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

CRISTINA PASCUAL RAMOS,1 ENEVOLD FALSEN,2 NEREA ALVAREZ,1 EVA AKERVALL,2 BERIT SJODEN,2 AND MATTHEW D. COLLINS*1

Department of Microbiology, BBSC Institute of Food Research, Reading Laboratory, Reading RG6 6BZ, United Kingdom, and Culture Collection, Department of Clinical Bacteriology, University of Goteborg, Goteborg, Sweden2

Four strains of a previously unknown, catalase-negative, facultatively anaerobic, gram-positive, rod-shaped organism originating from humans were characterized by biochemical, chemical, and molecular taxonomic methods. The four strains phenotypically closely resembled one another, and although they possessed characteristics consistent with membership in the genus Actinomyces, they differed from all previously recognized species of this genus. The results of comparative 16S rRNA gene sequencing studies demonstrated that the unknown human bacterium was phylogenetically a member of the genus Actinomyces. Within the genus Actinomyces, the unidentified bacterium formed a loose, but statistically significant, association with a subgroup which included Actinomyces bovis, the type species of the genus. 16S rRNA sequence divergence values of >6%, however, unequivocally demonstrated that the unidentified bacterium represents a new subline of the genus Actinomyces. A new species, Actinomyces graevenitzii, is proposed for the four new isolates. The type strain of A. graevenitzii is CCUG 27294.

The genus Actinomyces as presently defined (10) is a group of facultatively anaerobic, asporogenous, gram-positive, non-acid-fast, rod-shaped organisms which generally exhibit various degrees of branching. Species of the genus Actinomyces have a broad range of biochemical and physiological characteristics and are primarily found in association with humans and animals (10, 11). In Bergey's Manual of Systematic Bacteriology (10), 12 Actinomyces species (2 of which were designated species incertae sedis) were listed. In recent years there has been a growing interest in the role of Actinomyces spp. and related gram-positive, high-G+C-content, asporogenous organisms as opportunistic pathogens of humans. As a result of this increased clinical interest, combined with improvements in the taxonomic methods used for characterizing such organisms, a plethora of new Actinomyces spp. from human sources have been described in the past few years; these new species include Actinomyces bernardiae (5), Actinomyces georgiae (7), Actinomyces gerencseriae (7), Actinomyces neuii (4), Actinomyces turgi- censis (13), and Actinomyces radingae (13). In this paper we report the phenotypic and phylogenetic characterization of four strains of a previously unknown Actinomyces-like bacterium from human sources. Based on the taxonomic results presented below, a new species, Actinomyces graevenitzii, is described.

Four human clinical isolates (CCUG 27294T, CCUG 29516, CCUG 32536, and CCUG 35697) were referred to the Culture Collection of the University of Goteborg (CCUG), Goteborg, Sweden, for identification. Strain CCUG 27294T originated from bronchus brush from a 57-year-old female, strain CCUG 29516 originated from bronchial secretion from a 44-year-old female, strain CCUG 32536 originated from sputum from a 46-year-old patient, and strain CCUG 35697 originated from the jaw of a 27-year-old male patient with osteitis. The isolates were biochemically characterized by using the API ZYM (enzymatic profiling), API Coryne (identification of coryneform bacteria), API 50CH (carbohydrate fermentation), and Rapid ID 32A (identification of anaerobes) systems according to the instructions of the manufacturer (API bioMérieux, Marcy l’Etoile, France). End products of glucose metabolism were determined as described by Funke et al. (3). Cellular fatty acid analyses were conducted by preparing fatty acid methyl esters and analyzing them by high-resolution capillary gas chromatography as described by Vandamme et al. (12). For polycrylamide gel electrophoresis (PAGE) of whole-cell proteins, most of the strains examined were grown on horse blood agar (Columbia base) at 37°C in the presence of 5% CO2; the only exception was strain CCUG 18307T (Actinomyces israelii), which was grown under anaerobic conditions. The PAGE analysis was performed as described previously (9). For densitometric analysis, normalization, and interpretation of protein patterns the Gelcompar GCW 3.0 software package (Applied Maths, Kortrijk, Belgium) was used. A phylogenetic analysis was performed by comparing 16S rRNA gene sequences. A large fragment of the 16S rRNA gene was amplified by PCR with universal primers pA (5’-AGGTTTGTACCTGTCGTCAG) and pPH (5’-AAGGATGATCCAGCGCA). The PCR products were purified with a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.) according to the manufacturer’s instructions and were directly sequenced by using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolates were determined by performing a database search with the program FASTA of the Genetics Computer Group package (1). These sequences and those of other known related strains were retrieved from the EMBL and Ribosomal Database Project data libraries and were aligned with the newly determined sequences by using the program PILEUP (1). The resulting multiple sequence alignment was corrected manually, and approximately 100 bases at the 5’ end of the rRNA were omitted from further analyses because of alignment ambiguities. A continuous stretch of 1,320 bases was used for the distance matrix analysis. A distance matrix was calculated by using the programs PRETTY (1) and DNADIST (using the
Kimura-2 correction parameter) (2). A phylogenetic tree was constructed by using the neighbor-joining method with the program NEIGHBOR (2). The stability of the groups was estimated by performing a bootstrap analysis (500 replications) with the programs DNABOOT, DNADIST, NEIGHBOR, and CONSENSE (2). A parsimony analysis was also performed by using the same data set (2).

