

Actinomyces europaeus sp. nov., Isolated from Human Clinical Specimens

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Ten strains of a hitherto undescribed catalase-negative, facultatively anaerobic, coryneform bacterium were isolated or collected by workers at three European clinical bacteriology laboratories or reference centers. These strains were isolated from humans, and most came from abscess material. Biochemical and chemotaxonomic characterization revealed that the strains belonged to the genus *Actinomyces*. The phenotypic features of the 10 strains were incompatible with the descriptions of the previously established *Actinomyces* species. A comparative 16S rRNA gene sequence analysis demonstrated that the previously undescribed strains constitute a new line in the genus *Actinomyces*. The name *Actinomyces europaeus* sp. nov. is proposed for these clinical isolates. The type strain is CCUG 32789A.

In the 1990s, there has been increasing recognition of the importance of coryneform bacteria (i.e., aerobic, asporogenous, irregular, non-partially acid-fast, gram-positive rods) as opportunistic human pathogens (8). As a result of increased medical interest in such organisms, combined with intensified taxonomic investigations, a number of new coryneform and related high-G+C-content bacteria have been described in recent years (8). The recognition of new coryneform pathogens in laboratories is complicated by the absence of reliable commercial identification systems with in-depth databases and the heterogeneity of the organisms encountered. Recent studies have shown, however, that the use of phenotypic approaches (biochemical profiles, lipids, cell walls) and molecular taxonomic approaches (e.g., 16S rRNA sequencing) in concert (polyphasic taxonomy) provides not only a powerful means for recognizing and delineating such organisms, but also the foundation for developing improved identification schemes. In this article we report the use of a polyphasic approach to characterize a group of 10 catalase-negative, facultatively anaerobic, coryneform bacterial strains obtained from human clinical specimens, which could not be assigned to any previously established taxon. Based on the results of the present study, a new species, *Actinomyces europaeus*, is proposed.

MATERIALS AND METHODS

Bacterial strains. The origins of the strains studied and their clinical sources are given in Table 1. Four strains were isolated by workers at the Department of Medical Microbiology, University of Zürich, Zürich, Switzerland, and three strains were isolated by workers at the Stichting Streeklaboratorium, Goes, The Netherlands, by standard methods (5). The three remaining isolates were referred to the Culture Collection of the University of Göteborg, Göteborg, Sweden.

Morphological and biochemical characteristics. The strains were grown aerobically at 37°C in a 5% CO₂-enriched atmosphere on Columbia agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% sheep blood. The same

medium was used to assess growth in a strictly anaerobic atmosphere (10% H₂, 10% CO₂, 80% N₂). The methods used to determine the biochemical profiles of the bacteria have been described in detail previously (5). Using the commercial API Coryne system (bioMérieux, Marcy l'Etoile, France), we read enzymatic reactions after 24 h of incubation at 37°C, whereas fermentation reactions were observed after 72 h of incubation. Additional enzymatic reactions were studied by using the API ZYM system (bioMérieux). The API 50CH system (carbohydrate fermentations) was used in conjunction with 50CHE medium (both obtained from bioMérieux), and reactions were read after 120 h of incubation at 37°C.

Antimicrobial agent susceptibility tests. MICs of 24 antimicrobial agents were determined by a microdilution method; 96-well microtiter plates contained the antibiotics in lyophilized form with one starting concentration and 11 twofold dilutions (i.e., there were eight antimicrobial agents per plate) (Merlin Diagnostics, Bornheim-Hersel, Germany). A bacterial suspension (McFarland standard 05) was prepared in 0.9% NaCl, 200 µl of the suspension was transferred into 10 ml of H-medium (Merlin), and 100 µl of this preparation was added to each well of the microtiter plate. The plates were incubated in a 5% CO₂-enriched atmosphere at 35°C. Bacterial growth was observed after 48 h by reading the microtiter plates with an automated Multiskan MS reader (Labsystems Oy, Helsinki, Finland) at a wavelength of 620 nm. An emission adsorbance value of ≥ 0.130 indicated growth. All of the plates were also examined by eye for bacterial growth, but no discrepancies were observed.

MIC₅₀ was the MIC of an antimicrobial agent at which 50% of the isolates were inhibited, and MIC₉₀ was the MIC at which 90% of the isolates were inhibited.

