Actinomyces bowdenii sp. nov., isolated from canine and feline clinical specimens

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Four strains of a previously undescribed Actinomyces-like bacterium were isolated from canine and feline clinical specimens. Phenotypic studies indicated the strains were members of the genus Actinomyces, and most closely resembled Actinomyces viscosus serotype I and Actinomyces slackii. Comparative 16S rRNA gene sequencing studies demonstrated the unknown bacterium constitutes a new subline within a group of Actinomyces species, which includes Actinomyces bovis, the type species of the genus. Based on phylogenetic and phenotypic evidence it is proposed that the unknown bacterium be classified as Actinomyces bowdenii sp. nov. The type strain of Actinomyces bowdenii is CCUG 37421T.

Keywords: taxonomy, phylogeny, Actinomyces bowdenii, 16S rRNA

The genus Actinomyces comprises a phenotypically heterogeneous group of anaerobic and facultatively anaerobic, asporogenous, Gram-positive, non-acid-fast, filamentous or diphtheroidal rod-shaped organisms (Schaal, 1986). In Bergey's Manual of Systematic Bacteriology (Schaal, 1986), 10 species were included in the genus Actinomyces, with two additional organisms listed as species incertae sedis. Since Bergey's Manual of Systematic Bacteriology was published, knowledge of the phylogenetic interrelationships of members of the genus Actinomyces has improved considerably (Pascual Ramos et al., 1997). In particular, comparative 16S rRNA gene sequencing has shown that the genus Actinomyces is phylogenetically very heterogeneous. It is now clear that the genus Actinomyces is phylogenetically intermixed with the genus Arcanobacterium, and actually consists of several species groups or lineages worthy of separate generic status (Pascual Ramos et al., 1997; Lawson et al., 1997). In addition to providing new insights into the phylogenetic complexities of the genus Actinomyces, 16S rRNA gene sequencing has provided systematists with a new and immensely powerful diagnostic tool for discerning new diversity within this problematic group of organisms and related taxa. Indeed in the past few years, the use of 16S rRNA gene sequencing in combination with improved phenotypic approaches, has resulted in the recognition of a plethora of new Actinomyces and Actinomyces-like taxa from human and animal sources [e.g. Actinomyces europaeus (Funke et al., 1997); Actinomyces neuii subsp. anitatus and neuii (Funke et al., 1994); Actinomyces hyovaginalis (Collins et al., 1993); Actinomyces radin-gae (Wüst et al., 1995); Actinomyces turicensis (Wüst et al., 1995); Actinobaculum schaaliae (Lawson et al., 1997); Arcanobacterium bernardiae (Funke et al., 1995); Arcanobacterium phocae (Pascual Ramos et al., 1997)]. In this article, we report the use of such a polyphasic taxonomic approach for the characterization of four strains of a hitherto unknown Actinomyces-like bacterium from canine and feline sources. On the basis of the taxonomic findings we describe yet another new member of the genus Actinomyces, Actinomyces bowdenii sp. nov.

Strain M1327/96/1T (CCUG 37421T; CCUG Culture Collection of the University of Göteborg) was isolated from an abscess under the mandible of a dog in a mixed culture with Prevotella oris and Bacteroides fragilis. Strain M1956/95/1 (CCUG 37422) was recovered from feline pleural fluid as part of a mixed culture which included Pasteurella multocida and Fusobacterium mortiferum. Strain CCUG 39734 was isolated in pure culture from pus of a canine abscess. The dog (male Irish setter) had a swollen neck and an
examination revealed a hard, massive swelling ventral to the mandible. Strain CCUG 34477 was isolated from a subcutaneous foreign body pyogranuloma of a dog (female pointer) in mixed culture with a Pasteurella sp. and some obligate anaerobes. The pyogranuloma was approximately 15x15x5 cm, located distally on the side of the thorax. The unidentified Actinomyces-like isolates were cultured on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C, in air plus 5% CO₂. The strains were biochemically characterized by using the API rapid ID32Strep, API CORYNE, and API ZYM systems according to the manufacturer's instructions (API bioMérieux). PAGE analysis of whole-cell proteins was performed as described by Pot et al. (1994). For densitometric analysis, normalization and interpretation of protein patterns the GCW 3.0 software package (Applied Maths, Kortrijk, Belgium) was used. The similarity between all pairs of traces was expressed by the Pearson product–moment correlation coefficient converted for convenience to a percentage similarity. The 16S rRNA genes of representative isolates were amplified by PCR and directly sequenced using a Taq dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A, Applied Biosystems). The closest known relatives of the new isolates were determined by performing database searches. These sequences and those of other known related strains were retrieved from the GenBank or Ribosomal Database Project (RDP) databases and aligned with the newly determined sequences using the program PILEUP (Devereux et al., 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated using the programs PRETTY and DNADIST (using the Kimura-two-correction parameter) (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). Parsimony analysis was also performed using the same package.

