Corynebacterium canis sp. nov., isolated from a wound infection caused by a dog bite

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A non-lipophilic, coryneform bacterium isolated from a patient's wound caused by a dog bite was characterized by phenotypic, chemotaxonomic and molecular genetic methods. Chemotaxonomic features suggested assignment of the unknown bacterium to the genus *Corynebacterium*. The isolate exhibited the following unusual features, which made it possible to phenotypically differentiate it from all other medically relevant corynebacteria: the Gram stain showed some very filamentous rods (>15 µm in length); some cells exhibited branching; colonies were domed and adherent to agar; the micro-organism was positive for pyrazinamidase, β-glucosidase, α-glucosidase and trypsin but negative for β-galactosidase. 16S rRNA gene sequencing and partial *rpoB* gene sequencing showed that the closest phylogenetic relative, *Corynebacterium freiburgense*, exhibited more than 1.9% and 17.9% divergence with the unknown bacterium, respectively. Based on both phenotypic and molecular genetic data, it is proposed that the isolate should be classified as a novel species, *Corynebacterium canis* sp. nov., with the type strain 1170^T (=CCUG 58627^T =DSM 45402^T).

During the 1990s, a plethora of new species of the genus *Corynebacterium* isolated from human clinical specimens were defined (Funke & Bernard, 2007). Within the last few years, microbiologists have also focused on the descriptions of new *Corynebacterium* species derived from animals (Collins *et al.*, 1999b, 2001a, b, 2004; Fernández-Garayzábal *et al.*, 2003, 2004; Funke *et al.*, 2010; Goyache *et al.*, 2003a, b; Vela *et al.*, 2003; Yassin, 2009). It is generally agreed that the most frequently found species of the genus *Corynebacterium* in human clinical materials have already been defined. Transmission of corynebacteria from animals to humans has not been systematically investigated. We have recently described a novel species, *Corynebacterium freiburgense*, which was probably transmitted from a dog to a human (Funke *et al.*, 2009). The present report outlines, again, the characteristics of an unusual *Corynebacterium* strain (1170^T) which might have been transmitted from an animal to a human. Applying a

polyphasic taxonomic approach, we demonstrate that this strain represents another new species of the genus *Corynebacterium* for which the name *Corynebacterium canis* sp. nov. is proposed.

The isolate (strain 1170^T) was aerobically cultured in August 2009 from a wound swab of a 47-year-old female who had been bitten by her dog in her forearm. Strain 1170^T grew together with anaerobically growing *Bacteroides* sp. and *Prevotella* sp. We did not attempt to culture strain 1170^T from the dog's mouth.

The Gram stain of strain 1170^T showed some coryneform bacteria arranged in single cells or clusters, but filamentous forms (>15 µm in length) were also observed; some cells even exhibited branching. Filamentous forms are very unusual for medically relevant corynebacteria but can be observed in *Corynebacterium durum* (Riegel *et al.*, 1997), *Corynebacterium sundsvallense* (Collins *et al.*, 1999a) and *Corynebacterium matruchotii*, the latter with the typical whip-handle appearance at the end of the cells (Funke & Bernard, 2007) which was not observed in strain 1170^T. Branching is, to the best of our knowledge, not observed in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene and partial *rpoB* gene sequences of strain 1170^T are GQ871934 and GQ871935, respectively.

other medically relevant corynebacteria. Strain 1170^T did not stain partially acid-fast (modified Kinyoun stain). Colonies on Columbia sheep blood agar plates (BD) were beige–whitish, showed irregular margins and were 1–2 mm in diameter after 48 h of incubation. Supplementation of Columbia sheep blood agar plates with Tween 80 (Merck) (Funke & Bernard, 2007) did not significantly enhance the colony size, i.e. strain 1170^T was non-lipophilic. The colonies were domed and adherent to blood agar; for the corynebacteria, adherence to agar is infrequently found but can be observed in some strains of *C. durum* (Riegel *et al.*, 1997), *C. sundsvallense* (Collins *et al.*, 1999a), *C. thomssenii* (Zimmermann *et al.*, 1998) and *C. freiburgense* (Funke *et al.*, 2009).

Strain 1170^T was further screened for chemotaxonomical features and biochemical reactions applying methods outlined previously (Funke *et al.*, 1993). Chemotaxonomic investigations revealed the presence of *meso*-diaminopimelic acid as diamino acid of the peptidoglycan as well as of mycolic acids, which was, together with the negative reaction for partial acid-fastness, compatible with the assignment of strain 1170^T to the genus *Corynebacterium* (Funke & Bernard, 2007). The main straight-chain saturated fatty acids were palmitic and stearic acids; oleic acid was the predominant unsaturated fatty acid; tuberculostearic acid was not detected.

