NOTE

Characterization of a *Gemella*-like organism from the oral cavity of a dog: description of *Gemella palaticanis* sp. nov.

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A hitherto unknown Gram-positive, catalase-negative, facultatively anaerobic coccus isolated from a vesicle on the gum of a dog was characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing studies demonstrated that the isolate represents a new subline within the genus *Gemella*. The unknown bacterium was readily distinguished from all currently described members of this genus, *Gemella haemolysans*, *Gemella bergeri*, *Gemella morbillorum* and *Gemella sanguinis*, by biochemical tests and electrophoretic analysis of whole-cell proteins. On the basis of phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Gemella palaticanis* sp. nov. The type strain of *Gemella palaticanis* is CCUG 39489^T.

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

Species of the genus *Gemella* consist of catalase-negative, facultatively anaerobic, Gram-positive, coccoid-shaped organisms that are either arranged in pairs, often with adjacent sides flattened, or arranged in tetrads, short-chains or small, irregular clusters. Until recently, only two species of the genus were recognized, *Gemella haemolysans* and *Gemella morbillorum* (Berger, 1992). *G. haemolysans*, the type species of the genus, was originally classified as a representative of the Gram-negative genus *Neisseria* (Thjötta & Böe, 1938). However, numerous studies showed that the organism was biochemically incompatible with the genus *Neisseria*, and a new genus, *Gemella*, was created (Berger, 1961). *G. morbillorum*, the second species to be assigned to the genus, was originally classified as *Diplococcus morbillorum* (Prévot, 1933) and subsequently transferred to *Peptostreptococcus* and then to *Streptococcus* before its placement in the genus *Gemella* (Kilpper-Bälz & Schleifer, 1988). Recently, two additional species of the genus have been described, *Gemella bergeri* (Collins *et al.*, 1998a) and *Gemella sanguinis* (Collins *et al.*, 1998b), from human clinical specimens. Both *G. haemolysans* and *G. morbillorum* are residents of the

mucous membranes of humans and of certain warm-blooded animals and are recognized opportunistic pathogens. Although the habitats of *G. bergeri* and *G. sanguinis* are unknown, it seems probable that these species also form part of the normal human microbiota. In this article, we report the characteristics of a novel *Gemella*-like organism isolated from the oral cavity of a dog. On the basis of the results of a polyphasic taxonomic study, we describe another new species of the genus, *Gemella palaticanis* sp. nov.

Strain M663-98-1^T was recovered from a swab of a vesicle on the gum of a 1-year-old male Labrador dog that was diagnosed as suffering from a long-standing mild inflammatory lesion. The strain was isolated along with *Pasteurella* spp. on Columbia sheep-blood agar at 37 °C and has been deposited in the Culture Collection of the University of Göteborg, Sweden, under accession number CCUG 39489^T. The isolate was biochemically characterized by using the API Rapid ID32S and API ZYM 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 GELCOMP GCW 3.0 software package (Applied Maths) was used for densitometric analysis, normalization and interpretation of protein patterns. The G + C content of DNA of strain CCUG 39489^T was determined as described

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 39489^T is Y17280.

Fig. 1. Similarity dendrogram based on whole-cell protein pattern of *Gemella palaticanis* sp. nov. and related species. Levels of correlation are expressed as percentages of similarity for convenience.

by Garvie (1978). The 16S rRNA gene(s) of the isolate was amplified by PCR and directly sequenced 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 new isolate was determined by performing database searches. These sequences and those of other known related strains were retrieved from the GenBank or the Ribosomal Database Project (RDP) databases 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 Kimura's two-parameter correction) (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR and the stability of the groupings was estimated by bootstrap analysis (200 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The isolate from the gum of a dog consisted of Gram-positive, ovoid-shaped cells that were catalase- and oxidase-negative. On blood-agar plates after 72 h, the strain formed small pin-head colonies. Using commercial API systems, the isolate produced acid from glucose, lactose, maltose, sucrose (weak reaction) and trehalose. The organism failed to produce acid from D-arabitol, L-arabinose, cyclodextrin, glycogen, manitol, melibiose, melezitose, methyl β -D-glucopyranoside, pullulan, raffinose, ribose, tagatose or sorbitol.