The four isolates consisted of Gram-positive straight to slightly curved rods some of which displayed branching. Cells were non-acid-fast and non-spore-forming. The strains grew under aerobic and anaerobic conditions and were catalase-positive. The four isolates phenotypically closely resembled each other producing acid from glucose, maltose, melibiose, melezitose, methyl-β-D-glucopyranoside, lactose, D-ribofuranose, sucrose and trehalose. None of the isolates produced acid from t-arabinose, D-arabitol, cyclo-dextrin, mannitol, glycerol, pullulan, sorbitol or D-xylene. Acid phosphatase, alanine phenylalanine proline arylamidase, β-glucosidase, β-galactosidase, β-galactosidase, β-galacturonidase and leucine arylamidase were produced by the isolates but tests for N-acetyl-β-glucosaminidase, arginine dihydrolase, chymotrypsin, cystine arylamidase, esterase C4, ester lipase C8, α-fucosidase, α-galactosidase, β-glucuronidase, glycerol acid phosphatase, arylamidase, α-mannosidase, β-mannosidase, lipase C14, pyroglutamic acid arylamidase, trypsin, valine arylamidase and urease were negative. Variable reactions were observed for alkaline phosphatase, pyrazinamidase and α-glucosidase. All of the isolates hydrolysed aesculin but not gelatin or hippurate. The cellular morphology and biochemical reactions of the isolates were consistent with their assignment to the genus Actinomyces. The results of a comparative analysis of whole-cell protein profiles of the unknown strains and other Actinomyces species by SDS-PAGE is shown in Fig. 1. All four isolates clustered together and formed a distinct group with a within-group correlation level of 75% or more. The nearest species to the unknown isolates was Actinomyces slackii followed by Actinomyces viscosus serotype I (Fig. 1). The PAGE findings confirmed the four unidentified strains represent a phenotypically homogeneous group of organisms and that they are distinct from all Actinomyces species and close relatives described to date. To ascertain the phylogenetic position of the unknown bacterium, comparative 16S rRNA gene sequencing was conducted. The almost complete gene sequence (> 1500 nucleotides) of a representative isolate from a dog (CCUG 37421) and cat (CCUG 37422) was determined and pairwise analysis showed these to be identical. Sequence database searches confirmed the unknown bacterium was most closely related to species of the genus Actinomyces (results not shown). Highest sequence relatedness was shown with Actinomyces sp. serotype WVA 963 (97.2% 16S sequence similarity), Actinomyces naeslundii genospecies 1 (96.8% similarity) Actinomyces viscosus serotype I (96.9% similarity) and Actinomyces slackii. The type strain of this latter species contains two distinct 16S rRNA gene sequences. A comparison of the two sequences of Actinomyces slackii with the unknown bacterium revealed 96.7–96.8% sequence similarity. The results of neighbour-joining analysis showed Actinomyces viscosus serotype I, Actinomyces naeslundii and Actinomyces sp. serotype WVA 963 (Johnson et al., 1990) to be the nearest phenotypic relatives of the unidentified bacterium (Fig. 2). The association of the unknown bacterium with this subcluster of three Actinomyces species was supported in 85% of tree replicates. The placement of the unknown bacterium, inferred from neighbour-joining, was confirmed by parsimony analysis.

It is apparent from both phenotypic and phylogenetic evidence that the unidentified isolates recovered from canine and feline clinical material represents a hitherto unknown Actinomyces species. Earlier 16S rRNA sequencing studies (Pascual Ramos et al., 1997) have shown that the genus Actinomyces is not monophyletic and consists of several lineages worthy of separate generic status. It is clear from the present 16S rRNA study that the novel bacterium reported here forms a
Fig. 1. Similarity dendogram based on whole-cell protein patterns of Actinomyces bowdenii sp. nov. and related species. Levels of correlation are expressed as percentages of similarity for convenience.

