Actinomyces weissii sp. nov., isolated from dogs

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Two Gram-positive, rod-shaped, non-spore-forming bacteria were isolated from the oral cavities of two dogs. On the basis of 16S rRNA gene sequence similarities both strains were shown to belong to the genus Actinomyces and were most closely related to Actinomyces bovis (97.3% and 97.5%, respectively). The polyamine profile of the two isolates and Actinomyces bovis DSM 43014T was composed of spermidine and spermine as the major components. Menaquinone MK-9 was the major compound in the quinone system of the two strains and Actinomyces bovis. The polar lipid profiles of strains 2298T and 4321 were almost identical, containing diphosphatidylglycerol as the major compound, and moderate to trace amounts of phosphatidylcholine, phosphatidylinositol, phosphatidylinositol-mannoside, phosphatidylglycerol and several unidentified lipids. A highly similar polar lipid profile was detected in Actinomyces bovis DSM 43014T supporting the affiliation of strains 2298T and 4321 to the genus Actinomyces. The typical major fatty acids were C16:0, C18:0 and C18:1ω9c. Fatty acids C14:0 and C18:2ω6,9c were found in minor amounts. The results of physiological and biochemical analyses revealed clear differences between both strains and the most closely related species of the genus Actinomyces. Thus, strains 2298T and 4321 represent a novel species, for which the name Actinomyces weissii sp. nov., is proposed, with strain 2298T (=CIP 110333T=LMG 26472T=CCM 7951T=CCUG 61299T) as the type strain.

The genus Actinomyces is one of the largest genera within the class Actinobacteria and comprises a broad spectrum of anaerobic or facultatively anaerobic, asporogenous, non-acid-fast, Gram-positive, filamentous or diphtheroid, rod-shaped organisms with a high DNA G+C content (Schaal, 1986). Several species of the genus Actinomyces are long-established pathogens of humans and animals, and in
recent years, various novel species of the genus *Actinomyces* and the related taxon *Arcanobacterium* have been described (Hoyles et al., 2000; An et al., 2006; Lehnen et al., 2006; Azuma et al., 2009; Hensse et al., 2009; Renvoise et al., 2009, 2010; Funke et al., 2010).

At the time of writing, the genus *Actinomyces* comprised 44 recognized species, including *Actinomyces canis*, *Actinomyces cathul*, *Actinomyces coleocanis*, *Actinomyces hordeoovulnis* and *Actinomyces bowdenii* of canine origin, and the genus *Arcanobacterium* contained four species (see under http://www.bacterio.cict.fr/a/actinomyces.html, http://www.bacterio.cict.fr/a/arcanobacterium.html) and http://www.dsmz.de).

Typical culture properties of bacteria of the genus *Arcanobacterium* are a synergistic or CAMP-like haemolytic activity displayed on sheep blood agar in the presence of staphylococcal β-haemolysin and in the presence of various other indicator strains, and the reverse CAMP reaction of *Arcanobacterium haemolyticum* and *Arcanobacterium phocae*, characterized by an arc-shaped zone of inhibition of the staphylococcal β-haemolysin (Ülberg-Mohyla et al., 2009).

During routine microbiological diagnostic procedures, two strains displaying CAMP-like reactions and a reverse CAMP reaction were identified. Neither strain had characteristics of *Arcanobacterium haemolyticum* or *Arcanobacterium phocae*. In this taxonomic study using a polyphasic approach, it was found that both strains represent a novel species of the genus *Actinomyces*.