Gas-liquid chromatography. Cells were grown under anaerobic conditions in PRAS chopped meat carbohydrate broth (9) for 48 h at 37°C to determine the end products of fermentation. The detection method used has been described previously (5). Cellular fatty acid patterns were generated by using the Sherlock Microbial Identification System (Microbial ID, Inc., Newark, Del.) (21).

Cell wall analysis. The peptidoglycan structure was determined by the method of Schleifer and Kandler (18), except that ascending thin-layer chromatography on cellulose sheets (Merck, Darmstadt, Germany) was used.

PAGE of whole-cell proteins. Most strains were grown on horse blood agar (Columbia base) at 37°C in the presence of 5% CO₂; the only exception was *Actinomyces israelii* CCUG 18307^T, which was grown under anaerobic conditions. Polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins was performed as described previously (15). For densitometric analysis, normalization, and interpretation of protein patterns, the Gelcompar 3.1 software package (Applied Maths, Kortrijk, Belgium) was used. Levels of correlation are presented below as percentages of similarity.

DNA base composition. DNAs were prepared and G+C contents were determined as described previously (6).

Determination of 16S rRNA gene sequences and phylogenetic analyses. A large fragment of the 16S rRNA gene was amplified by PCR by using universal primers pA (5'-AGAGTTTGATCCTGGCTCAG; positions 8 to 27 [*Escherichia coli* numbering]) and pH* (5'-AAGGAGGTGATCCAGCCGA; positions

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TABLE 1. Origins of the strains studied

Strain ^a	Year isolated	Patient		Source
		Sex ^b	Age (yr)	
DMMZ 747 (= DSM 11076)	1993	F	50	Abscess
DMMZ 1113 (= DSM 11077 = CCUG 35467)	1994	F	54	Breast abscess
DMMZ 1284 (= DSM 11078 = CCUG 35470)	1995	M	27	Suprapubic abscess
DMMZ 2233	1996	M	35	Perianal abscess
CCUG 32789A ^T	1994	M	63	Femur tissue
CCUG 33355	1994	NK ^c	65	Abscess
CCUG 34687 (= CDC F7662)	1986	F	NK	Labial abscess
LMG 16599 (= CCUG 35554)	1986	M	57	Perianal abscess
LMG 16600 (= CCUG 35548)	1987	M	64	Decubital ulcer
LMG 16601 (= CCUG 35549)	1988	F	62	Atherom cyst

^a DMMZ, Department of Medical Microbiology, University of Zürich; DSM, German Collection of Microorganisms and Cell Cultures; CCUG, Culture Collection, Department of Clinical Bacteriology, University of Göteborg; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; LMG, Microbiology Laboratory, University of Ghent.

^b F, female; M, male.

^c NK, not known.

1541 to 1522) as previously described (11). The PCR products were purified by using a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions and were sequenced by using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, Calif.) and a model 373A automatic sequencer (Applied Biosystems, Inc.). The sequences determined and the sequences of other high-G+C-content actinomycetes (obtained from the EMBL Data Library) were aligned by using the program PILEUP (2), and the alignment was corrected manually. Distance matrices were produced by using the DNADIST program of the PHYLIP package (3). Unrooted phylogenetic trees were constructed with the algorithm of Fitch and Margoliash (4) and by using the neighbor-joining method of Saitou and Nei (16). The statistical significance of the groups obtained was assessed by bootstrapping (500 replicates) by using the programs SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE (3).

Nucleotide sequence accession number. The 16S rRNA gene sequence of strain CCUG 32789A^T which we determined has been deposited in the EMBL Data Library under accession no. Y08828.

RESULTS AND DISCUSSION

Seven of the 10 clinical strains were isolated from human abscesses (Table 1). In the majority of the cases the coryneform organism was the only aerobically growing bacterium, but on anaerobically incubated culture plates a mixed anaerobic flora (i.e., at least three morphologically different anaerobes) was detected. Therefore, the etiologic role of the unidentified coryneform bacterium as a pathogen remained unclear. Similar clinical sources have been reported for *Arcanobacterium bernardiae* (7), as well as for *Actinomyces radingae* and *Actinomyces turicensis* strains (13, 22, 23).

Colonies of the strains were translucent, grayish, and less than 0.5 mm in diameter after 48 h of incubation in a 5% CO₂-enriched atmosphere at 37°C. The growth rate under strictly anaerobic conditions was similar to the growth rate described above. Some strains exhibited very slight beta-hemolysis on sheep blood agar plates. Gram staining of the organisms revealed relatively short gram-positive rods with irregular morphology and no filament formation.

