed that two of these nonfermenter lipid-requiring strains (T30014 and T71705) developed mucoid colonies on the Tween 80-supplemented medium. The nonlipophilic *Corynebacterium* group ANF-1 strains show widespread growth on either sheep blood agar or 1% Tween 80-supplemented sheep blood agar (Fig. 1).

By a high-performance liquid chromatography method which has been validated as a rapid and reliable method for the identification of coryneform bacteria (7), the esters of mycolic acids from the seven nonlipophilic *Corynebacterium* group ANF-1 strains and two of the lipid-requiring strains, T18502 and T88593, exhibited characteristic patterns which differentiated these strains from the mucoid *Corynebacte-*

FIG. 1. Growth of *Corynebacterium* group ANF-1 strains on 5% sheep blood agar (A and C) and 1% Tween 80-supplemented sheep blood agar (B and D) for 72 h at 37°C. (A and B) Nonlipophilic strain LCDC 88199^T; (C and D) lipid-requiring strain T18502^T.

rium group ANF-1 strains and the other *Corynebacterium* species.

Cell wall components and DNA base composition. The cell walls of the 11 strains identified as *Corynebacterium* group ANF-1 were found to contain cell wall component type IV (*meso*-diaminopimelic acid, arabinose, and galactose) and mycolic acids of short chain lengths (C_{26} to C_{36}). These results support the assignment of these strains to the genus *Corynebacterium* (4). The G+C contents of all strains studied are listed in Table 1. The values for nonlipophilic and Tween-requiring *Corynebacterium* group ANF-1 strains show a tight cluster (66 to 68 mol%) and are similar to those for *C. urealyticum* and *C. mycetoides*. The other *Corynebacterium* species and related organisms, notably *Corynebacterium* group ANF-3, showed quite different values.

DNA-DNA hybridization. As shown in Table 1, DNA-DNA hybridization of DNAs from *Corynebacterium* group ANF-1 strains with labeled DNAs from strains LCDC 88199^T and T18502^T of the two proposed subspecies, respectively, revealed the division of group ANF-1 into two genomic groups. The DNA relatedness between strain LCDC 88199^T and the other six nonlipophilic *Corynebacterium* group ANF-1 strains ranged from 86 to 100%, with a difference in melting temperature ΔT_m range of from 1 to 3°C (mean = 1.7°C). The DNA relatedness between strain LCDC 88199^T and two of the Tween-requiring strains, T18502^T and T88593, was 73 and 76%, respectively, and the ΔT_m was 3 and 4°C, respectively. Conversely, strain T18502^T exhibited 98% DNA relatedness with another Tween-requiring strain, T88593 (ΔT_m = 2°C), and 71 to 77% similarity with typical

FIG. 2. Schematic patterns of the different *Pvu*II ribotypes according to the length of each DNA fragment (in kilobases). Lanes: 1 and 2, lipid-requiring *Corynebacterium* group ANF-1 strains T18502^T and T88593, respectively; 3 to 9, nonlipophilic *Corynebacterium* group ANF-1 strains (lane 3, LCDC 88199^T); 10, *Corynebacterium* group ANF-1-like T30014; A, *C. diphtheriae* NCTC 380; B, *C. jeikeium* CIP 82.51; C, *C. urealyticum* ATCC 43042^T; D, *C. xerosis* ATCC 373^T; E, *C. pseudodiphtheriticum* CIP 102940; F, *C. striatum* ATCC 6940^T. The arrows indicate the bands differentiating the species and subspecies.

strains belonging to *Corynebacterium* group ANF-1 (ΔT_m range of from 2 to 5°C; mean = 3.6°C).

Corynebacterium species and mycolic acid-containing coryneform groups were 1 to 24% related to strain LCDC 88199^T at 65°C, and *Corynebacterium* species with G+C contents near 66% were 12 to 40% related to Tween-requiring *Corynebacterium* group ANF-1 strain T18502^T. The mucoid *Corynebacterium* group ANF-1 strains T30014 and T71705 exhibited 30 to 39% hybridization with DNA from either LCDC 88199^T or T18502^T. Further studies should allow the determination of the taxonomic status of these mucoid, lipid-requiring strains.

Ribotyping. In order to further characterize the *Corynebacterium* group ANF-1 strains, we determined the rRNA gene restriction patterns of these strains and those of some medically important *Corynebacterium* species. After *Pvu*II cleavage of DNA, heterogeneous but closely related patterns were observed for the seven nonlipophilic strains and for the two lipid-requiring strains T18502^T and T88593.

The DNA band profiles of these nine strains for this DNA restriction hydrolysis showed the presence of at least three common bands among the four bands of approximately 3.9, 4.9, 5.9, and 6.4 kb, which differentiate between the mucoid *Corynebacterium* group ANF-1 strain T30014 (two bands) and the other *Corynebacterium* species (one or no bands) (Fig. 2). The profiles of the two Tween-requiring strains T18502^T and T88593 showed an additional band of 4.6 kb that was not found in the other strains. The two mucoid *Corynebacterium* group ANF-1 strains T30014 and T71705 were clearly differentiated from the other *Corynebacterium* group ANF-1 strains by *Eco*RI endonuclease digestion of total DNAs (Fig. 3).