Strain 2298T was isolated by routine procedures together with α-haemolytic streptococci, aerobic spore-forming bacilli, *Corynebacterium* spp., and *Prevotella melaninogenica* from a wound infection caused by a foreign body in the oral cavity of a four-year-old, male Labrador Retriever. Strain 4321 was isolated with cavity of a four-year-old, male Labrador Retriever. Strain bacilli, *Corynebacterium* 1756

Phylogenetic analysis was performed using the ARB software packages (Ludwig et al., 2004) and the corresponding 16S rRNA LTP-ARB database (release 102, September 2010, Yarza et al., 2008). The 16S rRNA gene sequences of strains 2298T and 4321 were aligned according to the seed alignment in the ARB database. The alignment was corrected manually based on secondary structure information. Nucleotide sequences between *Escherichia coli* 16S rRNA gene positions 128 and 1367 (Brosius et al., 1978) were used for phylogenetic analysis and all positions containing gaps were removed from the analysis. Phylogenetic trees were constructed with the maximum-likelihood methods using RAxML v7.04 (Stamatakis, 2006) with GTR-CAT and rapid bootstrap analysis, and the neighbour-joining method based on the Jukes–Cantor model (Jukes & Cantor, 1969). Sequence similarities were calculated in ARB without an evolutionary substitution model. The 16S rRNA gene sequences of the strains 2298T and 4321 were a continuous stretch of 1379 bp and 1347 bp, respectively. Sequence similarity calculations revealed that the strains had a similarity of 99.8 % to each other and that the closest relative of both strains was *Actinomyces bovis* (97.3 % and 97.5 %). Sequence similarities to all other recognized species of the genus *Actinomyces* were between 88.1 and 96.9 % and were between 88.1 and 89.7 % with species of the genus *Arcanobacterium*. Construction of phylogenetic trees including all genera of the family *Actinomyceae* clearly placed strains 2298T and 4321 within a distinct cluster of species of the genus *Actinomyces*, and within the cluster with *Actinomyces bovis* DSM 43014T, both supported by high bootstrap values independent of the tree method used (Fig. 1 and Fig. S1, available in IJSEM Online). Sequence similarities of strains 2298T and 4321 to other species of the genus *Actinomyces* isolated from dogs ranged from 89.6 % for *Actinomyces canis* CCUG 41706T to 96 % for *Actinomyces bovedenii* M1327/96/1T. Type strains of *Actinomyces bovedenii* and *Actinomyces cathul* (95 % sequence similarity) clustered with strains 2298T and 4321, whereas type strains of *Actinomyces canis*, *Actinomyces coleocanis* and *Actinomyces hordeoovulnis* clustered with other species of the genus *Actinomyces* (Figs 1 and S1).

*Actinomyces bovis* DSM 43014T and the two isolates shared a similar polyamine profile, containing spermidine and spermine as the predominant compounds in the following quantities [μmol (g dry weight)]−1): *Actinomyces bovis* DSM 43014T, spermidine 0.14 and spermine 0.09; strain 2298T, spermidine 0.15 and spermine 0.13; strain 4321, spermidine 0.17 and spermine 0.18. Strains 2298T and 4321 shared a highly similar quinone system that was composed of the major menaquinone MK-9 (65.2 and 64.7 %, respectively), moderate amounts of MK-8 (17.4 and 16.5 %, respectively), and MK-10 (12.3 and 15.4 %, respectively) and minor quantities of MK-7, MK-6, MK-5 and MK-4. The strains were grown in BHI at 37 °C for 72 h without agitation before extraction. Analysis was performed with a gas chromatograph (6890, Hewlett Packard) with Sherlock MIDI software version 2.11 and TSBA peak naming table version 4.1.

