NOTE

***Actinomyces canis* sp. nov., isolated from dogs**

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Three strains of a previously undescribed catalase-positive *Actinomyces*-like bacterium were isolated from dogs. Biochemical testing and PAGE analysis of whole-cell proteins indicated that the strains were phenotypically highly related to each other but different from previously described *Actinomyces* and *Arcanobacterium* species. Sequencing of 16S rRNA showed that the unknown bacterium represents a new subline within a cluster of species which includes *Actinomyces hyovaginalis*, *Actinomyces georgiae*, *Actinomyces meyeri*, *Actinomyces odontolyticus*, *Actinomyces radingae* and *Actinomyces turicensis*. On the basis of phenotypic evidence and 16S rRNA sequence divergence levels (greater than 5% with recognized *Actinomyces* species) it is proposed that the unknown strains from canine sources be classified as a new species with the name *Actinomyces canis* sp. nov. The type strain of *Actinomyces canis* is CCUG 41706^T (= CIP 106351^T).

Keywords: *Actinomyces canis*, dog isolate, taxonomy, phylogeny, 16S rRNA

The genus *Actinomyces* comprises a broad group of anaerobic and facultatively anaerobic, asporogenous, non-acid-fast, Gram-positive, rod-shaped organisms with a high G+C DNA content (Schaal, 1986). The taxonomic inter-relationships of members of the genus *Actinomyces* and related taxa have been greatly clarified in the past few years with the use of molecular chemical and molecular genetic taxonomic methods. In particular, 16S rRNA gene sequencing has shown the genus *Actinomyces* to be very heterogeneous and phylogenetically intermixed with other genera such as *Arcanobacterium* and *Mobiluncus* (Pascual *et al.*, 1997a; Lawson *et al.*, 1997). Presently, the genus *Actinomyces* consists of 18 species, most of which are known to be indigenous to mucous membranes, particularly the oral cavity, of humans and animals. Many of these species have only recently been delineated [e.g. *Actinomyces europaeus* (Funke *et al.*, 1997), *Actinomyces graevenitzii* (Pascual *et al.*, 1997b), *Actinomyces neuii* subsp. *anitratus* and *neuui* (Funke *et al.*, 1994); *Actinomyces radingae* (Wüst *et al.*, 1995), *Actinomyces turicensis* (Wüst *et al.*, 1995), *Actinobaculum schaalii* (Lawson *et al.*, 1997) and *Arcanobacterium bernardiae* (Funke *et al.*, 1995)] and have come to light because of the use of much-improved

taxonomic criteria, particularly the highly discriminatory sequence data derived from 16S rRNA. Currently relatively little is known about the natural habitat, prevalence and pathogenic potential of many *Actinomyces* species, especially those from veterinary sources. There is no doubt, however, that more extensive application of molecular-based tools for characterizing *Actinomyces* species will result in a better understanding of their occurrence and significance and will facilitate the recognition of even more diversity. During the course of a study of taxonomically problematic *Actinomyces* from domesticated animals, we have used a combination of phenotypic and molecular taxonomic methods to characterize three unusual *Actinomyces*-like organisms from dogs. On the basis of the results of this study, we describe yet another new species of the genus *Actinomyces*, i.e. *Actinomyces canis* sp. nov.

Strain M2289/98/2^T (CCUG 41706^T) was isolated from the vagina of a dog with a history of infertility. The organism was obtained in heavy growth together with *Pasteurella* spp. and *Streptococcus* spp. Strain M1429/95/1 (CCUG 42597) was isolated from purulent material from the chest of a 3-year-old foxhound bitch with fatal pyogranulomatous pleurisy, along with *Streptococcus canis* and *Bacteroides* spp. Strain CCUG 34479 was isolated from a subcutaneous abscess in a 9-month-old female Schiller Stovare hunting dog. The organism was isolated in mixed

Abbreviation: RDP, Ribosomal Database Project.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CCUG 41706^T is AJ243891.

Fig. 1. Unrooted tree showing the phylogenetic relationships of *Actinomyces canis* 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 the branching points. Bar, 1% sequence divergence.

culture with *Bacteroides* spp. and *Fusobacterium* spp. The abscess was found to contain a foreign body in the form of a piece of grass awn. 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 ID32 Strep, API CORYNE and API ZYM systems according to the instructions of the manufacturer (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) 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 gene sequence of the isolates was amplified by the PCR and directly sequenced using a *Taq* DyeDeoxy 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-parameter model) (Felsenstein, 1989). A phylogenetic tree was constructed (Fig. 1) 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 DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). Parsimony analysis was also performed using the same package (Felsenstein, 1989).