FIG. 3. rRNA gene restriction patterns after cleavage by *Eco*RI. The lengths of DNA fragments (in kilobases) were obtained by comparison with 5'-³²P-labeled *Bst*EII fragments of lambda DNA (lane 8). Lanes: 1 to 6, nonlipophilic *Corynebacterium* group ANF-1 strains (lane 1, LCDC 88199^T); 7 and 9, lipid-requiring *Corynebacterium* group ANF-1 strains T18502^T and T88593, respectively; 10 and 11, mucoid *Corynebacterium* group ANF-1 strains.

These results agree with DNA-DNA hybridization values and indicate that these two digestions with restriction enzymes may serve as a discriminatory genomic marker for studying rRNA genes of the *Corynebacterium* group ANF-1 strains within the genus *Corynebacterium*.

DISCUSSION

A species is considered to include strains with 70% or greater DNA-DNA relatedness and with 5°C or less ΔT_m (21). According to this generally accepted criterion defining a genomic species, the seven strains of nonlipophilic *Corynebacterium* group ANF-1 and the Tween-requiring strains T18502^T and T88593 constitute a new genomic species in the genus *Corynebacterium* for which we propose the name *C. afermentans*.

Thus, this chemotaxonomic and genomic study has confirmed that *Corynebacterium* group ANF-1, as described by Hollis and Weaver (12), is a distinct taxon group in the genus *Corynebacterium*. Moreover, we have found that lipid-requiring strains should be included in this group. In the genus *Corynebacterium*, a variant intermedius is described for lipophilic strains of *C. diphtheriae*, and Smith considered some lipid-requiring diphtheroids to be identical to *C. xerosis* on the basis of biochemical tests (18). Here, we have found that a single genomic group can contain both lipophilic and nonlipophilic strains.

The reassociation values and ΔT_m values indicate significant sequence divergence in this genomic species between the typical strains and two of the Tween-requiring strains. On the basis of frequency of distribution of divergence values, Grimont found that a ΔT_m of approximately 3°C corresponds to divergence values occurring among different

TABLE 2. Characteristics used for differentiation of two subspecies of *C. afermentans* from medically relevant *Corynebacterium* species^a

Species	Value ^b for the following characteristic:					
	Urease	Nitrate reductase	Lipid requirement	Acid from:		
				Glucose	Sucrose	Maltose
<i>C. afermentans</i> subsp. <i>afermentans</i>	—	—	—	—	—	—
<i>C. afermentans</i> subsp. <i>lipophilum</i>	—	—	+	—	—	—
<i>C. diphtheriae</i>	—	+	d	+	—	+
<i>C. bovis</i>	—	—	+	+	—	—
<i>C. jeikeium</i>	—	—	+	+	—	d
<i>C. minutissimum</i>	—	—	—	+	d	+
<i>C. pseudodiphtheriticum</i>	+	+	—	—	—	—
<i>C. pseudotuberculosis</i>	+	d	+	+	d	+
<i>C. renale</i>	+	—	—	+	—	d
<i>C. striatum</i>	—	+	—	+	+	—
<i>C. urealyticum</i>	+	—	+	—	—	—
<i>C. xerosis</i>	—	+	—	+	+	+

^a Data are from references 4 and 12 and our observations.^b +, 90% or more positive strains; —, 10% or less positive strains; d, 11 to 89% of positive strains.

subspecies within a species (10). Our results show a 3.6°C ΔT_m with levels of DNA-DNA relatedness of 70 to 80% between nonlipophilic strains and the Tween-requiring strains. Thus, we believe that these DNA-DNA hybridization and ΔT_m values provide sufficient genomic evidence to recognize these two closely related genomic groups at the subspecies level in this new species.

C. afermentans can be distinguished from other *Corynebacterium* species by its biochemical characteristics (Table 2). However, some difficulties in identifying coryneform isolates at the genus level have been reported (5). Thus, cell wall component analysis, including mycolic acid determination, may provide the identification at the genus level for coryneform strains which do not produce acid from sugars.

On the basis of culture, biochemical, and nucleic acid characteristics, we propose a new species, *C. afermentans* sp. nov., which contains two subspecies: *C. afermentans* subsp. *afermentans* subsp. nov., formerly nonlipophilic *Corynebacterium* group ANF-1 strains, and *C. afermentans* subsp. *lipophilum* subsp. nov., formerly lipid-requiring *Corynebacterium* group ANF-1 strains.

C. afermentans subsp. *afermentans* sp. nov. *C. afermentans* (a.fer.men'tans. Gr. pref. *a*, not; L. part. adj. *fermentans*, leavening; M.L. adj. *afermentans*, nonfermenting carbohydrates).

This description is based on observations of the authors and descriptions of the literature of *Corynebacterium* group ANF-1 strains (2, 4, 7, 12, 13).

