Assignment of Human-Derived CDC Group 1 Coryneform Bacteria and CDC Group 1-Like Coryneform Bacteria to the Genus *Actinomyces* as *Actinomyces neuui* subsp. *neuui* sp. nov., subsp. nov., and *Actinomyces neuui* subsp. *anitratus* subsp. nov.

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Almost the entire 16S rRNA gene sequences of some strains of CDC group 1 and group 1-like coryneform bacteria, isolated from human sources, were determined. Comparative analysis of the rRNA sequence data revealed that both groups of coryneforms belong to the genus *Actinomyces*. On the basis of the present molecular findings and previous biochemical studies, we propose a new *Actinomyces* species, *Actinomyces neuui* sp. nov., containing *Actinomyces neuui* subsp. *neuui* subsp. nov. for CDC group 1 coryneform bacteria and *Actinomyces neuui* subsp. *anitratus* subsp. nov. for CDC group 1-like coryneform bacteria.

In 1987, Na'was et al. (12) described some aerobically growing coryneform bacteria, isolated from clinical specimens, which were designated CDC (Centers for Disease Control) fermentative coryneform group 1. These nonmotile coryneforms are catalase positive; reduce nitrate; do not hydrolyze urea, esculin, or gelatin; and are characterized by their ability to ferment many carbohydrates (e.g., glucose, maltose, sucrose, lactose, mannitol, and xylose) used in the routine panel for identification of coryneforms. Recently, we reported the isolation, from human sources, of biochemically similar but nevertheless different (e.g., unable to reduce nitrate) bacteria which were referred to as CDC group 1-like coryneform bacteria (7). Both CDC group 1 and group 1-like coryneform bacteria were frequently isolated from abscesses in association with mixed anaerobic flora. Studies of volatile and nonvolatile metabolic and cellular fatty acid patterns as well as the composition of cell wall and membrane components (lack of *meso*-diaminopimelic acid and mycolic acids) (14) prompted us to suggest that CDC group 1 and group 1-like coryneform bacteria belong to the genus *Actinomyces* (7). In this report, we have determined the nucleotide sequence of the gene encoding the 16S rRNA of CDC group 1 and group 1-like coryneform bacteria in order to establish their exact phylogenetic position.

MATERIALS AND METHODS

Bacterial strains. The CDC group 1 coryneform bacterium (strain 97/90^T) was isolated from an infected mammary hematoma, and the CDC group 1-like bacterium (strain 50/90^T) was obtained from a blood culture. Both isolates were from patients hospitalized in Zürich, Switzerland.

Growth conditions and biochemical tests. Bacteria were grown on Columbia agar base (Becton Dickinson, Cockeysville, Md.) supplemented with 5% sheep blood at 37°C in a

5% CO₂ atmosphere. Cells were harvested after 48 h of incubation. The biochemical testing methods have been described in detail previously (7). In addition, fermentation of carbohydrates was determined with the API 50CH system (API bioMérieux, Marcy l'Etoile, France). Fermentation reactions were recorded after 5 days of incubation at 37°C.

Sequence determination of rRNA genes. Total genomic DNA was isolated with a standard miniprep method outlined by Ausubel et al. (1). A large fragment of the 16S rRNA gene was amplified by PCR using the universal primers pA (8 to 28, *Escherichia coli* numbering [3]) and pH* (1542 to 1522) (2). Direct sequencing of the amplified product was performed with primers to conserved regions of the rRNA and the Sequenase version 2.0 sequencing kit (U.S. Biochemical Corp.) as described previously (10). The rRNA sequences determined were aligned with those of other published high-G+C-content actinomycetes (EMBL Data Library), and the similarity values were determined with the Beckman Microgenie program (13). Nucleotide substitution rates were calculated (9), and an unrooted phylogenetic tree was produced with the algorithm of Fitch and Margoliash (5, 6).

Nucleotide sequence accession number. The nucleotide sequences of the 16S rRNA of strains 97/90^T and 50/90^T have been deposited with GenBank (EMBL) under accession no. X71861 and X71862, respectively.