According to the general typing scheme for coryneform bacteria encountered in clinical specimens outlined by von Graevenitz and Funke (20), we observed the following biochemical characteristics: catalase-negative, fermentative, nonmotile rods; negative for nitrate reduction and urea hydrolysis; variable for hydrolysis of esculin; positive for fermentation of glucose and maltose but not for fermentation of mannitol and xylose; variable for fermentation of sucrose; and negative for the CAMP reaction (Table 2). All of the strains produced large amounts of succinic acid as the end product of glucose fermentation. The strains were, therefore, considered members of an *Actinomyces* species. The biochemical profile study was extended by using the API Coryne system. The results of the initial biochemical screening reactions were confirmed, and the organisms were tentatively designated Enevold Falsen (EF) group 43 bacteria, *Arcanobacterium bernardiae*-like bacteria, or CDC coryneform group E-like bacteria (10) by the different collaborators who participated in this joint project as the biochemical profile was incompatible with the profiles of previ-

TABLE 2. Characteristics that differentiate *Actinomyces europaeus* from other human-derived, aerobically growing *Actinomyces* and *Arcanobacterium* species^a

Species	Catalase activity	Nitrate reduction	Urease activity	Esculin hydrolysis	Fermentation of:					CAMP reaction	Pyrazinamidase activity	β-Galactosidase activity	α-Glucosidase activity	β-N-Acetylglucosaminidase activity	
					Glucose	Maltose	Sucrose	Mannitol	Xylose						Glycogen
<i>Actinomyces europaeus</i>	- ^b	-	-	V	+	+	V	-	-	V	-	-	+	+	-
<i>Arcanobacterium bernardiae</i>	-	-	-	-	+	+	-	-	-	+	-	+	-	+	-
<i>Arcanobacterium haemolyticum</i> ^c	-	-	-	-	+	+	V	-	-	-	REV	+	+	+	+
<i>Actinomyces naeslundii</i>	V	V	+	+	+	+	+	-	V	V	-	ND	V	V	-
<i>Actinomyces neuii</i>	+	V	-	-	+	+	+	+	+	-	+	+	+	+	-
<i>Actinomyces odontolyticus</i> ^d	-	+	-	V	+	V	+	-	V	V	-	ND	-	-	-
<i>Arcanobacterium pyogenes</i> ^c	-	-	-	V	+	V	V	V	+	+	-	-	+	+	-
<i>Actinomyces radingae</i>	-	-	-	+	+	+	+	V	+	-	-	+	+	+	+
<i>Actinomyces turicensis</i>	-	-	-	-	+	+	+	+	+	-	-	-	+	+	+
<i>Actinomyces viscosus</i>	+	+	V	V	+	+	+	-	V	V	-	ND	V	V	-

^a Data from references 5, 7, 8, 10, 12, 17, and 23.

^b -, negative; +, positive; V, variable; REV, reverse CAMP reaction; ND, no data available.

^c This organism exhibits beta-hemolysis on sheep blood agar.

^d A brown-red pigment is formed in older colonies.

ously established *Actinomyces* species (Table 2). The strains were initially designated *Arcanobacterium bernardiae*-like because they fermented maltose more rapidly than they fermented glucose, like *Arcanobacterium bernardiae* (8), and because their biochemical profile was similar to that of *Arcanobacterium bernardiae* (7), which ferments glycogen but not sucrose and which does not hydrolyze esculin (Table 2). The unknown coryneform bacteria also phenotypically resembled CDC coryneform group E bacteria (10, 13) (i.e., *Actinomyces radingae* and *Actinomyces turicensis* [23]) except that they did not ferment xylose (Table 2). If hydrolysis of esculin was positive, it was delayed (occurring only after 72 to 96 h of incubation), as was hydrolysis of gelatin (as determined by the API Coryne system), which was positive only after 5 days of incubation at 37°C.

