Characterization of a *Gemella*-like organism isolated from an abscess of a rabbit: description of *Gemella cuniculi* sp. nov.

L. Hoyles,¹ G. Foster,² E. Falsen³ and M. D. Collins¹

Author for correspondence: M. D. Collins. Tel: +44 1189 357226. Fax: +44 1189 26 7917.
e-mail: m.d.collins@reading.ac.uk

¹ Department of Food Science and Technology, University of Reading, UK

² SAC Veterinary Science Division, Drummond Hill, Inverness, UK

³ Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Sweden

An unknown Gram-positive, catalase-negative, ovoid-shaped bacterium isolated from the submandibular abscess of a rabbit was subjected to a polyphasic taxonomic analysis. Comparative 16S rRNA gene sequencing demonstrated the unknown coccus represents a new subline within the genus *Gemella*. The unknown isolate was readily distinguished from other recognized members of the genus *Gemella*, namely *Gemella haemolysans*, *Gemella bergeri*, *Gemella morbillorum*, *Gemella palaticanis* and *Gemella sanguinis*, by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on both phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium is classified in the genus *Gemella* as *Gemella cuniculi* sp. nov. The type strain is CCUG 42726^T.

Keywords: *Gemella cuniculi* sp. nov., taxonomy, phylogeny, 16S rRNA

INTRODUCTION

The genus *Gemella* embraces a group of catalase-negative, facultatively anaerobic, Gram-positive, coccoid-shaped (elongated and rod-shaped forms also occur) organisms which occur in pairs, tetrads or short-chains (Berger, 1992). The genus originally contained the single species *Gemella haemolysans* (formerly *Neisseria haemolysans*) and a second species, *Gemella morbillorum* (formerly *Streptococcus morbillorum*), was later assigned to the genus (Berger, 1961; Kilpper-Bälz & Schleifer, 1988). *Gemella haemolysans* and *Gemella morbillorum* are commensals of the mucous membranes of humans and some warm-blooded animals and have been shown to cause severe localized and generalized infections in humans, particularly immunocompromised patients (e.g. Berger, 1992; Eggelmeier *et al.*, 1992; Kaufhold *et al.*, 1989; Mitchell & Teddy, 1986; Petit *et al.*, 1993). During a study of *Gemella*-like organisms from human and animal sources, we recently described three other species of the genus, namely *Gemella bergeri*, *Gemella sanguinis* and *Gemella palaticanis*. *Gemella bergeri* and *Gemella sanguinis* were recovered from human clinical specimens (Collins *et al.*, 1998a, b), whereas *Gemella palaticanis* was isolated from a dog (Collins *et al.*,

1999). Although the pathogenic potential of these new species is not known, it seems likely that these are also residents of mucous membranes. In the course of this ongoing investigation, we have performed a polyphasic taxonomic study on a *Gemella*-like organism isolated from a submandibular abscess of a rabbit which does not correspond to any of the recognized species of this genus. In this article we report the phenotypic characteristics of the organism and the results of a phylogenetic analysis. Based on the results presented, a new species is described for which the name *Gemella cuniculi* sp. nov. is proposed.

METHODS

Culture and biochemical characterization. Strain M60449/99/1^T (CCUG 42726^T) was isolated from a submandibular abscess of a lop-eared pet rabbit. The bacterium was recovered from a mixed infection along with *Fusobacterium necrophorum*, *Prevotella melaninogenica* and a *Peptostreptococcus* species. The *Gemella*-like isolate, CCUG 42726^T, was cultured in air plus 5% CO₂ on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C. The strain was biochemically characterized by using the API rapid ID32 strep, API CORYNE and API ZYM systems, according to the manufacturer's instructions (API bioMérieux). Tests were performed in duplicate and the type strains of reference *Gemella* species, i.e. *Gemella bergeri* CCUG 37817^T, *Gemella morbillorum* CCUG 18164^T, *Gemella haemolysans* CCUG 37985^T, *Gemella palaticanis* CCUG 39489^T and *Gemella sanguinis* CCUG 27820^T, were included.

The GenBank accession number for the 16S rRNA sequence of isolate CCUG 42726^T is AJ251987.

PAGE analysis of whole-cell proteins. 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). The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage similarity value.

16S rRNA gene sequence analysis. The 16S rRNA genes of the isolate 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 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 CLUSTAL W (Thompson *et al.*, 1994). 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 correction; 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).

RESULTS AND DISCUSSION

The unidentified isolate consisted of Gram-positive, non-motile, ovoid-shaped cells. The organism grew on blood agar producing a β -haemolytic reaction and was non-pigmented. It was facultatively anaerobic and catalase-negative. Using commercially available API systems the isolate produced acid from glucose, mannitol and sorbitol. Acid production from maltose and sucrose was variable. The strain failed to produce acid from L-arabinose, D-arabitol, cyclodextrin, glycogen,