RESULTS AND DISCUSSION

The 16S rRNA genes of the human isolates 97/90^T (CDC group 1 coryneform bacteria) and 50/90^T (CDC group 1-like coryneform bacteria) were amplified in vitro, and their nucleotide sequences were determined directly. Figure 1 shows the aligned primary structure of the 16S rRNA of the two strains. The sequences determined consisted of 1,487 to 1,489 nucleotides ranging from positions 31 to 1519 (*E. coli* numbering). The two new 16S rRNA sequences were compared with those of other high-G+C-content gram-positive bacteria available from the EMBL Data Library. The highest degree of sequence relatedness was shown with members of

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 97/90 CGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATTGGTGC. . TTGCACTTTTGGTGAGAGTGGCGAACGGGTGAGTAACACGTGAGT
 50/90 CGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATTAGCGCTTTTGTGTTTTTGGTGAGAGTGGCGAACGGGTGAGTAACACGTGAGT

97/90 AACCTGCCCTTTTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACTGGATGTTCCGNCCTCCTCGCATGGGGTTGTTGGGAAAGGTTTTTCTGGATT
 50/90 AACCTGCCCTTTTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACTGGATGTTCCGNCCTCCTCGCATGGGGTTGTTGGGAAAGGTTTTTCTGGATT

97/90 GGGATGGGCTCGCGGCTTATCAGCTTGTGGTGGGGTGATGGCTTACCAAGGCTTTGACGGGTAGCCGGCCTGAGAGGGTGGTCCGTCACACTGGGACTG
 50/90 GGGATGGGCTCGCGGCTTATCAGCTTGTGGTGGGGTGATGGCTTACCAAGGCTTTGACGGGTAGCCGGCCTGAGAGGGTGGTCCGTCACACTGGGACTG

97/90 AGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGGATATTGCACAATGGACGCAAGTCTGATGCACCGACGTCGTGTGGGGGATGAAGGCCCTTCGG
 50/90 AGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGGATATTGCACAATGGACGCAAGTCTGATGCACCGACGTCGTGTGGGGGATGAAGGCCCTTCGG

97/90 GTTGTAACCTCCTTTCGCGCTGCGGAAAAGGCAGGGTTTTGGCCTTGTGTGATGGTAGTGGGTAAAGAAGCGCCGGCTCACTACGTGCCAGCAGCCGCGGTA
 50/90 GTTGTAACCTCCTTTCGCGCGCGGAAAAGGCAGGGTTTTGGCCTTGTGTGATGGTAGTGGGTAAAGAAGCGCCGGCTCACTACGTGCCAGCAGCCGCGGTA

97/90 ATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGTAGGCGGTTTGTGCGCTCTAGCGTTAAAGGCTCGGGCTTAACCCGGGTTTG
 50/90 ATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGTAGGCGGTTTGTGCGCTCTAGCGTTAAAGGCTCGGGCTTAACCCGGGTTTG

97/90 CGTTGGGTACGGGCGAGGCTTGAAGTGCAGTGGGGTAACTGGAATTCCTGGTGTAGCGGTGAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCG
 50/90 CGTTGGGTACGGGCGAGGCTTGAAGTGCAGTGGGGTAACTGGAATTCCTGGTGTAGCGGTGAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCG

97/90 GGTACTGGGCGCTTACTGACGCTGAGGAGCGAGAGCGTGGGGAGCGAACAGGATTAGATACCCCTGGTAGTCCATGCTGTAAACGTTGGGCACTAGGTGT
 50/90 GGTACTGGGCGCTTACTGACGCTGAGGAGCGAGAGCGTGGGGAGCGAACAGGATTAGATACCCCTGGTAGTCCATGCTGTAAACGTTGGGCACTAGGTGT

97/90 GGGGGCTGGTTTCATGGTTCCGCGCGTAGCTAACGCATTAAAGTGCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGGCC
 50/90 GGGGGCTGGTTTCATGGTTCCGCGCGTAGCTAACGCATTAAAGTGCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGGCC

97/90 CGCACAAAGCGCGGAGCATGCGGATTAAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCACCGCGAGACTGCAGAGATGTGGTTGCCCTTCG
 50/90 CGCACAAAGCGCGGAGCATGCGGATTAAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCACCGCGAGACTGCAGAGATGTGGTTGCCCTTCG

97/90 GGGTGGTGTGCAGGTGGTGCATGGTTGTGCTGAGCTCGTGTGCTGAGATGTTGGTTAAGTCCCGCAACGAGCGCAACCCCTTGTCTCATGTTGCCAGCACG
 50/90 GGGTGGTGTGCAGGTGGTGGTGCATGGTTGTGCTGAGCTCGTGTGCTGAGATGTTGGTTAAGTCCCGCAACGAGCGCAACCCCTTGTCTCATGTTGCCAGCACG

97/90 TTTGGGTGGGACTCGTGAGAGACTGCCGGGGTTAACTCGGAGGAAGGTGGGGATGACGTCAAATCATGATGCCCCATATGTCTTGGGCTTACGCGATGC
 50/90 TTATGGTGGGACTCGTGAGAGACTGCCGGGGTTAACTCGGAGGAAGGTGGGGATGACGTCAAATCATGATGCCCCATATGTCTTGGGCTTACGCGATGC

