NOTE

***Actinomyces coleocanis* sp. nov., from the vagina of a dog**

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A hitherto undescribed *Actinomyces*-like bacterium was isolated from the vagina of a dog. Biochemical testing and PAGE analysis of whole-cell proteins indicated that the isolate was phenotypically different from previously described *Actinomyces* species and related taxa. Sequencing of 16S rRNA showed that the unknown bacterium was distinct from all currently known *Actinomyces* species. Phylogenetically, the unidentified organism displayed a specific association with *Actinomyces europaeus*, but a sequence divergence of > 5% demonstrated that it represents a distinct species. Based on both phenotypic and 16S rRNA sequence considerations, it is proposed that the unknown strain from a dog be classified as a novel species, *Actinomyces coleocanis* sp. nov. The type strain is CCUG 41708^T (= CIP 106873^T).

Keywords: taxonomy, phylogeny, *Actinomyces coleocanis*, 16S rRNA

Actinomyces species primarily belong to the facultatively anaerobic indigenous microflora of human and animal mucous membranes (i.e. oral cavity, intestine and female genital tract), and several species are established pathogens (Schaal, 1986). Over the past decade, there has been a dramatic increase in the number of described species of the genus *Actinomyces*. The majority of these novel species have originated from human clinical specimens, where they occur as contaminants and/or represent hitherto unknown opportunistic pathogens, and have come to light primarily due to the implementation of improved molecular-based identification methodologies (e.g. 16S rRNA sequencing, PAGE protein profiling). Currently, much less is known about the diversity of *Actinomyces* species present in domestic and wild animals. There are, however, clear indications that the use of similar molecular diagnostic tools in veterinary microbiology will result in not only the recognition of much new diversity but also a better understanding of their host distribution and possible association with disease (Collins *et al.*, 1993; Pascual *et al.*, 1999; Hoyles *et al.*, 2000, 2001). During the course of an investigation of taxonomically problematic *Actinobacteria* from animal sources, we have characterized an unusual *Actinomyces*-like organism from the vagina

of a dog. Based on both phenotypic and phylogenetic evidence, we propose a novel species, *Actinomyces coleocanis* sp. nov.

Strain M343/98/2^T was isolated in mixed culture with *Corynebacterium genitalium* from the vagina of a cocker spaniel dog. The unidentified *Actinomyces*-like isolate was cultured on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C in air plus 5% CO₂. The strain was characterized biochemically by using the API Rapid ID32Strep 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). The GCW 3.0 software package (Applied Maths) was used for densitometric analysis, normalization and interpretation of protein patterns. The similarity between all pairs of traces was expressed by Pearson's product-moment correlation coefficient converted, for convenience, to a percentage similarity. The 16S rRNA gene sequence of the isolate was amplified by PCR and sequenced directly 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 novel isolate were determined by performing database searches. These sequences and those of other known related strains were retrieved from the GenBank or Ribosomal Database Project libraries and aligned with the newly determined sequence using

The GenBank accession number for the 16S rRNA sequence of strain CCUG 41708^T is AJ249326.

Fig. 1. Unrooted tree showing the phylogenetic relationships of *Actinomyces coleocanis* sp. nov. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1327 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are given at branching points. Bar, 1% sequence divergence.

the program DNATools (Rasmussen, 1995). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated using the programs PRETTY and DNADIST (using the Kimura-2 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 DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). Parsimony analysis was also performed using the same package (Felsenstein, 1989).

The unidentified isolate consisted of Gram-positive, straight to slightly curved rods, some of which displayed branching. Cells were non-acid-fast and non-spore-forming. The strain grew under aerobic and anaerobic conditions and was catalase-negative. Succinic and lactic acids were the major end products of glucose metabolism. Using API systems, the isolate produced acid from D-glucose, glycogen, maltose, lactose and pullulan. Activity was detected for alanine-phenylalanine-proline arylamidase, β -galactosidase, α -glucosidase, glycyl-tryptophan arylamidase and pyrazinamidase (weak reaction). All other tests on the API kits were negative. Based on its cellular morphology and staining characteristics, the absence of endospores, its facultatively anaerobic nature, negative catalase reaction and acid end products, we tentatively identified the organism as a member of the genus *Actinomyces*. However, the API biochemical profiles of the isolate did not correspond to any currently recognized species of the genus. In order to investigate