The MICs of all of the penicillins tested against the 10 new coryneform strains were very low. For amoxicillin the MIC range was ≤ 0.0625 to 0.125 $\mu\text{g/ml}$, the MIC₅₀ was ≤ 0.0625 $\mu\text{g/ml}$, and the MIC₉₀ was ≤ 0.0625 $\mu\text{g/ml}$; for oxacillin the MIC range was 0.25 to 1 $\mu\text{g/ml}$, the MIC₅₀ was 0.5 $\mu\text{g/ml}$, and the MIC₉₀ was 1 $\mu\text{g/ml}$; for penicillin G the MIC range was ≤ 0.016 to 0.125 $\mu\text{g/ml}$, the MIC₅₀ was 0.032 $\mu\text{g/ml}$, and the MIC₉₀ was 0.0625 $\mu\text{g/ml}$; and for piperacillin the MIC range was ≤ 0.125 to 0.5 $\mu\text{g/ml}$, the MIC₅₀ was 0.25 $\mu\text{g/ml}$, and the MIC₉₀ was 0.25 $\mu\text{g/ml}$. The MICs of most cephalosporins were also very low. For cefazolin the MIC range was ≤ 0.125 to 0.5 $\mu\text{g/ml}$, the MIC₅₀ was ≤ 0.125 $\mu\text{g/ml}$, and the MIC₉₀ was 0.25 $\mu\text{g/ml}$; for cefoxitin the MIC range was 0.125 to 0.5 $\mu\text{g/ml}$, the MIC₅₀ was 0.125 $\mu\text{g/ml}$, and the MIC₉₀ was 0.25 $\mu\text{g/ml}$; and for ceftriaxone the MIC range was ≤ 0.032 to 0.25 $\mu\text{g/ml}$, the MIC₅₀ was 0.0625 $\mu\text{g/ml}$, and the MIC₉₀ was 0.25 $\mu\text{g/ml}$. However, the MICs of some cephalosporins were significantly higher. For cefetamet the MIC range was 8 to >64 $\mu\text{g/ml}$, the MIC₅₀ was 32 $\mu\text{g/ml}$, and the MIC₉₀ was >64 $\mu\text{g/ml}$; and for cefibuten the MIC range was 4 to 16 $\mu\text{g/ml}$, the MIC₅₀ was 8 $\mu\text{g/ml}$, and the MIC₉₀ was 8 $\mu\text{g/ml}$. For the carbapenem imipenem the MIC range was 0.125 to 0.25 $\mu\text{g/ml}$, the MIC₅₀ was 0.125 $\mu\text{g/ml}$, and the MIC₉₀ was 0.25 $\mu\text{g/ml}$; and for the carbapenem meropenem the MIC range was 0.0625 to 0.125 $\mu\text{g/ml}$, the MIC₅₀ was 0.0625 $\mu\text{g/ml}$, and the MIC₉₀ was 0.125 $\mu\text{g/ml}$. For the macrolide erythromycin the MIC range was ≤ 0.032 to 4 $\mu\text{g/ml}$, the MIC₅₀ was ≤ 0.032 $\mu\text{g/ml}$, and the MIC₉₀ was ≤ 0.032 $\mu\text{g/ml}$ (only strain DMMZ 1284 was resistant); and for the macrolide clarithromycin the MIC range for all of the strains was ≤ 0.032 to 2 $\mu\text{g/ml}$, the MIC₅₀ was ≤ 0.032 $\mu\text{g/ml}$, and the MIC₉₀ was ≤ 0.032 $\mu\text{g/ml}$. For the tetracycline doxycycline the MIC range was 0.25 to 16 $\mu\text{g/ml}$, the MIC₅₀ was 0.5 $\mu\text{g/ml}$, and the MIC₉₀ was 0.5 $\mu\text{g/ml}$ (only strain LMG 16600 was resistant); and for tetracycline the MIC range for all of the strains was 0.5 to 32 $\mu\text{g/ml}$, the MIC₅₀ was 1 $\mu\text{g/ml}$, and the MIC₉₀ was 2 $\mu\text{g/ml}$. For the glycopeptide teicoplanin the MIC range was ≤ 0.0625 to 0.25 $\mu\text{g/ml}$, the MIC₅₀ was 0.125 $\mu\text{g/ml}$, and the MIC₉₀ was 0.25 $\mu\text{g/ml}$; and for the glycopeptide vancomycin the MIC range was 0.5 to 1 $\mu\text{g/ml}$, the MIC₅₀ was 1 $\mu\text{g/ml}$, and the MIC₉₀ was 1 $\mu\text{g/ml}$. For chloramphenicol the MIC range was 0.25 to 1 $\mu\text{g/ml}$, the MIC₅₀ was 0.5 $\mu\text{g/ml}$, and the MIC₉₀ was 1 $\mu\text{g/ml}$; for clindamycin the MIC range for all of the strains tested was ≤ 0.0156 to >32 $\mu\text{g/ml}$, the MIC₅₀ was 0.032 $\mu\text{g/ml}$, and the MIC₉₀ was 0.032 $\mu\text{g/ml}$ (only strain DMMZ 1284 was resistant); and for rifampin the MIC range was ≤ 0.016 to 0.032 $\mu\text{g/ml}$, the MIC₅₀ was ≤ 0.016 $\mu\text{g/ml}$, and the MIC₉₀ was ≤ 0.016 $\mu\text{g/ml}$. For the aminoglycoside amikacin the MIC range was 4 to 16 $\mu\text{g/ml}$, the MIC₅₀ was 8 $\mu\text{g/ml}$, and the MIC₉₀ was 16 $\mu\text{g/ml}$; and for the aminoglycoside tobramycin the MIC range was 1 to 4 $\mu\text{g/ml}$, the MIC₅₀ was 2 $\mu\text{g/ml}$, and the MIC₉₀ was 4 $\mu\text{g/ml}$. Two quinolones were also

