NOTE

***Corynebacterium capitovis* sp. nov., from a sheep**Matthew D. Collins,¹ Lesley Hoyles,¹ Geoffrey Foster,²
Berit Sjöden² and Enevold Falsen³¹ Department of Food
Science and Technology,
University of Reading,
Reading RG6 6AP, UK² SAC Veterinary Services,
Inverness, UK³ Culture Collection,
Department of Clinical
Bacteriology, University of
Göteborg, SwedenAuthor for correspondence: Matthew D. Collins. Tel: +44 118 935 7226. Fax: +44 118 926 7917.
e-mail: m.d.collins@reading.ac.uk

An unknown Gram-positive rod-shaped bacterium was isolated from skin scrapings from the infected head of a sheep and subjected to a polyphasic taxonomic analysis. Chemical analysis revealed the presence of straight-chain and monounsaturated fatty acids and short-chain (C₃₂–C₃₆) mycolic acids consistent with the genus *Corynebacterium*. Comparative 16S rRNA gene sequencing confirmed that the unknown rod was a member of the genus *Corynebacterium*, with the organism forming a distinct sub-line and displaying greater than 3% sequence divergence with established species. The unknown *Corynebacterium* isolate was readily distinguished from recognized species of the genus by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium from a sheep be classified in the genus *Corynebacterium*, as *Corynebacterium capitovis* sp. nov. The type strain of *Corynebacterium capitovis* is CCUG 39779^T (= CIP 106739^T).

Keywords: *Corynebacterium capitovis*, sheep, taxonomy, phylogeny, 16S rRNA

The genus *Corynebacterium* currently contains over 50 species, the vast majority of which have been isolated from man or other animals. During the past decade, the number of recognized *Corynebacterium* species has increased dramatically due in the main to the use of higher precision molecular-based diagnostic methodologies. In particular, 16S rRNA gene sequencing in conjunction with molecular chemical approaches such as whole-cell protein profiling have proved invaluable not only for the recognition of new diversity, but in combination with improved phenotyping for the delineation of novel taxa (see for example Collins *et al.*, 1999; Fernandez-Garayzabal *et al.*, 1997, 1998; Funke *et al.*, 1997, 1998; Riegel *et al.*, 1995). In this article, we have used molecular chemical and molecular genetic approaches to aid the characterization of an unusual *Corynebacterium*-like organism recovered from the infected head of a sheep. Based on the reported findings, we propose yet another new species of the genus *Corynebacterium*, *Corynebacterium capitovis* sp. nov.

An unidentified rod-shaped organism designated S108/98/2 (CCUG 39779^T) was isolated from skin

scrapings of the infected head of a sheep. The unknown organism was isolated along with *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Peptococcus magnus*. The unidentified rod was cultured on Columbia Blood agar base supplemented with 5% defibrinated horse blood at 37 °C, in air plus 5% CO₂. The strain was biochemically characterized by using the API CORYNE and API ZYM systems according to the manufacturer's instructions (API bioMérieux). Preparation of cellular protein extracts for PAGE analysis, densitometric analysis, normalization of the protein profiles and numerical analyses were performed as described by Pot *et al.* (1994) using the GelCompar 3.0 software package (Applied Maths, Kortrijk, Belgium). The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage similarity value. Cell wall murein was prepared by mechanical disruption of cells and complete acid hydrolysates were analysed as described by Schleifer & Kandler (1972). Fatty acid methyl esters were prepared and analysed as described by Kämpfer & Kroppenstedt (1996) and the presence of mycolic acids was investigated by GLC analysis of trimethylsilylated derivatives (TMS-MAME) (Klatte *et al.*, 1994). The 16S rRNA genes of the isolate were amplified by PCR and directly sequenced using a *Taq*

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 39779^T is AJ297402.

Dye-Deoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic 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 (RDP) Libraries and aligned with the newly determined sequence 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 DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). The 16S rRNA gene sequence of strain CCUG 39779^T has been deposited in GenBank under accession number AJ297402.

The unidentified isolate recovered from a polymicrobial infection of the head of a sheep consisted of Gram-positive, non-motile, non-spore-forming, diphtheroid-shaped cells. Using commercially available API systems, the isolate produced acid from glucose but not from lactose, maltose, mannitol, sucrose, *N*-acetyl- β -glucosamine, ribose or D-xylose. Reactions for alkaline phosphatase, acid phosphatase, ester lipase C8, esterase C4, leucine arylamidase and lipase C14 were positive. No activity was detected for α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -fucosidase, α -mannosidase, valine arylamidase, pyrazinamidase, pyrrolidonyl arylamidase, phosphoamidase, chymotrypsin, trypsin or urease. The organism did not hydrolyse aesculin or gelatin and did not reduce nitrate. In terms of its overall morphological and biochemical characteristics, the unidentified bacterium somewhat resembled corynebacteria but did not appear to correspond to any recognized species of this genus. Cell wall analysis revealed the presence of meso-diaminopimelic acid within the isolate, consistent with its provisional assignment to the genus *Corynebacterium*. The cellular fatty acids of the isolate consisted of C16:0 (23%), C16:1 ω 9c (3%), C18:0 (6.1%) and C18:1 ω 9c (67.8%), which again resembled corynebacteria. Tuberculostearic acid was not present. TLC analysis of whole-cell methanolytates and GLC analysis of trimethylsilylated derivatives revealed the presence of short-chain mycolic acids (C32:0, C32:1, C32:2, C34:1, C36:1 and C36:2) in the strain, confirming its identification as a true *Corynebacterium*. PAGE analysis of whole-cell proteins of the unknown sheep bacterium was performed and the generated profile was compared with a comprehensive database maintained by the Culture Collection of the University of Göteborg. The unidentified organism from sheep was found to be