When applying the commercial API Coryne (bioMérieux), we observed the numerical code 3050125 after 24 h of incubation at 35 °C which corresponded with an identification of the isolate as *Rothia dentocariosa*. However, the microscopic morphology of strain 1170^T was not compatible with *R. dentocariosa* in which extremely long cells are not seen. In addition, *R. dentocariosa* contains lysine instead of *meso*-diaminopimelic acid as diamino acid, lacks mycolic acids and has predominantly branched chain (anteiso-C_{15:0} and anteiso-C_{17:0}), not monosaturated and saturated, cellular fatty acids in its cell wall.

We also tested strain 1170^T for the presence of the diphtheria toxin gene using PCR-primers Cdipt-1 ATCCACTTT-TAGTGCAGAACCTTGGTCA and Cdipt-2 GAAAACT-TTCTCTCGTACCACGGGACTAA as outlined previously (Nakao & Popovic, 1997), but the isolate did not harbour this virulence gene.

Another unusual feature of strain 1170^T was the positive α -glucosidase reaction which is observed in the pyrazinamidase-positive species *Corynebacterium freneyi* and *Corynebacterium xerosis* (Funke & Bernard, 2007), both of which are not adherent to agar (Funke & Bernard, 2007; Funke & Frodl, 2008). The positive β -glucosidase (aesculinase) reaction (strong, turning positive within 6 h in the API Coryne system) is also highly unusual for clinically relevant non-lipophilic corynebacteria and is detected only in some strains of *Corynebacterium aurimucosum*, *Corynebacterium glucuronolyticum*, *C. durum* and *C. matruchotii* (Funke & Bernard, 2007). Finally, strain 1170^T, to the best of our knowledge, is the only medically

relevant member of the genus *Corynebacterium* expressing trypsin activity. This enzyme activity (cleavage of proteins) might be of advantage for strain 1170^T in its natural habitat (probably dogs' mouths).

In summary, strain 1170^T exhibited some very unusual phenotypic features not compatible with any of the established species of the genus *Corynebacterium*. Therefore, we decided to investigate the phylogenetic distinctiveness of strain 1170^T by sequencing the almost entire 16S rRNA gene (1497 bp) according to a published method (Beck *et al.*, 2008). For 16S rRNA gene sequence comparisons we used the EzTaxon software (Chun *et al.*, 2007) as well as the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/blast/Blast.cgi). Strain 1170^T clustered within the genus *Corynebacterium* with 16S rRNA gene similarities to the 79 species defined at the time of writing ranging from 91.87% for *Corynebacterium afermentans* subsp. *lipophilum* to 98.09% for *C. freiburgense*. The ten closest (including *C. freiburgense*) phylogenetic relatives of the novel strain are: *Corynebacterium ulcerans* (95.87% 16S rRNA gene sequence similarity), *C. pseudotuberculosis* (95.72%), *C. diphtheriae* (95.67%), *C. argentoratense* (95.58%), *C. spheniscorum* (95.54%), *C. falsenii* (95.41%), *C. vitaeruminis* (95.33%), *C. aquilae* (95.26%) and *C. felinum* (95.25%). The nearest other genera comprised *Mycobacterium*, *Dietzia*, *Tsukamurella* and *Rhodococcus* with 16S rRNA gene sequence similarities at a level of approximately 93%. It is evident from the molecular genetic data, showing a 16S rRNA gene divergence clearly above the 1.3% threshold (Stackebrandt & Ebers, 2006), that strain 1170^T represents a novel species of the genus *Corynebacterium*. Table 1 outlines phenotypic features that allow a clear differentiation between the novel species and its nearest phylogenetic relatives. A phylogenetic tree was reconstructed using the neighbour-joining method, included in the MEGA4 suite software (Tamura *et al.*, 2007), based on a comparison of approximately 1350 nt. Bootstrap values, expressed as percentages of 1000 replications, are

Table 1. Characteristics that differentiate strain 1170^T from its nearest phylogenetic relatives

Species: 1, *C. canis* sp. nov.; 2, *C. argentoratense*; 3, *C. diphtheriae*; 4, *C. freiburgense*; 5, *C. pseudotuberculosis*; 6, *C. ulcerans*. Data from Funke *et al.* (2009), Funke & Bernard (2007) and this study. +, Positive; –, negative.