tested. For ciprofloxacin the MIC range was 0.5 to 1 $\mu\text{g/ml}$, the MIC₅₀ was 1 $\mu\text{g/ml}$, and the MIC₉₀ was 1 $\mu\text{g/ml}$; and for ofloxacin the MIC range was 2 to 4 $\mu\text{g/ml}$, the MIC₅₀ was 2 $\mu\text{g/ml}$, and the MIC₉₀ was 4 $\mu\text{g/ml}$. The susceptibility pattern described above is consistent with the susceptibility patterns obtained for almost all previously described *Actinomyces* species (17).

An analysis of the cellular fatty acids (CFAs) revealed that C_{16:0} (33% \pm 2% of the total CFAs [mean \pm standard deviation]), C_{18:1 ω 9} (22% \pm 3%), and C_{18:0} (20% \pm 3%) were the main CFAs. Significantly, C_{10:0} (1% \pm 1%), C_{12:0} (1% \pm 1%), and C_{14:0} (3% \pm 1%) were also detected; these CFAs are indicators that the strains should be assigned to the genus *Actinomyces* or the genus *Arcanobacterium* (1). The CFA profiles of the new coryneform bacterium both qualitatively and quantitatively matched the CFA profiles of CDC coryneform group E strains as reported by Bernard et al. (1). An analysis of partial peptidoglycan hydrolysates revealed that the murein type was the L-lysine-L-lysine-D-glutamic acid type (type A5 α), as has been described for *Actinomyces neuui* and *Arcanobacterium haemolyticum* (21a). The G+C contents of four representative strains of the previously undescribed coryneform bacterium were found to be 61 to 63 mol% values which are within the range of values obtained for members of the genus *Actinomyces* (17).

The whole-cell protein profiles of 7 of the 10 strains listed in Table 1 were examined by sodium dodecyl sulfate-PAGE. A dendrogram derived from a numerical analysis of the protein profiles is shown in Fig. 1. All seven strains grouped together and formed a distinct branch with a within-group correlation level of more than 80%. This indicated that the strains examined represent a homogeneous group and that they are distinct from all other *Actinomyces* and *Arcanobacterium* species examined.

To investigate the genetic relationships of the new coryneform bacterium, the genes encoding the 16S rRNAs of six strains were amplified by PCR and subjected to a sequence analysis. The almost complete 16S rRNA gene sequences of two strains (DMMZ 1113 and CCUG 32789A^T) were determined. Each sequence consisted of approximately 1,430 nucleotides, and only a single base difference was found between the two strains (level of sequence similarity, $>99.9\%$). Comparative sequence searches of the EMBL and GenBank libraries by using the FASTA program (2) revealed that the newly determined sequences were most closely related to sequences of species belonging to the genus *Actinomyces* (data not shown). The sequence of strain CCUG 32789A^T was subjected to a pairwise analysis with the sequences of *Actinomyces* spp. and some close relatives, and derived evolutionary distances were used to determine phylogenetic relationships. A tree depicting the phylogenetic position of strain CCUG 32789A^T within the genus *Actinomyces* is shown in Fig. 2, and the levels of sequence similarity between the new *Actinomyces* species and other *Actinomyces* spp. are shown in Table 3. The new organism formed a relatively long line and did not exhibit close affinity with any previously recognized *Actinomyces* species. The sequence divergence values (Table 3), with the closest relative exhibiting $<92\%$ 16S rRNA gene homology, clearly demonstrated (19) that the new strain represents a hitherto unknown subline within the genus *Actinomyces*. To determine whether the sequences of strains CCUG 32789A^T and DMMZ 1113 were characteristic of the group of isolates, partial sequences of four other strains (DMMZ 747, DMMZ 1284, CCUG 33355, and CCUG 34687) were determined. Short fragments (approximately 700 bases; positions 40 to 760 [*E. coli* numbering]) which included highly diagnostic regions V1 to V4