The four human clinical isolates were gram-positive, non-motile, non-spore-forming, non-acid-fast bacteria. The cells were straight to slightly curved rods with some branching and sometimes had swollen ends. Colonies on horse blood agar were approximately 0.2 mm in diameter after 24 h of incubation at 37°C in an atmosphere containing 5 to 10% CO₂. During the initial cultivation all of the strains adhered very strongly to the agar surface. The adherence was less after repeated subculturing. The strains were facultatively anaerobic and produced acid from glucose and some other sugars. Lactic acid and smaller amounts of succinic acid were the major end products of glucose fermentation. The strains were catalase negative and did not hydrolyze hippurate, esculin, or urea. They did not reduce nitrate to nitrite. The whole-cell protein profiles of the four unknown strains were determined by sodium dodecyl sulfate-PAGE. A dendrogram derived from a numerical analysis of the protein profiles is shown in Fig. 1. All four strains grouped together and formed a distinct branch with a within-group correlation level of more than 80%. These data demonstrate that the unknown strains represent a phylogenetically homogeneous group and that they are distinct from all of the other *Actinomyces* and *Arcanobacterium* spp. examined.

To establish the phylogenetic affinities of the clinical isolates, partial 16S rRNA gene sequences were examined. The sequence of a large fragment (>1,400 nucleotides) from strain CCUG 27294T and the sequences of short fragments (>700 nucleotides) from strains CCUG 29516 and CCUG 32536 were determined. A comparative sequence analysis of approximately 650 nucleotides (which included diagnostic variable regions V1 to V3) revealed 100% similarity among the three strains, which demonstrated their genealogical homogeneity. To determine the generic position of the unidentified bacterium, the 16S rRNA sequence of strain CCUG 27294T was compared with the 16S rRNA sequences of other gram-positive bacteria with high G+C contents. The unidentified bacterium exhibited high levels of sequence similarity (generally >90%) with species of the genera *Actinomyces* and *Arcanobacterium* (Table 1). Significantly lower degrees of relatedness were observed with other actinomycete and coryneform taxa (data not shown). Figure 2 is a tree constructed by the neighbor-joining method and shows the phylogenetic relationships of strain CCUG 27294T with *Actinomyces* spp., *Arcanobacterium* spp., and some other related high-G+C-content organisms. From the phylogenetic analysis it is clear that the unidentified bacterium belongs to the *Actinomyces* genus.
bacterium represents a new subline within the genus *Actinomyces* (8). Although the new bacterium did not exhibit a particularly close phylogenetic affinity with any previously recognized species, it formed a loose and statistically significant association (bootstrap value, 100%) with a subcluster which included *Actinomyces bovis* and closely related organisms (Fig. 2). A parsimony analysis was also performed, and all significant groups (Fig. 2) were confirmed (data not shown).

It is evident from both the results of phenotypic and phylogenetic studies that the four clinical isolates which we studied belong to a new species in the genus *Actinomyces*. Phylogenetically, the new bacterium exhibits a significant association with the *Actinomyces bovis* cluster of species (8), although the tree topology and sequence divergence values of >6% unequivocally demonstrate that the new bacterium is distinct at the species level. The new bacterium from clinical sources can be readily distinguished from other aerobically growing human *Actinomyces* species by the tests shown in Table 2. Notable phenotypic features of the new bacterium include the tendency of its colonies to adhere strongly to agar, its production of reducing activity, its acid formation from mannitol, and xylose activity. Three species, *Actinomyces gruevenitzii*, is proposed.

**Description of *Actinomyces gruevenitzii* sp. nov.** *Actinomyces gruevenitzii* (N. L. gen. n. *gruevenitzii*, of Graevenitz, in honor of Alexander von Graevenitz, contemporary American microbiologist, for his many contributions to clinical microbiology) cells are straight or slightly curved rods that exhibit some branching and have swollen ends. Cells are gram positive, do not form spores, and are nonmotile. Colonies on blood agar incubated in the presence of 5% CO₂ are nonpigmented, opaque, and approximately 0.2 mm in diameter after 24 h of incubation. Colonies adhere to agar surfaces. Facultatively aerobic and catalase negative. Lactic acid and succinic acid are produced. Pyrrolidonyl arylamidase, lipase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, and α-fucosidase activities are not detected. The major cellular fatty acids are C₁₂:0 (3 to 4%), C₁₅:0 (10 to 11%), C₁₆:1ω₇c (4 to 5%), C₁₆:0 (38 to 40%), C₁₆:1ω₆c (28 to 30%), and C₁₈:0 (6 to 8%). Isolated from human clinical specimens (mainly respiratory tract secretions). Habitat is unknown. The type strain is CCUG 27294. The type strain has the characteristics of the species.

**Nucleotide sequence accession number.** The 16S rRNA gene sequence of strain CCUG 27294 has been deposited in the GenBank database under accession no. Y09589.

This work was supported in part by grants ERBCHR-C79-0194, BI02-C79-0119, and BI02-C79-3098 from the European Union.

We are grateful to G. Funke, University of Zurich, for his contributions to the description of the new species.

**REFERENCES**


---

### TABLE 2. Characteristics that differentiate *Actinomyces graevenitzii* from other aerobically growing, catalase-negative *Actinomyces* spp. isolated from humans

<table>
<thead>
<tr>
<th>Species</th>
<th>Nitrates reduction</th>
<th>Urease activity</th>
<th>Esculin hydrolysis</th>
<th>Acid produced from mannitol</th>
<th>Acid produced from xylose</th>
<th>β-Galactosidase activity</th>
<th>α-Glucosidase activity</th>
<th>N-Acetyl-β-glucosaminidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinomyces graevenitzii</em></td>
<td>-b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>Actinomyces georgiae</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinomyces gerencseriae</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinomyces israelii</em></td>
<td>V</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinomyces naeslundii</em></td>
<td>V</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinomyces odontolyticus</em></td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinomyces radingae</em></td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Actinomyces turicensis</em></td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Data from references 6, 7, 10, and 11.

- b = negative; + = positive; V = variable; ND, not determined.