distinct from other *Corynebacterium* species (data not shown) and failed to show a specific affinity with any recognized species. The nearest species on PAGE analysis to the unknown isolate corresponded to *Corynebacterium mastitidis*, with the isolate joining the latter at less than 50% correlation. To ascertain the phylogenetic position of the unknown isolate, its almost complete 16S rRNA gene sequence was determined and subjected to a comparative analysis. Sequence database searches revealed that the unknown bacterium was phylogenetically a member of the genus *Corynebacterium* but was distinct from all described species. A tree constructed using the neighbour-joining method depicting the phylogenetic placement of the unidentified organism within a subset of the genus *Corynebacterium* is shown in Fig. 1 and unequivocally shows that it represents a new species. The unknown sheep isolate formed a distinct sub-line, associated with a small group of species, which included *Corynebacterium auris*, *Corynebacterium mycetoides* and *Corynebacterium lipophiloflavum*.

The results of the polyphasic taxonomic analysis clearly show that the unknown rod-shaped bacterium isolated from the infected head of a sheep represents a hitherto unrecognized species within the genus *Corynebacterium*. Phylogenetically the bacterium forms a distinct sub-line, with *C. auris*, *C. mycetoides* and *C. lipophiloflavum* as its nearest relatives. However, bootstrap resampling did not reveal a particularly significant affinity with any of the aforementioned species and sequence divergence values of more than 3% unequivocally demonstrate that the sheep bacterium represents a distinct species. The distinctiveness of the unknown species was also very evident from the whole-cell protein profiling analysis and from its biochemical traits. Therefore, based on both phylogenetic and phenotypic evidence, we propose that the unknown sheep bacterium be classified in the genus *Corynebacterium*, as *Corynebacterium capitovis* sp. nov. Tests which are useful in distinguishing *C. capitovis* from its closest phylogenetic relatives and from some other veterinary and human clinically relevant *Corynebacterium* species are shown in Table 1.

Description of *Corynebacterium capitovis* sp. nov.

Corynebacterium capitovis (ca.pit.o'vis. L. gen. n. *capitis* of a head; L. gen. n. *ovis* of a sheep; N.L. gen. n. *capitovis* of a sheep's head).

Cells are Gram-positive, non-motile, non-spore-forming diphtheroids. Colonies are circular, entire, convex and approximately 0.5 mm diameter after 24 h at 37 °C on blood agar. Colonies are lemon-pigmented and non-haemolytic. Facultatively anaerobic and catalase-positive. Non-lipophilic. Acid is produced from glucose but not from lactose, maltose, mannitol, ribose, sucrose or D-xylose. Activity for alkaline phosphatase, acid phosphatase, esterase C4 (weak),

Fig. 1. Unrooted tree based on 16S rRNA showing the phylogenetic relationships of *Corynebacterium capitovis* sp. nov. Bar, 1% sequence divergence.

Table 1. Characteristics that differentiate *Corynebacterium capitovis* from other veterinary and human clinically relevant *Corynebacterium* species

Glu, glucose; Mal, maltose; Suc, sucrose; v, variable; +, positive; —, negative.

Species	Fermentation/ oxidation	Lipophilism	Nitrate	Urease	Pyrazinamidase	Alkaline phosphatase	Acid production			Other characteristics
							Glu	Mal	Suc	
<i>C. capitovis</i>	F	—	—	—	—	+	+	—	—	Acid phosphatase positive
<i>C. camporealensis</i>	F	—	—	—	+	+	v	—	—	Acid phosphatase and ribose negative
<i>C. striatum</i>	F	—	+	—	+	+	+	—	v	
<i>C. jeikeium</i>	O	+	—	—	+	+	+	v*	—	No growth anaerobically
<i>C. coyleae</i>	F	—	—	—	+	+	+	—	—	Acid phosphatase and ribose positive
<i>C. auris</i>	O	—	—	—	+	+	—	—	—	Acid phosphatase positive
<i>C. argenteoratense</i>	F	—	—	—	+	v	+	—	—	
<i>C. amycolatum</i>	F	—	v	v	+	+	+	v	v	No mycolic acids
<i>C. bovis</i>	O	+	—	+	v	+	+	—	—	
<i>C. pseudotuberculosis</i>	F	—	v	+	—	v	+	+	v	
<i>C. mastitidis</i>	O	+	—	v	v	+	—	v	v	
<i>C. cystitidis</i>	F	—	—	+	+	+	+	—	—	
<i>C. renale</i>	F	—	—	+	+	—	+	—	—	
<i>C. pilosum</i>	F	—	+	+	+	+	+	—	—	
<i>C. lipophiloflavum</i>	O	+	—	+	+	+	—	—	—	

* Negative according to API CORYNE system database.

ester lipase C8, lipase C14 (weak) and leucine arylamidase is detected. No activity is detected for cystine arylamidase, chymotrypsin, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -mannosidase, N -acetyl- β -glucosaminidase, valine arylamidase, pyrazinamidase, phosphoamidase, pyrrolidonyl arylamidase, urease or trypsin. Aesculin and gelatin are not hydrolysed. Nitrate is not reduced. Cell wall contains *meso*-diaminopimelic acid. Long-chain fatty acids are of the straight chain saturated and monounsaturated types. Tuberculostearic acid is not present. Mycolic acids are present (C_{32} – C_{36}). Isolated from infected head of a

sheep. Habitat is unknown. The type strain is CCUG 39779^T (= CIP 106739^T).

Acknowledgements

We are grateful to Lena Dahl for assistance with PAGE analyses and to Professor Hans Trüper (University of Bonn, Germany) for help in coining the species epithet.

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