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amounts of MK-7 (4.1 and 2.5 %, respectively) and MK-6 (0.9 and 1.0 %, respectively). A similar quinone system with the major compound MK-9, but with clear differences in quantitative amounts of the different components was detected in *Actinomyces bovis* DSM 43014T (84.1 % MK-9, 10.5 % MK-8, 3.1 % MK-6, 1.7 % MK-10 and 0.6 % MK-7). This result for *Actinomyces bovis* was not in agreement with data from Hess *et al.* (1979) who reported a quinone system of four components, exhibited little variability among strains of the genus *Actinomyces*. Assuming that the quinone data for *Actinomyces bovis* CCUG 1430 are correct, the differences seen when compared with *Actinomyces bovis* DSM 43014T might be explained by some heterogeneity within the species, or suggest that *Actinomyces bovis* CCUG 1430 is a representative of another species of the genus *Actinomyces*. Studies on the variability of the quinone system among reliably classified actinobacterial strains are rather limited. Recently, Buczolits *et al.* (2008) studied the variability in the quinone system of *Microbacterium paraoxydans* and showed that the quinone system, consisting of four components, exhibited little variability among four strains of this species. Also strains 2298 and 4321 differed only slightly in the composition of their quinone system and hence it can be assumed that generally strains of certain species of the genus *Actinomyces* do not show significant differences in their quinone system. In conclusion, the view is supported that the genus *Actinomyces* is not a single species, but rather a heterogeneous group of microorganisms.
quione systems were also reported for four other species of the genus *Actinomyces*, namely *Actinomyces viscosus*, *Actinomyces israelii*, *Actinomyces bovni* and *Actinomyces ruminicola*. The first three species were shown to contain predominantly MK-10(H4), whereas *Actinomyces ruminicola* contained MK-10 and MK-9 (7:3). Generally, species of a well-defined actinobacterial genus do not contain either completely unsaturated or partially saturated quinones, and considering the phylogenetic distance of these four species to *Actinomyces bovis* (Fig. 1), a dissection of the genus *Actinomyces* may be recommended but support from other data would be desirable for this reclassification. Nevertheless the highly similar quione system of *Actinomyces bovis* DSM 43014T, strains 2298T and 4321 (major quione MK-9) are in agreement with their very close phylogenetic relatedness and do support the assignment of strains 2298T and 4321 to the genus *Actinomyces* since *Actinomyces bovis* is the type species of this genus. The differences in minor quione components did not suggest placing the two novel strains as members of the species *Actinomyces bovis*. The polar lipid profiles of strains 2298T (Fig. 2) and 4321 (not shown) were almost identical, differing only in the relative quantities of certain lipids. The major polar lipid detected in the two strains was diphostidylglycerol. In addition, there were moderate amounts of phosphatidylinositol, phosphatidylinositol-mannoside, phosphatidylcholine, an unidentified aminolipid (AL1), an unidentified phosphoglycerolipid (PGL), an unidentified aminolipid (AL2) and trace amounts of phosphatidylycerol, two unidentified aminolipids (AL1, AL3), an unidentified glycolipid (GL) and an unidentified polar lipid (L) not reacting with any of the applied specific staining reagents. A highly similar polar lipid profile was detected in *Actinomyces bovis* DSM 43014T (Fig. 2) but the presence of minor amounts of another aminoglycerol (AGL2) and a fourth aminolipid (AL4) distinguished the type species of the genus *Actinomyces* from the two novel isolates. Since these two distinguishing lipids were only detected in minor amounts and so far no polar lipid data from other *Actinomyces bovis* strains are available supporting their species specificity, we do not propose that these traits are justification for the erection of a novel species. However, this polar lipid profile clearly supports the affiliation of the two isolates with the genus *Actinomyces*. The fatty acid profiles of both novel strains were very similar. The typical major fatty acids were C16:0, C18:0 and C18:1ω9c. Fatty acids C14:0 and C18:2ω6ω9c were found in minor amounts. The results of the physiological characterization tests are given in Table 1 and also in the species description. Based on this polyphasic approach, strains 2298T and 4321 represent a novel species of genus *Actinomyces*, for which the name *Actinomyces weissii* sp. nov. is proposed.

**Description of Actinomyces weissii** sp. nov.

*Actinomyces weissii* (weis’i.i. N.L. gen. masc. n. weissii of Weiss, to honour Reinhard Weiss, a contemporary German microbiologist, for his contributions to veterinary microbiology).