Activities for alanine-phenylalanine-proline arylamidase and glycyl-tryptophan arylamidase were detected but no activities were detected for alkaline phosphatase, arginine dihydrolase, *N*-acetyl- β -glucosaminidase, α -galactosidase, β -galactosidase, β -mannosidase, pyroglutamic acid arylamidase or urease. The isolate did not produce acetoin and did not hydrolyse hippurate. Based on the aforementioned characteristics, the isolate showed some resemblance to members of the genus *Gemella*. The results of PAGE analysis of whole-cell proteins depicted in Fig. 1, however, revealed that the isolate was distinct from the four currently recognized species of this genus and all reference Gram-positive, catalase-negative cocci examined. To investigate the phylogenetic position of the unknown isolate, comparative 16S rRNA gene sequence analysis was performed. The almost complete gene sequence (>1400 nucleotides) of the dog isolate was determined and sequence searches of GenBank and RDP databases revealed that the unknown isolate was phylogenetically most closely associated with species of the genus *Gemella*. A tree constructed by the neighbour-joining method depicting the phylogenetic affinity of the unknown coccus is shown in Fig. 2 and clearly shows that the bacterium represents a distinct subline within the genus *Gemella*.

The bacterium, which originated from a vesicle on the gum of a dog, exhibited >4% 16S rRNA sequence divergence from currently known *Gemella* species and clearly represents a new species of this genus. Biochemically, the unknown coccus does not correspond

Fig. 2. Unrooted tree showing the phylogenetic relationships of *Gemella palaticanis* sp. nov. and some other low-G+C-content, Gram-positive bacteria. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1320 nucleotides. Bootstrap values, expressed as a percentage of 200 replications, are given at branching points. Bar, 1% sequence divergence.

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

Test	<i>G. palaticanis</i>	<i>G. bergeri</i>	<i>G. haemolysans</i>	<i>G. morbillorum</i>	<i>G. sanguinis</i>
Acid from:					
Lactose	+	—	—	—	—
Mannitol	—	v	—	v	+
Sorbitol	—	—	—	—/(+)	+
Sucrose	+	—	v	+	+
Trehalose	+	—	—	—	—
Production of:					
Alkaline phosphatase	—	—	+	—	+
Alanine-phenylalanine-proline arylamidase	+	—	—	v	v
Glycyl-tryptophan arylamidase	+	—	v	v	—

v, Variable; —/(+), a few strains tested positive.

to any of the four recognized species and PAGE analysis of whole-cell proteins confirmed the phenotypic separateness of the bacterium. On the basis of both phenotypic and phylogenetic evidence, we therefore consider that the unknown bacterium should be classified as a new species of the genus *Gemella*, for which the name *Gemella palaticanis* sp. nov. is proposed. Tests that serve to distinguish the novel bacterium from other *Gemella* species are given in Table 1.

Description of *Gemella palaticanis* sp. nov.

Gemella palaticanis (pa.la.ti.ca'nis. L. neut. n. *palatum* gum; L. masc. n. *canis* dog; M.L. gen. masc. n. *palaticanis* of the gum of a dog).

Cells are Gram-positive, non-spore-forming cocci that occur in pairs, in clusters or in short chains. Cocci are sometimes elongate. Colonies on blood-agar plates after 72 h are small, circular, entire, low-convex, translucent to opaque and smooth. Non-pigmented and non-haemolytic. Facultatively anaerobic. Catalase- and oxidase-negative. Acid is produced from glucose, lactose, maltose, sucrose (weak reaction) and trehalose. Acid is not produced from D-arabitol, L-arabinose, cyclodextrin, glycogen,

mannitol, melibiose, melezitose, methyl β -D-glucopyranoside, pullulan, raffinose, ribose, tagatose or sorbitol. Alanine-phenylalanine-proline arylamidase and glycyl-tryptophan arylamidase are produced. Alkaline phosphatase, arginine dihydrolase, *N*-acetyl- β -glucosaminidase, α -galactosidase, β -galactosidase, β -galacturonidase, β -glucosidase, β -glucuronidase, β -mannosidase, pyroglutamic acid arylamidase and urease are not produced. Hippurate is not hydrolysed. Acetoin is not produced. The G+C content of DNA is 32 mol%. Isolated from a vesicle on the gum of a dog. Habitat is unknown. The type strain is M663-98-1^T (=CCUG 39489^T).

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