NOTE

***Actinomyces catuli* sp. nov., from dogs**

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An *Actinomyces*-like bacterium was recovered from two dogs. Based on cellular morphology and biochemical criteria, the unknown bacterium resembled the genus *Actinomyces* but it did not appear to correspond to any of the currently recognized species of this genus. PAGE analysis of whole-cell proteins confirmed that the strain was phenotypically distinct from all other *Actinomyces* species and comparative 16S rRNA gene sequencing showed that the bacterium represents an unknown sub-line within the genus. Based on phenotypic and phylogenetic evidence, it is proposed that the bacterium from dogs be classified as a new species of the genus *Actinomyces*, *Actinomyces catuli*. The type strain of *Actinomyces catuli* is CCUG 41709^T (= CIP 106507^T).

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The genus *Actinomyces* encompasses a phenotypically diverse group of anaerobic and facultatively anaerobic, asporogenous, Gram-positive, non-acid-fast, filamentous or diphtheroidal rod-shaped organisms, most of which are residents of the mucosal surfaces, particularly the oral cavity, of humans and some animals (Schaal, 1986). In recent years, there has been a rapid expansion in the number of described species of the genus *Actinomyces* [e.g. *Actinomyces europaeus* (Funke *et al.*, 1997); *Actinomyces graevenitzii* (Pascual *et al.*, 1997b); *Actinomyces neuui* subsp. *anitratus* and *neuui* (Funke *et al.*, 1994); *Actinomyces radingae* (Wüst *et al.*, 1995); *Actinomyces turicensis* (Wüst *et al.*, 1995)] and numerous taxa closely related to the genus [e.g. *Actinobaculum schaalii* (Lawson *et al.*, 1997); *Arcanobacterium bernardiae* (Funke *et al.*, 1995)] have been described. The vast majority of these newly described organisms have originated from human sources. Improvements in identification methods, in particular the use of molecular diagnostic tools such as protein profiling and 16S rRNA sequencing, have undoubtedly driven this increase in the number of recognized species and there is evidence that many more *Actinomyces* remain to be discovered from human sources. Much less is known about the range of *Actinomyces*-like organisms in domestic and wild animals. There are, however, indications (Pascual *et al.*, 1997a, 1999) that the implementation of more precise molecular methodologies for the identification of problematic

veterinary organisms will result in the recognition of much greater *Actinomyces* diversity, leading to a better understanding of their host distribution and clinical significance. In this article, we report the characteristics of two strains of an unusual *Actinomyces* species isolated from dogs. Based on the results of a molecular chemical and molecular genetic taxonomic study, yet another new species of the genus *Actinomyces*, *Actinomyces catuli* is described.

Strain M2231/94/1 (CCUG 42832) was isolated in 1994 from a polymicrobial infection of a dog with pneumonia and exudative pleurisy. It was isolated from mediastinal tissue and blood clot with *Bacteroides fragilis*, *Fusobacterium nucleatum*, *Peptostreptococcus anaerobius* and *Peptostreptococcus productus*. Strain M1192/98/1^T (CCUG 41709^T) was also isolated from a dog in 1998, but no other information on the source is available. The unidentified organisms 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 ZYM and API CORYNE 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 4.0 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

The GenBank/EMBL/DBJ accession number for the 16S rRNA sequence of CCUG 41709^T is AJ276805.

Fig. 1. Unrooted tree showing the phylogenetic relationships of *Actinomyces catuli* 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.

16S rRNA genes of the unknown isolates were amplified by 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) Libraries 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).