The three 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 three isolates closely resembled each other phenotypically, producing acid from glucose, L-arabinose, glycogen, maltose, pullulan, lactose, D-ribose and D-xylose. None of the isolates produced acid from D-arabitol, cyclodextrin, mannitol, melibiose, melezitose, methyl β -D-glucopyranoside, sorbitol, tagatose or trehalose. Acid production from sucrose and raffinose was variable. All of the isolates produced alanine phenylalanine proline arylamidase, α -glucosidase, α -galactosidase, β -galactosidase, β -galacturonidase, α -fucosidase, ester lipase C-8 (weak reaction), cystine arylamidase (weak reaction), pyrazinamidase, leucine arylamidase and *N*-acetyl- β -glucosaminidase. None of the strains produced arginine dihydrolase, acid phosphatase, alkaline phosphatase, chymotrypsin, esterase C-4, β -

Fig. 2. Similarity dendrogram based on whole-cell protein patterns of *Actinomyces canis* sp. nov. and related species. Levels of correlation are expressed as percentages of similarity for convenience.

glucosidase, β -glucuronidase, α -mannosidase, β -mannosidase, lipase C-14, phosphoamidase, pyroglutamic acid arylamidase, trypsin, valine arylamidase or urease. The production of glycyl tryptophan arylamidase was variable. None of the isolates hydrolysed aesculin, gelatin or hippurate. The API CORYNE code for the isolates was 2430766/7. 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. 2. The three isolates from dogs formed a distinct cluster, which was separate from all described *Actinomyces* species and related taxa. The nearest neighbour of the dog isolates based on the protein profiling analysis, corresponded to *Arcanobacterium bernardiae*, although this association was not particularly close,

joining the novel cluster at a correlation level of about 50 %.

The phylogenetic position of the unknown organisms was determined by comparative 16S rRNA gene sequencing. The almost complete gene sequences (> 1400 nucleotides) of the three isolates were determined and pairwise analysis showed them to be almost identical (CCUG 41706^T versus CCUG 42597, 99.9 % similarity; CCUG 41706^T versus CCUG 34479, 99.9 %; CCUG 34479 versus CCUG 42597, 99.8 %). Sequence database searches confirmed that the unknown bacterium was most closely related to species of the genus *Actinomyces*. The highest sequence similarity was shown with *Actinomyces hyovaginalis* (95 %), *Actinomyces turicensis* (94.9 %), *Actinomyces odontolyticus* (94.7 %) and *Actinomyces georgiae* (94 %). Treeing analysis confirmed the affinity of the unknown bacterium with the genus *Actinomyces*. Strain CCUG 41706^T formed a distinct subline branching at the base of a robust cluster of species which included *Actinomyces georgiae*, *Actinomyces hyovaginalis*, *Actinomyces meyeri*, *Actinomyces odontolyticus*, *Actinomyces radingae* and *Actinomyces turicensis*. The association of the unknown dog bacterium with this species was statistically significant (having a bootstrap resampling value of 91 % in 500 tree replications).

It is apparent from both phenotypic and phylogenetic evidence that the three unidentified isolates recovered from clinical material from dogs represent a hitherto unknown *Actinomyces* species. Both sequence divergence values and treeing analysis show that the bacterium represents a distinct subline within the genus *Actinomyces*. The nearest phylogenetic relatives of the unknown bacterium correspond to a distinct cluster of *Actinomyces* species (viz. *Actinomyces georgiae*, *Actinomyces hyovaginalis*, *Actinomyces meyeri*, *Actinomyces odontolyticus*, *Actinomyces radingae* and *Actinomyces turicensis*), although sequence divergence values of 5 % or more unequivocally show that the unknown canine bacterium represents a different species. Thus, on the basis of the results of the reported polyphasic taxonomic study, we consider that the unknown bacterium merits classification as a new species of the genus *Actinomyces*, for which the name *Actinomyces canis* is proposed. It is pertinent to note that *Actinomyces canis* can be readily distinguished from other *Actinomyces* species biochemically and by PAGE analysis of whole-cell proteins. Biochemical tests that are useful in distinguishing *Actinomyces canis* from other catalase-positive or -variable *Actinomyces* species are shown in Table 1.

Description of *Actinomyces canis* sp. nov.

Actinomyces canis (can.is. L. gen. n. *canis* of the dog).

Cells are straight to slightly curved rods, some of which exhibit branching. Cells stain Gram-positive, are non-acid-fast and are non-motile. Facultatively anaerobic and catalase-positive. With API systems,

Table 1. Characteristics useful in differentiating *Actinomyces canis* and some other catalase-positive or catalase-variable *Actinomyces* species

Abbreviations: D-Ara, D-arabitol; L-Ara, L-arabinose; Glu, glucose; Man, mannitol; Suc, sucrose; D-Xyl, D-xylose; α -MDG, methyl α -D-glucoside; Gly, glycogen; Pull, pullulan; Rib, ribose; Tre, trehalose; PAL, alkaline phosphatase; PYZ, pyrazinamidase; β -GLU, β -glucosidase; β -NAG, β -N-acetylglucosaminidase; +, positive; –, negative; w, weak reaction; v, variable; v(+), most strains positive; v(–), most strains negative.