97/90 TACAGTGGTGTCTACAGAGGGTTGCTATTCCGTGAGGTTGAGCGAATCTCTTAAAGGGCATCTTGGTTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAG
 50/90 TACAGTGGTGTCTACAGAGGGTTGCTATTCCGTGAGGTTGAGCGAATCTCTTAAAGGGCATCTTGGTTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAG

97/90 GTGGAGTCGCTAGTAATCGCAGATGAGCATTGCTGCGGTGAATACGTTCTCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTTGGTAACACCCGA
 50/90 GTGGAGTCGCTAGTAATCGCAGATGAGCATTGCTGCGGTGAATACGTTCTCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTTGGTAACACCCGA

97/90 AGCCTGTGGCCTAACCTTTTTGGGGGAGTGGTGAAGGTGGGGCTAGCGATTGGGACGAAAGTCGTAACAAGGTAGCCGTACCGGAAGG
 50/90 AGCCTGTGGCCTAACCTTTTTGGGGGAGTGGTGAAGGTGGGGCTAGCGATTGGGACGAAAGTCGTAACAAGGTAGCCGTACCGGAAGG

FIG. 1. Nucleotide sequences of derived 16S rRNA of *A. neuui* subsp. *neuui* and *A. neuui* subsp. *anitratus*.

the genus *Actinomyces*. Percent similarity values based on a continuous stretch of approximately 1,330 nucleotides (from positions 107 to 1464, *E. coli* numbering) of CDC group 1 and group 1-like strains and *Actinomyces* species are shown in Table 1. Approximately 100 nucleotides at the 5' end of the rRNA were excluded from these calculations because of incomplete sequence data for some *Actinomyces* species (15)

and to eliminate possible alignment errors because of variations in the length of region V1. A phylogenetic tree constructed from a matrix of derived evolutionary distances showing the interrelationships of the two coryneform isolates and species of the genus *Actinomyces* is shown in Fig. 2.

Comparative sequence analyses show that the CDC group

TABLE 1. 16S rRNA sequence similarity between *A. neuui* and related species

Taxon	% of sequence similarity to taxon ^a :									
	2	3	4	5	6	7	8	9	10	11
1 <i>Actinomyces bovis</i>	94.8	95.2	87.2	88.4	91.4	90.1	88.2	88.2	88.3	85.1
2 <i>Actinomyces naeslundii</i>		98.1	87.2	89.0	91.3	90.6	88.1	88.1	88.2	85.4
3 <i>Actinomyces viscosus</i>			87.6	89.0	91.7	90.3	88.1	88.1	88.3	85.2
4 " <i>Actinomyces suis</i> "				91.2	88.1	88.5	87.8	87.7	89.5	83.6
5 <i>Actinomyces pyogenes</i>					88.9	88.6	88.3	88.3	94.1	83.8
6 <i>Actinomyces odontolyticus</i>						95.3	89.3	89.5	88.7	86.2
7 <i>Actinomyces hyovaginalis</i>							90.0	90.2	88.5	85.4
8 <i>Actinomyces neuui</i> subsp. <i>neuui</i>								99.7	88.6	82.4
9 <i>Actinomyces neuui</i> subsp. <i>anitratus</i>									88.9	82.4
10 <i>Arcanobacterium haemolyticum</i>										83.8
11 <i>Bifidobacterium bifidum</i>										

^a Values are based on a comparison of ca. 1,340 nucleotides. With a larger set of data with 1,489 nucleotides (including variable region V1), *A. neuui* subsp. *neuui* and *A. neuui* subsp. *anitratus* strains exhibited 99.3% sequence similarity.

FIG. 2. Unrooted tree showing the phylogenetic interrelationships of *A. neuii* subsp. *neuii*, *A. neuii* subsp. *anitratus*, and some other *Actinomyces* species. The network is based on a comparison of ca. 1,330 nucleotides.