Fig. 2. Unrooted tree showing the phylogenetic relationships of Actinomyces bowdenii sp. nov. and some other high G + C content Gram-positive bacteria. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1327 nucleotides. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points. Scale bar, 1% sequence divergence.

distinct subline within a cluster of species which includes the type species Actinomyces bovis (bootstrap value 100%), and, therefore, can be regarded as an authentic Actinomyces species. Furthermore, sequence divergence values of approximately 3–8% with other members of this phylogenetic cluster unequivocally demonstrate that the bacterium represents a new species. Molecular chemical analysis and biochemical profiling also show the new bacterium is phenotypically distinct from all currently recognized species.
Table 1. Characteristics useful in differentiating Actinomyces bowdenii and some other catalase-positive or catalase-variable Actinomyces species

<table>
<thead>
<tr>
<th>Species</th>
<th>Catalase Nitrate reduction</th>
<th>Hydrolysis of:</th>
<th>Fermentation of:</th>
<th>Production of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amescin Gelatin Urea</td>
<td>d-Ara Glu</td>
<td>Man Suc d-Xyl</td>
<td>PAL PyZ β-GLU β-NAG</td>
</tr>
<tr>
<td>Actinomyces bowdenii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Actinomyces</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinomyces slackii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Actinomyces viscosus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arcanobacterium</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Fermentation of: d-Ara, D-arabitol; Glu, glucose; Man, mannitol; Mel, melezitose; Suc, sucrose; d-Xyl, d-xylose

Production of: PAL, alkaline phosphatase; PyZ, pyrazinamidase; β-GLU, β-glucosidase; β-NAG, β-N-acetylglucosaminidase; β-MDG, methyl β-D-glucoside.

Phenotypically the unknown organism most closely resembles Actinomyces viscosus serotype I (which includes the type strain of the species; Johnson et al., 1990). However, it can be distinguished from Actinomyces viscosus serotype I in producing acid from melezitose. In addition some strains of the unknown species form acid from ribose and produce pyrazinamidase. Actinomyces viscosus serotype I is invariably negative for these tests. The unknown bacterium can also be readily distinguished from Actinomyces slackii by not producing acid from d-xylose, and in displaying alanine phenylalanine proline arylamidase activity but not valine arylamidase activity. Thus, based on the results of the reported polyphasic taxonomic study, we consider the bacterium recovered from canine and feline clinical material merits classification as a new species of the genus Actinomyces, for which the name Actinomyces bowdenii is proposed.

Description of Actinomyces bowdenii sp. nov.

Actinomyces bowdenii (bow.den.i. i. N.L. gen. n. bowdenii of Bowden, to honour George Bowden, a contemporary British microbiologist, for his contributions to actinomycete microbiology).

Cells are straight to slightly curved rods (2-4 µm in length) some of which exhibit branching. Cells stain Gram-positive, are non-acid-fast and non-motile. Colonies on blood agar are non-haemolytic, greyish-white, rounded and approximately 2 mm in diameter after 24-48 h aerobic incubation at 37 °C. Growth is not enhanced by increased concentration of CO₂ 1.5-10%. Facultatively anaerobic and catalase-positive. Succinic acid is the major product of glucose metabolism, together with acetic and lactic acids. Using API systems acid is produced from d-glucose, maltose, melibiose, melezitose, methyl-β-D-glucopyranoside, lactose, D-riaffinose, ribose, sucrose and trehalose. Acid is not produced from L-arabinose, D-arabitol, cyclohexanone, mannitol, pullulan, sorbitol, tagatose or d-xylose. Acid may or may not be produced from tagatose. Aesculin is hydrolysed but gelatin and hippurate are not. Acid phosphatase, alanine phenylalanine proline arylamidase, β-glucosidase, may or may not be detected. Acetoin is not produced. Activities for alkaline phosphatase, pyrazinamidase and β-glucosidase, may or may not be detected. Actinomycin is not produced. Nitrate is reduced to nitrite. MK-10(H2) is the major menaquinone. Isolated from clinical material from dogs and a cat. Habitat is not known. The type strain is CCUG 37421T.

Tests which serve to distinguish Actinomyces bowdenii from other catalase-positive or catalase-variable Actinomyces spp. are given in Table 1.

Acknowledgements

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References