The two strains consisted of Gram-positive, straight to slightly curved rods, which were non-acid-fast and non-spore-forming. Strain CCUG 41709^T was catalase-negative although isolate CCUG 42832 was found to be weakly catalase-positive. Both strains produced acid from glucose, lactose, maltose, D-raffinose, ribose, sucrose, D-xylose and trehalose. Acid production from L-arabinose, melibiose and methyl β -D-glucopyranoside was either weakly positive or negative. Acid phosphatase, ester lipase C8, α -glucosidase, β -glucosidase, β -glucuronidase, α -galactosidase (weak), β -galactosidase, leucine arylamidase, pyrrolidonyl arylamidase (weak reaction) and pyrazinamidase were produced by both isolates. The two strains hydrolysed aesculin but failed to hydrolyse hippurate and gelatin. They reduced nitrate to nitrite and were Voges-Proskauer-negative. The cellular morphology and biochemical reactions of the isolates were consistent with their assignment to the genus *Actinomyces*. To determine the overall phenotypic resemblance of the isolates to each other and to recognized/reference *Actinomyces* species, a comparative analysis of whole-

cell protein profiles by SDS-PAGE was performed. The unknown dog isolates clustered together (correlation level > 90%) and formed a distinct group which was separate from all *Actinomyces* species described to date (data not shown). *Actinomyces urogenitalis* was the nearest neighbour of the dog isolates based on protein profiling, joining the latter cluster at about the 75% correlation level. Other species were more distantly related (correlation levels < 60%). To investigate the phylogenetic relationships of the dog isolates, their 16S rRNA gene sequences were determined and subjected to a comparative analysis. The two unidentified isolates displayed 99.9% sequence similarity with each other and sequence database searches confirmed that they were most closely related to species of the genus *Actinomyces* (results not shown). Highest sequence relatedness was shown with the *Actinomyces bovis* group of species (approx. 93–96% sequence similarity), with *Actinomyces slackii* (96% sequence similarity) and *Actinomyces urogenitalis* (96% sequence similarity) displaying highest relatedness. The results of treeing analyses are shown in Fig. 1 and confirmed the association of the unknown dog bacterium (as exemplified by strain CCUG 41709^T) with the *Actinomyces bovis* cluster of species.

The genus *Actinomyces* as presently defined is not monophyletic and consists of several rRNA lineages worthy of separate generic status (Pascual *et al.*, 1997a, 1999). The type species of the genus, *Actinomyces bovis*, falls into a distinct and robust rRNA cluster which embraces several species (viz: *Actinomyces bowdenii*, *Actinomyces denticolens*, *Actinomyces howellii*, *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces slackii*, *Actinomyces urogenitalis* and *Actinomyces viscosus*) which can be regarded as the genus *Actinomyces sensu stricto*. The recovery of the unknown bacterium from dogs in this cluster of species therefore unequivocally demonstrates that it is an authentic *Actinomyces* species. Furthermore, the results of treeing analyses combined with sequence divergence values of > 3% between the unknown bacterium and other members

Table 1 Some characteristics useful in differentiating *Actinomyces catuli* and some other catalase-positive or catalase-variable *Actinomyces* species

1, *Actinomyces catuli*; 2, *Actinomyces bowdenii*; 3, *Actinomyces canis*; 4, *Actinomyces hordeovulneris*; 5, *Actinomyces howellii*; 6, *Actinomyces naeslundii* (serotype I); 7, *Actinomyces naeslundii* (serotype II); 8, *Actinomyces neuui* subsp. *anitratus*; 9, *Actinomyces neuui* subsp. *neuui*; 10, *Actinomyces slackii*; 11, *Actinomyces viscosus* (serotype I); 12, *Actinomyces viscosus* (serotype II). All results based on API test systems. +, Positive; –, negative; w, weak reaction; v, variable; v(+), most strains positive; v(–), most strains negative.