The bacteria are gram-positive, irregular rods or coccobacilli that sometimes contain metachromatic granules. All are nonmotile and nonspore forming and are arranged in typical V-shaped forms or palisades. They are not acid fast, and no rod-coccus cycle is present. They are catalase positive and oxidase negative. They exhibit good growth under aerobic conditions and very slight growth under anaerobic conditions. Nonhemolytic colonies (1 to 2 mm in diameter) are grayish-white and smooth after 24 h at 37°C on fresh sheep blood agar. On 1% Tween 80-supplemented medium, bright colonies (2 mm in diameter) are beige, and Tween 80 esterase activity is detected. Acid is not produced from D-glucose, glycogen, lactose, sucrose, ribose, D-xylose, D-mannose, D-galactose, trehalose, and D-mannitol or on

triple sugar iron agar. Tyrosine, gelatin, DNA, starch, and urea are not degraded. Esculin is not hydrolyzed. The methyl red test is negative, and acetoin, indole, and H₂S (on triple sugar iron agar) are not produced. Hydrolysis of hippurate is variable. Growth is visible in 6.5% NaCl. Alkaline phosphatase, esterase, lipase, and acid phosphatase are produced. α -Galactosidase, β -galactosidase, α -glucuronidase, α -glucosidase, and β -glucosidase are not produced. Cells utilize acetate and lactate. Propionate, D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, and phenyl acetate are not utilized. The strains are susceptible to ampicillin, mezlocillin, piperacillin, ticarcillin, cephalotin, cefotaxime, gentamicin, netilmicin, amikacin, norfloxacin, ciprofloxacin, vancomycin, teicoplanin, rifampicin, pristinamycin, tetracycline, and fusidic acid, and some strains are susceptible to erythromycin, virginiamycin, and nitrofurantoin. All strains are resistant to fosfomycin.

The cell wall contains meso-diaminopimelic acid, arabinose, and galactose. Straight-chain saturated fatty acids are mainly palmitic and stearic acids. Branched saturated fatty acids are heptadecanoic acid and pentadecanoic acid. Straight-chain unsaturated fatty acids are mainly oleic acid and linoleic acid. The predominant types of mycolic acids are C_{30:0}, C_{30:1}, C_{32:1}, C_{33:1}, C_{34:2}, and C_{36:2}. The DNA base composition is 66 to 67 mol% G+C. *C. afermentans* subsp. *afermentans* strains were isolated from human clinical specimens such as blood samples, skin, and ears, but no pathogenic relationships were reported.

The type strain is LCDC 88199 (= CIP 103499). It was isolated in Canada from a human blood culture. Strain CIP 103499^T has all of the above properties for the species. Hippurate is not hydrolyzed. The G+C content of its DNA is 66 mol%.

C. afermentans subsp. *lipophilum* subsp. nov. The biochemical characteristics of *C. afermentans* subsp. *lipophilum* (li.po'phi.lum. Gr. n. *lipos*, animal fat; Gr. adj. *philos*, loving; M.L. adj. *lipophilus*, fat loving) are similar to those of *C. afermentans* subsp. *afermentans* except that growth is not visible in brain heart infusion broth and very small colonies appear after 48 h on sheep blood agar, but on 1% Tween 80-supplemented sheep blood agar, beige colonies

are large (2 mm in diameter) and without a mucoid consistency. Bacteria are pleomorphic gram-positive, nonspore-forming rods or coccobacilli that sometimes contain metachromatic granules. They are nonmotile and arranged in palisades and V shapes. They are not acid fast, and no rod-coccus cycle is present. They are catalase positive and oxidase negative. Acid is not produced from D-glucose, glycogen, lactose, sucrose, ribose, D-xylose, D-mannose, D-galactose, trehalose, and D-mannitol or on triple sugar iron. Tyrosine, gelatin, DNA, and urea are not degraded. Esculin is not hydrolyzed. The methyl red test is negative, and acetoin, indole, and H₂S (on triple sugar iron agar) are not produced. Hippurate is not hydrolyzed. Alkaline phosphatase, esterase, lipase, and acid phosphatase are produced. α -Galactosidase, β -galactosidase, α -glucuronidase, α -glucosidase, and β -glucosidase are not produced. Cells utilize acetate and lactate. Propionate, D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, and phenyl acetate are not utilized. The strains are susceptible to ampicillin, mezlocillin, piperacillin, ticarcillin, cephalotin, cefotaxime, gentamicin, netilmicin, amikacin, norfloxacin, ciprofloxacin, vancomycin, teicoplanin, rifampicin, pristinamycin, tetracycline, and fusidic acid, and some strains are susceptible to erythromycin. All strains are resistant to fosfomycin.

The cell wall contains *meso*-diaminopimelic acid, arabinose, galactose, and corynomycolic acids. Straight-chain saturated fatty acids are mainly palmitic and stearic acids. Branched saturated fatty acids are heptadecanoic acid. Straight-chain unsaturated fatty acids are mainly oleic acid, linoleic acid, and arachidonic acid. The DNA base composition is 68 mol% G+C. Bacteria were isolated from human blood cultures.

The type strain is strain T18502 (= CIP 103500). It was isolated at Strasbourg (France) from a human blood culture. Strain CIP 103500^T has the characteristics described for the species.

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