lactose, methyl- β -D-glucopyranoside, melibiose, mel-ezitose, *N*-acetylglucosamine, pullulan, trehalose, raffinose, ribose, tagatose and D-xylose. Tests for alkaline phosphatase, acid phosphatase, ester lipase C-8 (weak), phosphoamidase and pyrazinamidase (weak) were positive, whereas those for arginine dihydrolase, alanine-phenylalanine-proline arylamidase, chymotrypsin, cystine arylamidase, esterase C-4, α -fucosidase, α -galactosidase, β -galacturonidase, α -glucosidase, β -glucosidase, β -glucuronidase, lipase C14, α -mannosidase, β -mannosidase, leucine arylamidase, valine arylamidase, urease and trypsin were negative. The organism did not hydrolyse aesculin or gelatin and was Voges-Proskauer-negative. In terms of its cellular morphology and overall biochemical characteristics, the unidentified bacterium resembled the genus *Gemella*. PAGE analysis of whole-cell protein patterns, however, clearly demonstrated the unknown coccus was phenotypically distinct from currently described members of the genus *Gemella* and all other reference species examined. Indeed, the unknown organism failed to display a close affinity with any of the taxa studied (Fig. 1). The nearest species to the unidentified coccus based on protein profiling was *Gemella sanguinis*, although the association was very loose (joining at a correlation value of less than 60%). Other *Gemella* species were even more distantly related to the unknown isolate (Fig. 1). To investigate the phylogenetic position of the unknown bacterium its almost complete 16S rRNA gene sequence was determined and subjected to a comparative analysis. Sequence database searches revealed the unknown bacterium was phylogenetically most closely related to *Gemella* spp. (approx. 94.8–96.8% sequence similarity). Other low-G + C-containing taxa displayed much lower levels of sequence relatedness (data not shown). A tree constructed by the neighbour-joining method depicting the phylogenetic relationships of the

Table 1. Characteristics useful in differentiating *Gemella cuniculi* sp. nov. from other *Gemella* species

–, Negative; +, positive; v, variable; $\pm$, a few strains positive.

Test	<i>Gemella cuniculi</i>	<i>Gemella bergeri</i>	<i>Gemella haemolysans</i>	<i>Gemella morbillorum</i>	<i>Gemella palaticanis</i>	<i>Gemella sanguinis</i>
Acid from:						
Lactose	–	–	–	–	+	–
Mannitol	+	–	–	v	–	+
Maltose	–	–	+	+	+	+
Sucrose	–	–	v	+	+	+
Sorbitol	+	–	–	$\pm$	–	+
Trehalose	–	–	–	–	+	–
Production of:						
Alkaline phosphatase	+	–	+	–	–	+
Alanine-phenylalanine-proline arylamidase	–	–	–	v	+	+
Glycyl-tryptophan arylamidase	–	–	v	v	+	–

Fig. 1. Dendrogram derived from the unweighted pair group average linkage of correlation coefficients (expressed as percentage values) between whole-cell protein patterns of *Gemella cuniculi* sp. nov. and some relatives.

unidentified organism is shown in Fig. 2 and unequivocally demonstrates that the organism represents a new species within the genus *Gemella*. The bacterium formed a distinct subline and did not display a particularly significant affinity with any of the described species of the genus.

The polyphasic taxonomic analysis clearly shows the unknown ovoid-shaped bacterium isolated from a submandibular abscess of a rabbit represents a hitherto unrecognized member within the genus *Gemella*. Phylogenetically the bacterium forms a distinct subline clustering within the confines of the *Gemella* clade. 16S rRNA sequence divergence values of greater than 3% with other members of this genus unequivocally demonstrate the unknown coccus is worthy of separate

species status. In addition, the unidentified coccus can be phenotypically readily distinguished from all currently described *Gemella* species by biochemical tests (Table 1) and whole-cell protein profiling. Therefore, based on both phylogenetic and phenotypic evidence we consider the bacterium merits classification as a new species of the genus *Gemella*, for which the name *Gemella cuniculi* sp. nov. is proposed.

Description of *Gemella cuniculi* sp. nov.

Gemella cuniculi (cu.ni'cu.li. L. gen. masc. n. *cuniculi* of the rabbit).

Cells are Gram-positive, non-motile cocci. Produces β -haemolysis on blood agar. Non-pigmented. Facul-

Fig. 2. Unrooted tree based on 16S rRNA (accession numbers in parentheses) showing the phylogenetic relationships of *Gemella cuniculi* sp. nov. Bar, 1% sequence divergence. Bootstrap resampling values are shown at branching points.

tatively anaerobic and catalase-negative. Acid is produced from glucose, mannitol and sorbitol. Acid may be produced from maltose and sucrose. Acid is not produced from L-arabinose, D-arabitol, cyclodextrin, glycogen, melibiose, melezitose, N-acetylglucosamine, pullulan, raffinose, ribose, tagatose or D-xylose. Acid phosphatase, alkaline phosphatase, ester lipase C8 (weak), phosphoaminidase and pyrazinamidase are produced. Alanine-phenylalanine-proline arylamidase, arginine dihydrolase, chymotrypsin, cystine arylamidase, esterase C-4, α -fucosidase, α -galactosidase, β -galacturonidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -mannosidase, β -mannosidase, lipase C14, leucine arylamidase, valine arylamidase, urease and trypsin are not produced. Aesculin and gelatin are not hydrolysed and nitrate is not reduced to nitrite. Voges-Proskauer-negative. Isolated from a submandibular abscess of a rabbit. Habitat is unknown. The type strain is CCUG 42726^T (= CIP 106481^T).

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