further the phenotypic affinities of the unidentified strain, its whole-cell protein profile was determined and compared with those of other *Actinomyces* species using an extensive protein database maintained by the CCUG. Upon comparative analysis of SDS-PAGE whole-cell protein profiles, the unknown dog bacterium formed a distinct line and did not exhibit a particularly close affinity with any recognized *Actinomyces* species (data not shown). The nearest species to the unknown isolate based on protein profiling corresponded to *Actinomyces europaeus*, joining at a correlation level of about 50%, with *Actinomyces marimammalium* the next closest relative, joining the aforementioned cluster at a correlation level of about 45%. All other described *Actinomyces* species were more distantly related. The PAGE findings were consistent with the biochemical distinctiveness of the unknown isolate and demonstrate clearly that it is different from all *Actinomyces* species recognized to date. To ascertain the phylogenetic position of the unknown organism, its almost complete 16S rRNA gene sequence (1437 nucleotides) was determined. Preliminary sequence database searches based on sequence similarities indicated that the unknown bacterium was most closely related to species of the genus *Actinomyces*. The highest sequence similarity (approx. 94.8%) was shown to *A. europaeus*, with other species showing substantially lower levels of relatedness (data not shown). Treeing analysis further demonstrated the placement of the unidentified bacterium within the *Actinomyces* group of species, with the novel isolate displaying a specific phylogenetic association to *A. europaeus* (bootstrap value 100% for 500 tree replicates) (Fig. 1). The clustering

Table 1. Tests that are useful in distinguishing *A. coleocanis* sp. nov. from *A. europaeus*

Tests were performed using API Rapid ID32S and API CORYNE systems. +, Positive; –, negative; –(+), a few strains positive; v, variable.

Characteristic	<i>A. coleocanis</i>	<i>A. europaeus</i>
Production of acid from:		
Glycogen	+	v
Lactose	+	–
Ribose	–	v
Production of:		
α-Galactosidase	–	+
Glycyl-tryptophan arylamidase	+	–(+)
Pyrazinamidase	+	–
Hydrolysis of aesculin	–	+

of the unidentified organism with *A. europaeus* was confirmed by parsimony analysis (data not shown).

It is apparent from both phenotypic and phylogenetic evidence that the unidentified isolate recovered from the vagina of a dog represents a hitherto unknown *Actinomyces* species. Both sequence divergence values and treeing analysis show that the bacterium represents a novel subline within the genus *Actinomyces* and displays a specific association with *A. europaeus* (Funke *et al.*, 1997). However, sequence divergence of > 5% between the unidentified isolate and *A. europaeus* shows that this affinity is not particularly close and that the dog bacterium merits separate species status. The separateness of the dog bacterium was also very evident from biochemical testing and protein profiling analysis. The API CORYNE profile 2410162 resembles the profiles of some *Gardnerella vaginalis* strains, whereas the API Rapid ID32Strep profile 40016461000 mimics those of some *Streptococcus mitis* and *Actinomyces odontolyticus* strains. The use of the two commercial systems in concert serves to distinguish the novel organism readily from all described *Actinomyces* species. Thus, based on the results of the reported polyphasic taxonomic study, we propose that the unidentified organism be classified in the genus *Actinomyces* as *Actinomyces coleocanis* sp. nov. Tests that are useful in distinguishing *A. coleocanis* from its nearest phylogenetic relative, *A. europaeus*, are shown in Table 1.

Description of *Actinomyces coleocanis* sp. nov.

Actinomyces coleocanis (co.le.o.can'is. Gr. n. *koleos* vagina; L. gen. n. *canis* of the dog; N.L. gen. n. *coleocanis* of the vagina of a dog).

Cells are straight to slightly curved rods, some of which exhibit branching. Cells stain Gram-positive and are non-acid-fast and non-motile. Facultatively anaerobic and catalase-negative. Succinic acid and lactic acid are the main end products of glucose metabolism. Using API systems, acid is produced from D-glucose, glycogen, maltose, lactose and pullulan. Acid is not produced from L-arabinose, D-arabitol, cyclodextrin, mannitol, melibiose, melezitose, methyl β-D-glucopyranoside, N-acetyl β-glucosamine, raffinose, ribose, sorbitol, sucrose, tagatose, trehalose or D-xylose. Aesculin, gelatin and hippurate are not hydrolysed. Alanine-phenylalanine-proline arylamidase, β-galactosidase, α-glucosidase, glycyl-tryptophan arylamidase and pyrazinamidase (weak reaction) are produced. Arginine dihydrolase, alkaline phosphatase, α-galactosidase, β-glucosidase, β-glucuronidase, pyroglutamic acid arylamidase, β-mannosidase, pyrrolidonyl arylamidase and urease are not produced. Acetoin is not produced. Nitrate is not reduced to nitrite. Respiratory menaquinones are absent.

The type strain is strain M343/98/2^T (= CCUG 41708^T = CIP 106873^T). Isolated from the vagina of a cocker spaniel dog. Habitat is not known.

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