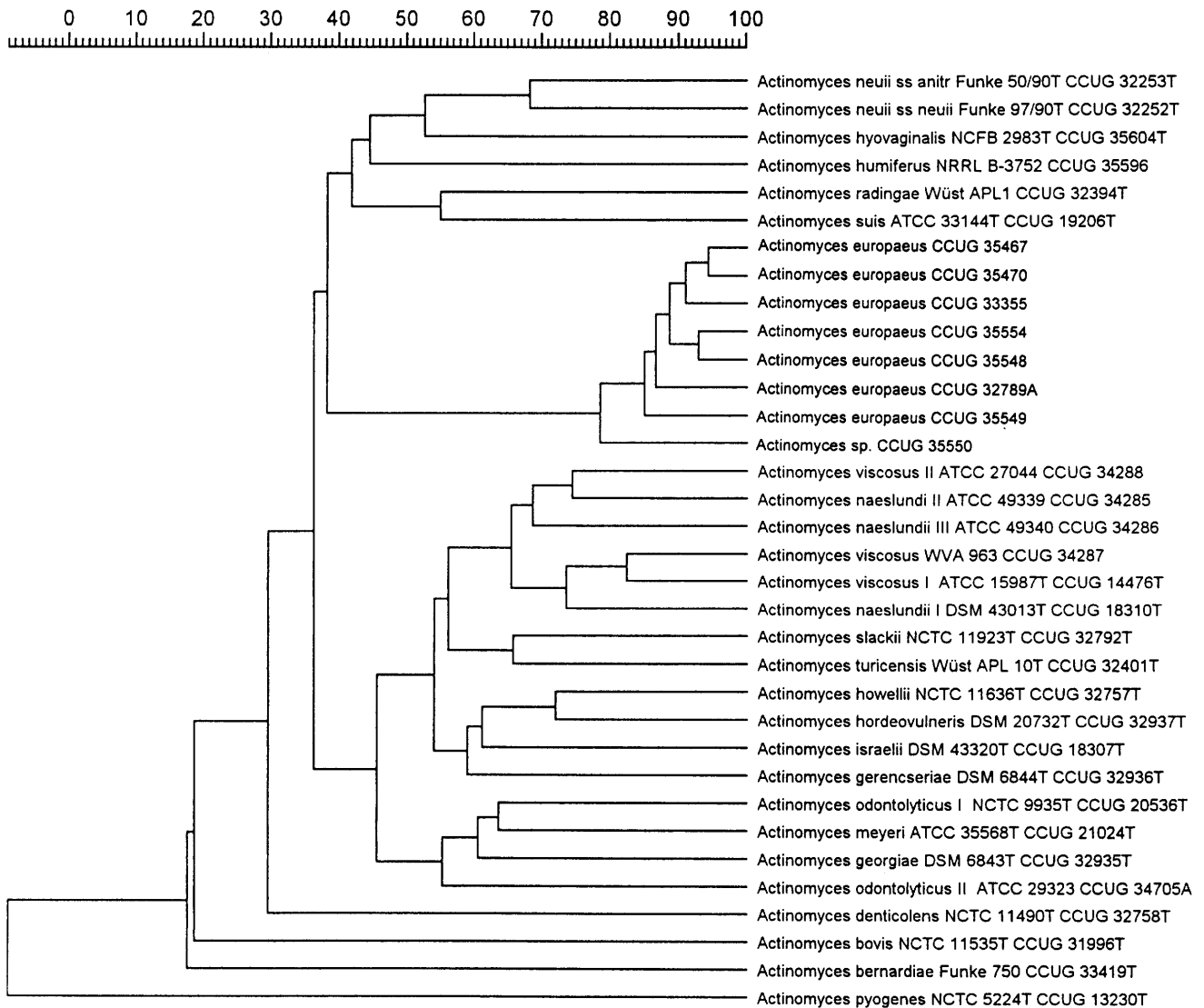


FIG. 1. Similarity dendrogram based on whole-cell protein patterns of *Actinomyces europaeus* and related species. Levels of correlation were expressed as percentages of similarity for convenience.

were sequenced, and the strains were found to be genealogically very homogeneous (zero to two differences were found).