Species	Catalase	Nitrate reduction	Hydrolysis of:			Fermentation of:											
			Aesculin	Gelatin	Urea	D-Ara	L-Ara	Glu	Man	Suc	D-Xyl	z-MDG	Mel	Gly	Pull	Rib	Tre
<i>Actinomyces canis</i>	+	—	—	—	—	—	+	+	—	v	+	—	—	+	+	+	—
<i>Actinomyces bowdenii</i>	+	+	+	—	—	—	—	+	—	+	—	—	+	—	—	+	+
<i>Actinomyces hordeovulneris</i>	+(w)	—	+	w	—	—	v	+	—	+	+	—	—	v	+	v	v
<i>Actinomyces howellii</i>	+	—	+	—	—	—	—	+	—	+	+	v	—	—	—	—	v
<i>Actinomyces naeshundii</i> (serotype I)	v(—)	+	v(+)	v(—)	w	—	—	v(+)	v(—)	v(+)	v(—)	+	v(—)	—	—	—	+
<i>Actinomyces naeshundii</i> (serotype II)	v(—)	+	v	—	w	—	—	+	—	+	—	+	+	—	—	—	+
<i>Actinomyces neuii</i> subsp. <i>antitratus</i>	+	—	—	—	—	+	—	+	+	+	v(+)	v	+	—	—	+	+
<i>Actinomyces neuii</i> subsp. <i>neuii</i>	+	+	—	—	—	+	—	+	+	+	+	—	v	—	—	v	v
<i>Actinomyces slackii</i>	+	+	+	v	v(—)	—	—	+	—	v	+	—	+	—	—	—	+
<i>Actinomyces viscosus</i> (serotype I)	+	+	+	—	v	—	—	+	—	+	—	v(+)	—	—	—	—	v
<i>Actinomyces viscosus</i> (serotype II)	+	+	+	—	v	—	—	+	—	+	—	+	v	—	—	—	v
<i>Arcanobacterium phocae</i>	v(+)	—	—	—	—	—	—	+	v(—)	+	v(—)	—	v(+)	+	—	+	v
Production of:																	
			PAL	PYZ	β-GLU	β-NAG											
<i>Actinomyces canis</i>			—	+(w)	—	+											
<i>Actinomyces bowdenii</i>			v(—)	v	+(w)	—											
<i>Actinomyces hordeovulneris</i>			—	v	v	+											
<i>Actinomyces howellii</i>			+	+	+	+											
<i>Actinomyces naeshundii</i> (serotype I)			—	+	+	—											
<i>Actinomyces naeshundii</i> (serotype II)			+(w)	+	+	—											
<i>Actinomyces neuii</i> subsp. <i>antitratus</i>			v	+	—	—											
<i>Actinomyces neuii</i> subsp. <i>neuii</i>			—	+	—	—											
<i>Actinomyces slackii</i>			—	v	+	—											
<i>Actinomyces viscosus</i> (serotype I)			+	—	+	—											
<i>Actinomyces viscosus</i> (serotype II)			+	+	+	—											
<i>Arcanobacterium phocae</i>			+	+	—	—											

acid is produced from D-glucose, L-arabinose, glycogen, maltose, lactose, pullulan, D-ribose and D-xylose. Acid is not produced from D-arabitol, cyclodextrin, mannitol, melibiose, melezitose, methyl β -D-glucopyranoside, sorbitol, tagatose or trehalose. Aesculin, gelatin and hippurate are not hydrolysed. Acid may or may not be produced from sucrose and raffinose. Alanine phenylalanine proline arylamidase, α -galactosidase, β -galactosidase, β -galacturonidase, α -glucosidase, α -fucosidase, ester lipase C-8 (weak), cystine arylamidase (weak), leucine arylamidase, N-acetyl- β -glucosaminidase and pyrazinamidase (weak reaction) are produced. Arginine dihydrolase, acid phosphatase, alkaline phosphatase, chymotrypsin, esterase C-4, β -glucosidase, β -glucuronidase, pyroglutamic acid arylamidase, α -mannosidase, β -mannosidase, lipase C-14, phosphoamidase, trypsin, valine arylamidase, pyrrolydonyl arylamidase and urease are not produced. Glycyl tryptophan arylamidase may or may not be produced. Acetoin is not produced. Nitrate

is not reduced to nitrite. The type strain is CCUG 41706^T (= CIP 106351^T). Isolated from clinical specimens from dogs. Habitat unknown. The type strain shows the characteristics of the species and produces acid from sucrose and raffinose.

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