Test	1	2	3	4	5	6	7	8	9	10	11	12
Catalase	v	+	+	+(w)	+	v(–)	v(–)	+	+	+	+	+
Nitrate reduction	+	+	–	–	–	+	+	–	+	+	+	+
Hydrolysis of:												
Aesculin	+	+	–	+	+	v(+)	v	–	–	+	+	+
Gelatin	–	–	–	w	–	v(–)	–	–	–	v	–	–
Urea	–	–	–	–	–	w	w	–	–	v(–)	v	v
Fermentation of:												
D-Arabitol	–	–	–	–	–	–	–	+	+	–	–	–
L-Arabinose	v	–	+	v	–	–	–	–	–	–	–	–
Glucose	+	+	+	+	+	v(+)	+	+	+	+	+	+
Mannitol	–	–	–	–	–	v(–)	–	+	+	–	–	–
Sucrose	+	+	v	+	+	v(+)	+	+	+	v	+	+
D-Xylose	+	–	+	+	+	v(–)	–	v(+)	+	+	–	–
Melezitose	v	+	–	–	v	v(–)	+	+	v	+	–	v
Glycogen	–	–	+	v	–	–	–	–	–	–	–	–
Pullulan	–	–	+	+	–	–	–	–	–	–	–	–
Ribose	+	+	+	v	–	–	–	+	v	–	–	–
Trehalose	+	+	–	v	v	+	+	+	v	+	v	v
Production of:*												
PAL	–	v(–)	–	–	+	v(–)	+(w)	v	–	–	+	+
PYZ	+	v	+(w)	v	+	+	+	+	+	v	–	+
β -GLU	+	+(w)	–	v	+	+	+	–	–	+	+	+
β -NAG	v	–	+	+	+	–	–	–	–	–	–	–
β -GUR	+	–	–	–	–	–	–	–	–	–	–	–

* Production of: PAL, alkaline phosphatase; PYZ, pyrazinamidase; β -GLU, β -glucosidase; β -NAG, *N*-acetyl- β -glucosaminidase; β -GUR, β -glucuronidase.

of this phylogenetic cluster show that it represents a new sub-line worthy of separate species status. Phenotypically, the unidentified bacterium can be readily distinguished from all members of the *Actinomyces bovis* cluster of organisms and other described *Actinomyces* species. Thus we consider the unknown bacterium from dogs merits classification as a new species of the genus *Actinomyces*, for which the name *Actinomyces catuli* is proposed. Tests which are useful in differentiating *Actinomyces catuli* from other catalase-positive or catalase-variable *Actinomyces* species are shown in Table 1.

Description of *Actinomyces catuli* sp. nov.

Actinomyces catuli (ca'tu.li. L. masc. n. *catulus* small dog; L. masc. gen. n. *catuli* of a small dog).

Cells are straight to slightly curved rods which stain Gram-positive, are non-acid-fast and non-motile. Colonies are tiny, irregular, convex, white and adherent to medium at colony base. Facultatively anaerobic and

catalase-variable. End products of glucose metabolism are acetic, lactic (major) and succinic acids. Using API systems, acid is produced from D-glucose, maltose, lactose, D-raffinose, ribose, sucrose, trehalose and D-xylose. Acid is not produced from D-arabitol, cyclo-dextrin, glycogen, mannitol, melezitose, pullulan, sorbitol or tagatose. Acid may or may not be produced from L-arabinose, melibiose and methyl β -D-glucopyranoside. Aesculin is hydrolysed but gelatin and hippurate are not. Acid phosphatase, ester lipase C8, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, leucine arylamidase, pyrrolidonyl arylamidase and pyrazinamidase are produced. Alanine phenylalanine proline arylamidase, alkaline phosphatase, cystine arylamidase, α -fucosidase, glycyl tryptophan arylamidase, lipase C14, α -mannosidase, β -mannosidase, pyroglutamic acid arylamidase, chymotrypsin, trypsin, valine arylamidase and urease are not produced. Activities for arginine dihydrolase, *N*-acetyl- β -glucosaminidase, esterase C4 and α -galactosidase may or may not be detected. Nitrate is

reduced to nitrite. Acetoin is not produced. Isolated from dogs. Habitat is not known. The type strain is CCUG 41709^T (= CIP 106507^T).

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