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NOTE

# *Arcanobacterium hippocoleae* sp. nov., from the vagina of a horse

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**A polyphasic taxonomic study was performed on a previously unidentified Gram-positive, facultatively anaerobic, diphtheroid-shaped organism isolated from a vaginal discharge of a horse. Comparative 16S rRNA gene sequencing demonstrated that the strain was a member of the genus *Arcanobacterium*, but sequence divergence values of >4% with described species of this genus (viz: *Arcanobacterium haemolyticum*, *Arcanobacterium bernardiae*, *Arcanobacterium phocae*, *Arcanobacterium pluranimalium* and *Arcanobacterium pyogenes*) demonstrated that the isolate represented a novel species. The unknown bacterium was readily distinguished from other *Arcanobacterium* species by biochemical tests. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Arcanobacterium hippocoleae* sp. nov. The type strain of *A. hippocoleae* is CCUG 44697$^T$ (= CIP 106850$^T$).**

**Keywords:** taxonomy, phylogeny, *Arcanobacterium hippocoleae* sp. nov., 16S rRNA

The genus *Arcanobacterium* (Collins *et al.*, 1982) consists of a group of facultatively anaerobic, asporogenous, non-acid-fast, Gram-positive diphtheroid-shaped organisms within the *Actinobacteria*. The genus originally consisted of a single species, *Arcanobacterium haemolyticum*, but in recent years, four other species have been assigned to the genus: *Arcanobacterium bernardiae* (formerly *Actinomyces bernardiae*); *Arcanobacterium pyogenes* (formerly *Actinomyces pyogenes*); *Arcanobacterium phocae*; and *Arcanobacterium pluranimalium*. All described *Arcanobacterium* species have been recovered from human and/or animal sources and *A. haemolyticum* and *A. pyogenes* are long-established pathogens (Funke *et al.*, 1995). *A. bernardiae* has also been recovered from a variety of human clinical specimens (e.g. abscesses, blood; see Funke *et al.*, 1995) and may be an opportunistic pathogen. *A. pluranimalium* has been recovered from deer and porpoise (Lawson *et al.*, 2001), whereas *A. phocae* has been isolated from clinical specimens of seals and other marine animals (e.g. Ramos *et al.*, 1997). These latter two species are of unknown pathological significance. During the course of a study of taxonomically problematic *Actinobacteria* from veterinary sources, an unusual *Arcanobacterium*-like organism from the vagina of a horse has been characterized. Based on the results of a polyphasic taxonomic study, a novel species of the genus *Arcanobacterium*, *Arcanobacterium hippocoleae* sp. nov., is described.

Strain M401624/00/2$^T$ (= CCUG 44697$^T$ = CIP 106850$^T$) was isolated from a 3-year-old female Arab cross horse with vaginal discharge. The vaginitis was considered to be bacterial in origin. However, it was not possible to assign pathological significance to M401624/00/2$^T$ as this strain was isolated in mixed culture together with an unidentified *Corynebacterium* species and coagulase-negative staphylococci. Strain M401624/00/2$^T$ was cultivated on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C in air + 5% $CO_2$ and was biochemically characterized by using the API rapid ID32 Strep, API CORYNE and API ZYM systems according to the manufacturer's instructions (API bioMérieux). The type strains and other reference strains used in the investigation were all maintained by the Culture Collection of the University of Göteborg, Sweden. All biochemical tests were performed in duplicate. The 16S rRNA gene of the isolate was amplified by PCR and directly sequenced using a *Taq* dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 44697$^T$ is AJ300767.

DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new 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 databases and aligned with the newly determined sequences using the program DNATOOLS version 5.01.696 (Rasmussen, 1993). The resulting multiple sequence alignment was corrected manually using the program GENDOC (Nicholas *et al*., 1997) and a distance matrix was calculated using the program DNADIST (using the Kimura-2 correction parameter). 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 isolate recovered from the horse vagina consisted of Gram-positive, irregular-shaped, non-branching rods. Using commercially available API systems, the organism produced acid from glucose and lactose. Results for acid production from maltose differed between the different API systems used. Using the API CORYNE kit, acid was produced from maltose, but using the API rapid ID32 Strep system, this substrate was not fermented. The isolate gave positive reactions for α-glucosidase, β-galactosidase and β-glucuronidase. Depending on the test system used, different results were observed for *N*-acetyl-β-glucosaminidase and alkaline phosphatase production. Using API CORYNE, both enzymes were detected, but not with the API rapid ID32 Strep system. Both enzymes were also detected using the API ZYM kit. Alanine phenylalanine proline arylamidase, pyrolydonyl arylamidase, pyroglutamic acid arylamidase, arginine dihydrolase, β-glucosidase, α-galactosidase, β-mannosidase, pyrazinamidase, urease and glycyl tryptophan arylamidase were not detected. The isolate hydrolysed aesculin (weak reaction) and hippurate. Cellular morphology and biochemical reactions of the isolate were consistent with its assignment to the genus *Arcanobacterium*, although it did not appear to correspond to any described species of the genus. To ascertain the phylogenetic position of the unknown isolate, its 16S rRNA gene was sequenced and subjected to comparative analysis. The almost complete gene sequence (1492 nt) of the strain was determined and sequence database searches confirmed that the unknown bacterium was most closely related to species of the genus *Arcanobacterium* (range 94·8–95·7 %) with related genera displaying significantly lower levels of relatedness (data not shown). The results of neighbour-joining treeing analysis are shown in Fig. 1 and clearly confirmed the placement of the unknown bacterium with the genus *Arcanobacterium*.