1 and group 1-like strains are members of the genus *Actinomyces* (Table 1). A high degree of 16S rRNA sequence similarity was also found with *Arcanobacterium haemolyticum*, although two *Actinomyces* species (*A. hyovaginalis* and *A. odontolyticus*) showed higher degrees of similarity to CDC group 1 and group 1-like strains than *A. haemolyticum*. At present, the generic placement of *A. haemolyticum* is critical and needs further investigation. However, CDC group 1 and group 1-like strains are clearly clustered within the confines of the genus *Actinomyces*. It is evident from sequence similarity values and the treeing program that these two coryneform groups show no specific affinity with other *Actinomyces* species. In a recent investigation (7), qualitative chromosomal DNA-DNA pairing indicated that CDC group 1 and CDC group 1-like coryneform bacteria might represent distinct genospecies. The finding of a high degree of 16S rRNA sequence similarity (99.3%) between these groups is not inconsistent with these findings but simply demonstrates the close genealogical relatedness of these species. Despite this high overall degree of similarity, the two coryneform isolates could be distinguished by sequences in variable region V1 of their 16S rRNA (five mismatches, two unmatched) (Fig. 1). Partial sequence analysis of additional isolates (viz., CDC group 1 strains 222/92 and 268/92 and CDC group 1-like strains 219/92, 246/92, and 256/92) showed that the sequences of this region were indeed characteristic of the two groups and supports their genospecific distinctiveness. However, at present no quantitative DNA-DNA hybridization data support the proposal of two different species (16). Nevertheless, CDC group 1 and CDC group 1-like strains can be distinguished phenotypically (e.g., by their ability to reduce nitrate) (Table 2). On the basis of the present phylogenetic analysis and phenotypic studies (7), we consider that the CDC group 1 and CDC group 1-like bacteria should be classified as a new species of the genus *Actinomyces* containing two subspecies. The name *Actinomyces neuii* subsp. *neuii* is proposed for CDC group 1 coryneform bacteria, and *Actinomyces*

TABLE 2. Characteristics for differentiation of *A. neuii* from other *Actinomyces* species and *A. haemolyticum*^a

Species	Catalase	Beta-hemolysis on sheep blood	Nitrate reduction	Urease	Esculin hydrolysis	Fermentation of ^b :					CAMP reaction
						Glu	Mal	Suc	Man	Xyl	
<i>A. neuii</i> subsp. <i>neuii</i>	+	—	+	—	—	+	+	+	+	+	+
<i>A. neuii</i> subsp. <i>anitratus</i>	+	—	—	—	—	+	+	+	+	+	+
<i>A. bovis</i>	—	V ^c	—	—	V	+	V	+	—	—	ND ^d
<i>A. naeslundii</i> ^e	V	—	V	+	+	+	+	+	V	V	—
<i>A. viscosus</i>	+	—	+	V	V	+	+	+	—	V	—
" <i>A. suis</i> "	—	ND	+	ND	ND	+	+	+	V	—	ND
<i>A. pyogenes</i>	—	+	—	—	—	+	V	V	V	+	—
<i>A. odontolyticus</i>	—	—	+	—	V	+	V	+	—	V	—
<i>A. hyovaginalis</i>	—	—	+	—	+	+	+	+	ND	+	ND
<i>A. israelii</i>	—	—	V	—	+	+	+	+	V	+	—
<i>A. gerencseriae</i>	—	—	V	—	+	+	+	+	V	V	ND
<i>A. georgiae</i>	—	—	V	—	V	+	+	+	V	+	ND
<i>A. meyeri</i>	—	—	—	V	—	+	V	+	—	+	ND
<i>A. hordeovulneris</i>	(+)	ND	—	—	+	+	+	ND	—	+	ND
<i>A. haemolyticum</i>	—	+	—	—	—	+	+	V	—	—	Rev ^g

^a Data were compiled from reference 4, 8, 11, and 14 and unpublished data.

^b Abbreviations: Glu, glucose; Mal, maltose; Suc, sucrose; Man, mannitol; Xyl, xylose.

^c V, variable.

^d ND, no data.

^e Genospecies 1 and 2.

^f Often weak or delayed reaction.

^g Rev, reverse CAMP reaction.

neuii subsp. *anitratus* is proposed for CDC group 1-like strains.

***Actinomyces neuii* subsp. *neuii* sp. nov.** *A. neuii* (neu' .ii. N. L. gen. n. *neuii*, of Neu, named to honor Harold Neu, an authority in antimicrobial chemotherapy and infectious diseases). This description of morphological and physiological features is based on a study of 17 strains (references 7 and 12 and unpublished data).