Characteristic	1	2	3	4	5	6
Filamentous rods	+	–	–	–	–	–
Adherence to agar	+	–	–	+	–	–
Pyrazinamidase	+	+	–	–	–	–
β -Glucosidase	+	–	–	+	–	–
α -Glucosidase	+	–	+	–	+	+
β -Galactosidase	–	–	–	+	–	–
α -Chymotrypsin	–	+	–	–	–	–
Trypsin	+	–	–	–	–	–

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences (approximately 1300 bp), showing the position of strain 1170^T among its closest phylogenetic neighbours within the genus *Corynebacterium*. *Rhodococcus equi* was used as a distant outlier and *Corynebacterium amycolatum* as a near outlier. Bar, 0.01 substitutions per nucleotide position. Bootstrap values, expressed as a percentage of 1000 replications, are given at the branching points. Type strains used and their corresponding GenBank 16S rRNA gene sequence accession numbers are given.

given at each branching point in Fig. 1. From the treeing analysis, it is evident that strain 1170^T represents a distinct species of the genus *Corynebacterium*.

To further outline the distinctiveness of strain 1170^T we sequenced a part of the *rpoB* gene using primers as described by Khamis *et al.* (2004). Within a stretch of 406 bp (GenBank accession number GQ871935), *C. freiburgense* was, again, one of the closest phylogenetic neighbours of strain 1170^T with 18.0% sequence divergence; this was clearly above the 5% threshold suggested for distinct species of the genus *Corynebacterium* (Khamis *et al.*, 2004). Other related corynebacteria were *C. argentoratense* (16.3% divergence), *C. durum* (16.7%), *C. vitaeruminis* (17.9%), *C. mastitidis* (18.0%), *C. matruchotii* (18.5%), *C. testudinoris* (18.8%), *C. ulcerans* (18.8%), *C. timonense* (19.5%) and *C. efficiens* (19.8%). A phylogenetic tree of the partial *rpoB* gene sequences, reconstructed by applying the same methods as for Fig. 1, is shown in Fig. 2.

Antimicrobial susceptibility testing was performed by applying the microdilution method of the Clinical and Laboratory Standards Institute (CLSI) as well as the interpretation guidelines of this organization (CLSI, 2006). Strain 1170^T was susceptible to cefotaxime, ciprofloxacin, doxycycline, erythromycin, gentamicin, linezolid, meropenem, penicillin, rifampicin and vancomycin. The strain was also susceptible to the vibriocidal compound O/129 (Funke *et al.*, 1996).

It is interesting to note that among the species of the genus *Corynebacterium* only a few species, like *Corynebacterium auriscanis* (Collins *et al.*, 1999b) and *C. ulcerans* (Lartigue *et al.*, 2005), have so far been isolated from dogs. We would like to emphasize that it is not unlikely that many other species of the genus *Corynebacterium* (and even some novel species) might be detected in clinical materials from dogs if those are more systematically screened for the presence of corynebacteria.

Based on the results of the polyphasic taxonomic study outlined, we propose strain 1170^T as a novel species of the genus *Corynebacterium*, for which the name *Corynebacterium canis* sp. nov. is proposed.

Description of *Corynebacterium canis* sp. nov.

Corynebacterium canis (ca'nis. L. gen. n. *canis* of a dog).

Cells are Gram-positive, non-spore-forming and non-motile. Some cells are typically club-shaped rods but some are filamentous and some even show branching. Colonies are beige-whitish, dryish with irregular edges, convoluted, up to 1–2 mm in diameter after 48 h of incubation and adherent to sheep blood agar. Facultatively anaerobic. Catalase-positive. Acid is produced from glycerol, galactose, glucose, fructose, mannose, arbutin, salicin, maltose, sucrose, trehalose, starch, glycogen, tagatose and 5-keto-gluconate but not from erythritol, arabinose, ribose, xylose, adonitol, methyl β -D-xylopyranoside, L-sorbose, inositol,

Fig. 2. Phylogenetic tree based on partial *rpoB* gene sequences (approximately 400 bp), showing the position of strain 1170^T among its closest phylogenetic neighbours within the genus *Corynebacterium*. *C. diphtheriae* was used as an outlier. Bar, 0.02 substitutions per nucleotide position. Bootstrap values, expressed as a percentage of 1000 replications, are given at the branching points. Type strains used and their corresponding GenBank *rpoB* gene sequence accession numbers are given.

D-mannitol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamine, amygdalin, cellobiose, lactose, melibiose, inulin, melezitose, xylitol, gentiobiose, turanose, D-lyxose, fucose, arabinol, gluconate or 2-keto-gluconate. Activities of the following enzymes can be detected: nitrate reductase, pyrazinamidase, β -glucosidase, α -glucosidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin and phosphoamidase. Activities of urease, pyrrolidonyl arylamidase, gelatinase, lipase, cystine arylamidase, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are not detected. The CAMP reaction is negative. The cell wall contains meso-diaminopimelic acid and mycolic acids are also present. The main straight-chain saturated fatty acids are palmitic and stearic acids; oleic acid is the predominant unsaturated fatty acid.

The type strain, 1170^T (=CCUG 58627^T =DSM 45402^T), was isolated from a patient's wound following a dog bite.

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