It is apparent from both phylogenetic and phenotypic evidence that the unidentified isolate recovered from a horse vagina represents a hitherto unknown *Arcanobacterium* species. Both sequence divergence and treeing analysis showed that the unidentified bacterium represents a distinct subline within the genus *Arcanobacterium*. *A. phocae*, a species recovered from clinical specimens of common seals and grey seals (Ramos *et al*., 1997), displayed highest sequence relatedness (95·7 %) with the unidentified organism. Although there is no precise correlation between the amount of 16S rRNA sequence divergence and species delineation, it is generally recognized that divergence values of 3 % or more are significant (Stackebrandt & Goebel, 1994). The observed 4·3 % divergence between the unknown bacterium and *A. phocae*, and greater divergence values with other *Arcanobacterium* species, is consistent with separate species status. The separate-

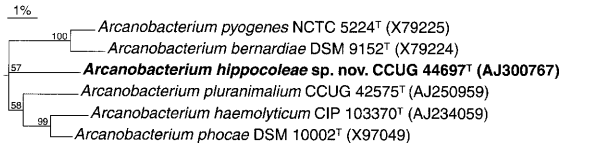


**Fig. 1.** Unrooted tree showing the phylogenetic relationships of *Arcanobacterium hippocoleae* sp. nov. and some closely related species. The tree, constructed using the neighbour-joining method, was based on a comparison of approx. 1327 nt. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points. Bar, 1 % sequence divergence.

**Table 1.** Tests useful for distinguishing *Arcanobacterium hippocoleae* from other *Arcanobacterium* species

Species (no. of strains tested): 1, *A. hippocoleae* (*n* = 1); 2, *A. bernardiae* (*n* = 15); 3, *A. haemolyticum* (*n* = 19); 4, *A. phocae* (*n* = 3); 5, *A. pyogenes* (*n* = 14). Tests performed using API Rapid ID32S, API CORYNE and API ZYM. Abbreviations: +, 90 % or more strains positive; −, 90 % or more strains negative; v, 11–89 % of strains positive.

| Character | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Acid from:** | | | | | |
| D-Arabitol | − | v | − | − | − |
| Glycogen | − | v | − | + | v |
| Lactose | + | − | + | − | + |
| Pullulan | − | + | − | − | − |
| Ribose | − | + | v | v | v |
| Sucrose | − | − | v | + | v |
| D-Xylose | − | − | − | v | + |
| **Hydrolysis of:** | | | | | |
| Gelatin | − | v | − | − | + |
| Hippurate | + | − | − | − | v |
| **Production of:** | | | | | |
| Acid phosphatase | − | − | + | + | − |
| Alanyl phenylalanine proline arylamidase | − | + | + | + | + |
| β-Glucuronidase | + | − | v | − | + |
| Pyrazinamidase | − | + | + | + | − |
| Pyrolydonyl arylamidase | − | v | v | + | + |
| Phosphoamidase | + | − | − | − | − |

ness of the unknown bacterium was also very evident from biochemical profiling. In particular, the unknown bacterium could be readily distinguished from other *Arcanobacterium* species by the traits shown in Table 1. Therefore, based on both phylogenetic and phenotypic evidence, it is proposed that the unidentified isolate be classified as a novel species of the genus *Arcanobacterium*, *Arcanobacterium hippocoleae* sp. nov.

### Description of *Arcanobacterium hippocoleae* sp. nov.

*Arcanobacterium hippocoleae* (hip.po.co′le.ae. Gr. n. *hippos* horse; Gr. n. *colea* vagina; M.L. fem. gen. n. *hippocoleae* of the horse vagina).

Cells are non-branching, irregular-shaped rods which stain Gram-positive, are non-acid-fast and non-motile. Colonies on blood agar are convex, circular, entire, shiny, opaque and grey. Weakly haemolytic. Facultatively anaerobic and catalase-negative. Using API systems, acid is produced from D-glucose and lactose, but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, pullulan, mannitol, melibiose, melezitose, methyl β-D-glucopyranoside, D-ribose, D-raffinose, sucrose, sorbitol, tagatose, trehalose or D-xylose. Acid may or may not be produced from maltose depending on the test system used. α-Glucosidase, β-galactosidase, β-glucuronidase, leucine arylamidase and phosphoamidase are detected, but not acid phosphatase, alanine phenylalanine proline arylamidase, arginine dihydrolase, chymotrypsin, esterase C-4, ester lipase C8, α-fucosidase, α-galactosidase, β-glucosidase, lipase C14, α-mannosidase, β-mannosidase, pyrolydonyl arylamidase, pyroglutamic acid arylamidase, pyrazinamidase, trypsin, valine arylamidase, urease or glycyl tryptophan arylamidase. *N*-Acetyl-β-glucosaminidase and alkaline phosphatase may or may not be detected depending on the test system used. Aesculin (weak reaction) and hippurate are hydrolysed, but not gelatin. Acetoin is not produced. Nitrate is not reduced to nitrite. Isolated from vaginal discharge from a horse. Habitat is not known. The type strain is CCUG 44697$^T$ (= CIP 106850$^T$).

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