The results of both phenotypic and phylogenetic studies demonstrate that the EF group 43 bacteria (*Arcanobacterium bernardiae*-like bacteria, CDC coryneform group E-like bacteria) described above constitute a new species. Phylogenetically, the new species forms a long isolated line within the genus *Actinomyces*. Pascual Ramos et al. (14) recently conducted a detailed comparative 16S rRNA analysis of *Actinomyces* spp. and showed that the genus *Actinomyces* (as presently defined) is not monophyletic. Both sequence divergence and tree topology considerations indicate that the genus *Actinomyces* is in urgent need of taxonomic revision and should be subdivided into several genera (14). Assignment of the EF group 43 bacteria to the genus *Actinomyces* is the most appropriate assignment at present, although we recognize that in the future this assignment may require emendation as the taxonomy of the genus *Actinomyces* is revised. Based on the results of the present findings, we propose that the EF group 43 bacteria (*Arcanobacterium bernardiae*-like bacteria, CDC coryneform

group E-like bacteria) described above should be classified in a new species of the genus *Actinomyces*, for which we propose the name *Actinomyces europaeus*.

Description of *Actinomyces europaeus* sp. nov. *Actinomyces europaeus* (eu.ro.pae'us. L. adj. *europaeus*, European, referring to the fact that six different European laboratories contributed to this study). The following description is based on the results of a study of 10 strains.

Cells are short (length, 0.5 to 1.5 μ m) gram-positive rods that sometimes are arranged in clusters. Cells are nonmotile and do not form spores. Colonies are circular and smooth with a translucent grayish appearance and are not more than 0.5 mm in diameter after 48 h of incubation in a 5% CO₂-enriched atmosphere at 37°C. Facultatively anaerobic. Catalase is not produced. Acid is produced from glucose, maltose, galactose, and D-fructose. Fermentation of sucrose, ribose, adonitol, D-mannose, α -methyl-D-mannoside, α -methyl-D-glucoside, melibiose, trehalose, melezitose, amido, glycogen, and D-turanose is variable. Acid is not produced from mannitol, xylose, L-arabinose, β -methyl-xyloside, rhamnose, dulcitol, sorbitol,

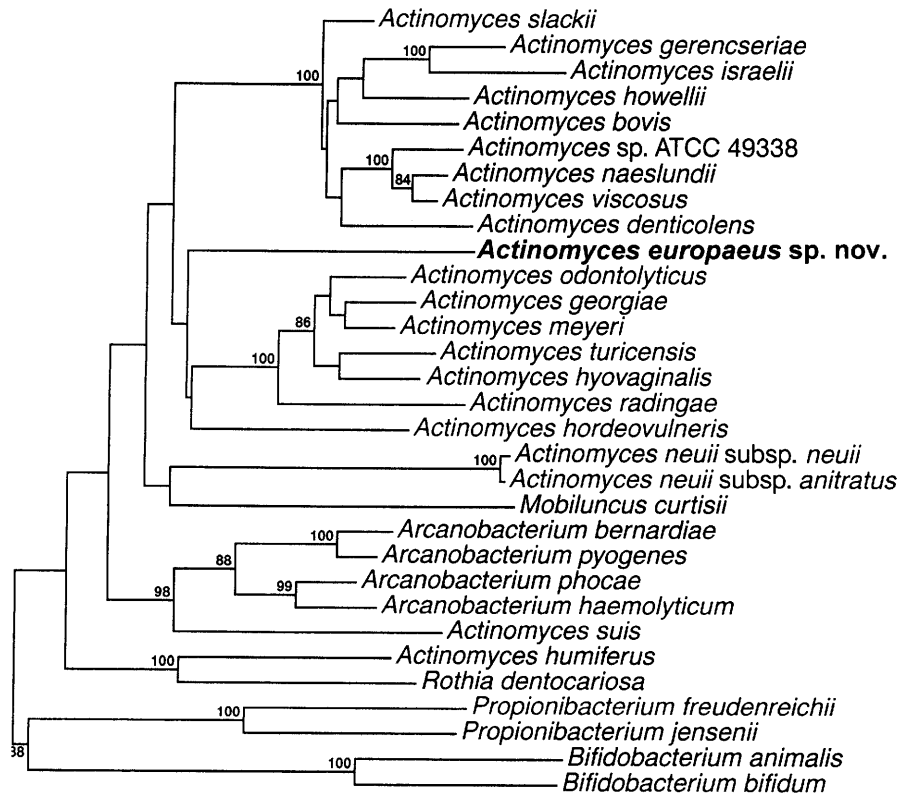


FIG. 2. Unrooted tree showing the phylogenetic relationships of *Actinomyces europaeus*, other members of the genus *Actinomyces*, and related taxa. The tree was constructed by using the neighbor-joining method and was based on a comparison of approximately 1,320 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at the branch points.