Cells are gram positive and predominantly diphtheroidal, but coccobacilli may occur; the rods are mainly arranged in clusters or V or Y forms. Cells are nonmotile and non-spore forming. Colonies are circular, smooth, convex, opaque, more white than creamy in color, and with entire edges. Colony sizes range from 0.5 to 1.5 mm in diameter after 48 h of incubation in 5% CO₂ on sheep blood agar. Cells are facultatively anaerobic. Alpha-hemolysis is observed for most strains on sheep blood agar and for all strains on human blood agar. Cells are catalase positive. Acid is produced from D-arabitol, erythritol, D-fructose, D-galactose, 5-ketogluconate, D-glucose, glycerol, inositol, lactose, D-lyxose, maltose, D-mannitol, D-mannose, melezitose, melibiose, raffinose, D-ribose, sucrose, trehalose, and D-xylose. Some strains produce acid from *N*-acetylglucosamine, adonitol, L-arabitol, L-arabinose, cellobiose, dulcitol, D-fucose, L-fucose, gentiobiose, gluconate, α -methyl-D-glucoside, glycogen, inulin, α -methyl-D-mannoside, D-tagatose, and xylitol. Acid is not produced from D-arabinose, 2-ketogluconate, L-rhamnose, salicin, D-glucitol, L-sorbose, and L-xylose. Leucine arylamidase, α -galactosidase, β -galactosidase, α -glucosidase, α -mannosidase, and pyrazinamidase are positive. Alkaline phosphatase, valine arylamidase, trypsin, chymotrypsin, acid phosphatase, β -glucuronidase, and β -glucosidase are negative. Esculin, urea and gelatin are not hydrolyzed. Nitrate is reduced to nitrite. Straight-chain saturated fatty acids are mainly palmitic and stearic acids. Oleic acid is the predominant unsaturated fatty acid. DNA base composition is 55 to 58 mol% G+C (midpoint temperature). The strains are isolated mainly from abscesses in association with mixed anaerobic flora as well as from human blood cultures. The type strain 97/90 has been deposited in the Deutsche Sammlung für Mikroorganismen, Braunschweig, Germany, under no. DSM 8576. Strain 97/90^T has the characteristics described for the species, except that, in the variable carbohydrate fermentation tests, only gentiobiose, α -methyl-D-glucoside, and xylitol are positive. The G+C content of strain 97/90^T's DNA is 55 mol%.

***Actinomyces neuii* subsp. *anitratus* subsp. nov.** *A. neuii* subsp. *anitratus* (a.ni.tra'.tus. Gr. pref. *a*, not; L.n. *nitratus*, nitrate; M.L. adj. *anitratus*, not reducing nitrate). The following description of morphological and physiological features is based on nine strains (reference 7 and unpublished data).

The morphological characteristics of *A. neuii* subsp. *anitratus* are similar to those of *A. neuii* subsp. *neuii*, except that *A. neuii* subsp. *anitratus* is nonhemolytic. Cells are catalase positive. Acid is produced from adonitol, D-arabitol, erythritol, D-fructose, D-galactose, 5-ketogluconate, D-glucose, α -methyl-D-glucoside, glycerol, inositol, lactose, D-lyxose, maltose, D-mannitol, D-mannose, melezitose, melibiose, raffinose, D-ribose, sucrose, trehalose, and D-xylose. Some strains produce acid from *N*-acetylglucosamine, L-arabitol, cellobiose, dulcitol, D-fucose, L-fucose, gentiobiose, gluconate, glycogen, salicin, and D-tagatose. Acid is not produced from D-arabinose, L-arabinose, 2-ketogluconate, inulin, α -methyl-D-mannoside, L-rhamnose, D-glucitol, L-sorbose, xylitol, and L-xylose. Leucine arylamidase, α -

lactosidase, β -galactosidase, α -glucosidase, α -mannosidase, and pyrazinamidase are positive. Alkaline phosphatase activity can be observed in some strains. Valine arylamidase, trypsin, chymotrypsin, acid phosphatase, β -glucuronidase, and β -glucosidase are negative. Esculin, urea, and gelatin are not hydrolyzed. Nitrate is not reduced to nitrite. Straight-chain saturated fatty acids are mainly palmitic and stearic acids. Oleic acid is the predominant unsaturated fatty acid. The DNA base composition is 55 to 58 mol% G+C (midpoint temperature). The strains are isolated mainly from abscesses in association with mixed anaerobic flora as well as from human blood cultures. The type strain is strain 50/90 and has been deposited in the Deutsche Sammlung für Mikroorganismen under no. DSM 8577. Strain 50/90^T has the characteristics described for the species, except that, in the variable carbohydrate fermentation tests, only *N*-acetylglucosamine, gluconate, and salicin are positive. The G+C content of its DNA is 55 mol%.

ACKNOWLEDGMENT

M.D.C. and S.S. were supported by a grant from the EEC (HRAMI project, BIOT-CT91-0294 SSMA).

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