Cells are irregular rods 2–10 μm in length and 1–1.5 μm in width. Gram-positive, and negative result in oxidase and Voges-Proskauer tests. Non-motile on Hitchens medium (Merck). Growth occurs on sheep blood agar with a weak zone of haemolysis under microaerobic conditions, this is less pronounced under aerobic and anaerobic conditions. Colonies are white–greyish with a diameter of 1 mm after 72 h incubation on sheep blood agar at 35°C in a 5% CO2-enriched atmosphere. CAMP-like reactions occur with various indicator strains and a reverse CAMP reaction can be observed. Acid is produced from D-glucose, glycogen, lactose, maltose and sucrose, but not from D-mannitol, D-ribose or D-xylene. Enzymatic activity is observed for alkaline phosphatase, z-mannosidase, β-galactosidase, z-glucosidase, N-acetyl-β-glucosaminidase, asacolin (β-glucosidase), catalase and DNase, but not for nitrate reductase, pyrazinamidase, pyrrolidonyl arylamidase, β-glucuronidase, urease, gelatinase and hyaluronidase. The polyamine profile contains spermidine and spermine as major compounds. The quione system is comprised of the major menaquinone MK-9, moderate amounts of MK-8 and MK-10 and minor amounts of MK-7 and MK-6. The major polar lipid is diphostidylglycerol. Moderate amounts of phosphatidylinositol, phosphatidylinositol-mannoside, phosphatidylcholine, an unidentified

![Fig. 2. Polar lipid profile of strain 2298T (a) and Actinomyces bovis DSM 43014T (b) following two-dimensional TLC and staining with molybdotrophosphoric acid. DPG, diphostidylglycerol; PC, phosphatidylcholine; PGL, phosphatidylycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol-mannoside; GL, unidentified glycolipid; PGL, unidentified phosphoglycerolipid; AL1–4, unidentified aminolipids AGL1–2, unidentified aminoglycerol; L, unidentified polar lipid not reacting with any specific staining reagent.](image-url)
Table 1. Physiological characteristics of strains 2298\textsuperscript{T} and 4321, *Actinomyces bovis*, and various species of the genus *Actinomyces* isolated from dogs and *Arcanobacterium haemolyticum*

<table>
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\*CAMP-like reactions with *Streptococcus agalactiae, Rhodococcus equi, Psychrobacter phenylpyruvicus, Arcanobacterium hippoceleae, Arcanobacterium plurianimalium, Arcanobacterium abortisuis* and *Arcanobacterium pyogenes* as indicator strains.

†Reverse CAMP reaction in the zone of staphylococcal β-haemolysin.

§Data for strains 1–4 and 9 obtained with: a, API Coryne test system (bioMérieux); b, tablets containing substrates (Inverness Medical); c, 4-Methylumbelliferyl-conjugated substrates (Sigma).

Data for strains 1–4 and 9 obtained with: a, API Coryne test system (bioMérieux); b, tablets containing substrates (Inverness Medical).

aminoglycolipid (AGL1), an unidentified phosphoglycolipid (PGL), an unidentified aminolipid (AL2) and trace amounts of phosphatidylglycerol, two unidentified aminolipids (AL1, AL3), an unidentified glycolipid (GL) and an unidentified polar lipid (L) are also present. Major fatty acids (for strain 2298\textsuperscript{T}) are C\textsubscript{14}:0, C\textsubscript{18}:1ω9c and C\textsubscript{18}:1ω6c. Fatty acids C\textsubscript{14}:0 and C\textsubscript{18}:1ω6c are found in minor amounts.

The type strain is 2298\textsuperscript{T} (=CIP 110333\textsuperscript{T}=LMG 26472\textsuperscript{T} =CCM 7951\textsuperscript{T}=CCUG 61299\textsuperscript{T}). The type strain and strain 4321 were isolated from the oral cavities of two dogs in Gießen, Germany.

**References**