TABLE 3. Levels of 16S rRNA gene sequence similarity between *Actinomyces europaeus* sp. nov. and related species

Species, subspecies, or strain (EMBL no.) ^a	% 16S rRNA sequence similarity with <i>Actinomyces europaeus</i> sp. nov.
<i>Actinomyces bovis</i> (X81061).....	90.2
<i>Actinomyces denticolens</i> (X80412).....	89.6
<i>Actinomyces georgiae</i> (X80413).....	89.4
<i>Actinomyces gerencseriae</i> (X80414).....	89.6
<i>Actinomyces hordeovulneris</i> (X82448).....	90.7
<i>Actinomyces howellii</i> (X80411).....	89.8
<i>Actinomyces humiferus</i> (X82449).....	87.3
<i>Actinomyces hyovaginalis</i> (X69616).....	90.3
<i>Actinomyces israelii</i> (X82450).....	89.0
<i>Actinomyces meyeri</i> (X82451).....	90.2
<i>Actinomyces naeslundii</i> (X81062).....	91.0
<i>Actinomyces neuii</i> subsp. <i>anitratus</i> (X71862).....	89.2
<i>Actinomyces neuii</i> subsp. <i>neuii</i> (X71861).....	89.4
<i>Actinomyces odontolyticus</i> (X80504).....	90.7
<i>Actinomyces radingae</i> (X78719).....	89.7
<i>Actinomyces slackii</i> (X82452).....	91.6
<i>Actinomyces</i> sp. strain ATCC 49338 (X81063).....	90.3
<i>Actinomyces suis</i> (X83623).....	87.7
<i>Actinomyces turicensis</i> (X78720).....	90.5
<i>Actinomyces viscosus</i> (X82453).....	91.4
<i>Arcanobacterium bernardiae</i> (X79224).....	87.8
<i>Arcanobacterium haemolyticum</i> (X73952).....	88.1
<i>Arcanobacterium phocae</i> (X97049).....	88.6
<i>Arcanobacterium pyogenes</i> (X79225).....	87.6

^a The numbers in parentheses are EMBL 16S rRNA nucleotide sequence accession numbers.

amygdalin, arbutin, salicin, lactose, inulin, D-raffinose, β-gentiobiose, and 2-ketogluconate. Nitrate is not reduced to nitrite. Urea is not hydrolyzed, and esculin hydrolysis is variable. β-Galactosidase, α-glucosidase, esterase, esterase-lipase, leucine arylamidase, valine arylamidase, and α-galactosidase activities are present, whereas pyrazinamidase, pyrrolidonyl arylamidase, β-glucuronidase, N-acetyl-glucosaminidase, alkaline phosphatase, trypsin, chymotrypsin, β-glucosidase, and α-mannosidase activities are not detected. Succinic acid is the main end product of fermentation. Palmitic and stearic acids are the main straight-chain cellular fatty acids, while oleic acid is the predominant unsaturated fatty acid. The interpeptide bridge in the peptidoglycan is L-lysine-L-lysine-D-glutamic acid (type A5α). The DNA base composition is 61 to 63 mol% G+C. Strains are isolated mainly from human abscesses. The habitat of the strains is unknown. Type strain CCUG 32789A has been deposited in Culture Collection of the University of Göteborg, Göteborg, Sweden. The type strain has the characteristics described above for the species. It does not hydrolyze esculin and does not ferment sucrose. The G+C content of the type strain is 62 mol%.

ACKNOWLEDGMENTS

We thank A. von Graevenitz for a careful review of the manuscript. We thank B. Sjöden for excellent technical assistance.

This work was supported by grants BIO-CT93-0119, BIO-CT94-3098, and CHRX-CT93-0194 from the European Union and by a grant from the Jubiläumsspende der Universität Zürich. E.F. gratefully acknowledges the financial support of H. Jungvid and N. Strömberg.

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