Corynebacterium freiburgense sp. nov., isolated from a wound obtained from a dog bite

Guido Funke,1 Reinhard Frodl,1 Kathryn A. Bernard2 and Ralf Englert3

1Department of Medical Microbiology and Hygiene, Gärtner and Colleagues Laboratories, Elisabethenstrasse 11, 88212 Ravensburg, Germany
2National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington Avenue, Winnipeg, MB R3E 3R2, Canada
3Department of Medical Microbiology, Labor Clotten, Bismarckallee 10, 79098 Freiburg/Breisgau, Germany

A non-lipophilic, coryneform bacterium, isolated from a patient’s wound obtained from 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 peculiar features which made it possible to differentiate it phenotypically from all other medically relevant corynebacteria: older colonies exhibited a ‘spoke-wheel’ macroscopic morphology, colonies were strongly adherent to blood agar and the strain did not have pyrazinamidase activity, but was positive for β-galactosidase. 16S rRNA gene sequencing showed that the closest phylogenetic relative exhibited more than 3.9% divergence from the unknown isolate. Based on phenotypic and molecular genetic data, it is proposed that the isolate should be classified as a representative of a novel species, Corynebacterium freiburgense sp. nov., with strain 1045T (=CCUG 56874T =DSM 45254T) as the type strain.

During the 1990s, a plethora of novel Corynebacterium species isolated from human clinical specimens was described (Funke & Bernard, 2007). Within the last few years, microbiologists have also focused on descriptions of novel Corynebacterium species obtained from animals (Collins et al., 1999b, 2001, 2004; Fernández-Garayzabal et al., 2004). Although it is generally agreed that the most frequently found Corynebacterium species in human clinical materials have already been defined, novel Corynebacterium species are still being described, often based, however, on single strains (Yassin et al., 2002; Yassin, 2007). The present report outlines the characteristics of a single, unusual Corynebacterium strain (1045T) which may have been transmitted from an animal to a human. Using a polyphasic taxonomic approach, it has been demonstrated that this strain represents another novel Corynebacterium species.

Strain 1045T was cultured in August 2008 from a wound swab of a 57-year-old female who had been bitten by her dog on her forearm. Strain 1045T grew together with Pasteurella multocida, α-haemolytic streptococci and Prevotella species. Gram staining of cells of strain 1045T showed coryneform bacteria arranged singly with typical club-shaped elements; filamentous forms were not observed. The isolate was negative for partial acid-fastness. Colonies on Columbia sheep blood agar plates (BD) were beige–whitish, showed irregular margins and were 1–2 mm in diameter. Supplementation of Columbia sheep blood agar plates with Tween 80 (Merck) (Funke & Bernard, 2007) did not enhance colony size significantly, i.e. strain 1045T was non-lipophilic. Interestingly, the colonies were strongly adherent to blood agar; of the coryneform bacteria, adherence to agar is also observed in some strains of Corynebacterium durum (Riegel et al., 1997), Corynebacterium sundsvallense (Collins et al., 1999a) and Corynebacterium thomssenii (Zimmermann et al., 1998). Another very peculiar feature was the ‘spoke-wheel’ macroscopic morphology of the colonies after 5 days of incubation at 37°C in a 5% CO2-enriched atmosphere. This type of morphology is not seen in other true corynebacteria, but may be observed in some Rothia dentocariosa strains (Funke & Bernard, 2007).

Strain 1045T was further screened for chemotaxonomic features and biochemical reactions using previously described methods (Funke et al., 1993). Chemotaxonomic investigations revealed the presence of meso-diaminopimelic acid as diamin acid of the peptidoglycan, as well as mycolic acids, features which, together with the negative reaction for partial acid-fastness, are compatible with the assignment of strain 1045T 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.

When applying the commercial API Coryne (bioMérieux), a negative pyrazinamidase reaction was observed (numerical API Coryne code: 1440365), which prompted us to consider that the isolate belonged to the Corynebacterium diphtheriae/C. ulcerans/C. pseudotuberculosis group of bacteria, since these bacteria are the only known large-colony-forming, medically relevant corynebacteria that do not express this particular enzyme continuously (Funke & Bernard, 2007). As a result of this and because of the clinical nature of the patient’s wound, the isolate was tested for the presence of the diphtheria toxin gene using PCR primers Cdipht-1 (5'-ATCCACCTTTTAGGGAAGACC TTGGTCA) and Cdipht-2 (5'-GAAAACCTTTCTTCG TACCAGGGACTAA), as outlined previously (Nakao & Bernard, 2007). As a result of this and because of the clinical nature of the patient’s wound, the isolate was tested for the presence of the diphtheria toxin gene using PCR primers Cdipht-1 (5'-ATCCACCTTTTAGGGAAGACC TTGGTCA) and Cdipht-2 (5'-GAAAACCTTTCTTCG TACCAGGGACTAA), as outlined previously (Nakao & Popovic, 1997). Results showed that strain 1045T did not harbour this virulence gene.

Another unusual feature for a medically relevant corynebacterium was the positive β-galactosidase reaction of strain 1045T in both the API Coryne (at pH 7.4) and the API ZYM (at pH 5.4) (bioMérieux) systems. C. durum (Rassoulian Barrett et al., 2001) and Corynebacterium glucuronolyticum (Funke et al., 1995) are the only other clinically significant true corynebacteria that express this enzyme. Two further features of strain 1045T are also not observed frequently in other clinical corynebacteria (Funke & Bernard, 2007), i.e. a positive aesculinease reaction (delayed, turning positive after 72 h incubation only) and the ability to ferment lactose [tested with both the API Coryne and the API 50CH (bioMérieux) systems].

In summary, strain 1045T exhibited some very unusual phenotypic features that are not compatible with any Corynebacterium species with validly published names. Therefore, the phylogenetic distinction of strain 1045T was investigated by sequencing the almost-entire 16S rRNA gene (1481 bp) according to a published method (Beck et al., 2008). Strain 1045T clustered with the type strains of the 70 currently recognized species of the genus Corynebacterium and 16S rRNA gene sequence similarities ranged from 91.54 % with C. durum to 96.06 % with C. pseudotuberculosis. The ten closest phylogenetic relatives of the isolate were the type strains of C. pseudotuberculosis (96.06 % 16S rRNA gene sequence similarity), Corynebacterium vitaeruminis (95.92 %), C. ulcerans (95.82 %), Corynebacterium felinus (95.81 %), Corynebacterium sphenisorum (95.60 %), Corynebacterium argentoratense (95.57 %), Corynebacterium variabile (95.57 %), Corynebacterium aquileae (95.55 %), C. diphtheriae (95.34 %) and Corynebacterium falsenii (95.19 %). As expected, the type strains of members of the genera Dietzia, Rhodococcus and Tsukamuraella were also related phylogenetically to the isolate, with 16S rRNA gene sequence similarities of approximately 92 %. It is evident from the molecular genetic data, which clearly shows 16S rRNA gene sequence divergence above the 3 % threshold (Stackebrandt & Goebel, 1994), that strain 1045T represents a novel Corynebacterium species. Table 1 outlines phenotypic features that enable the isolate to be differentiated clearly from its nearest phylogenetic relatives. A phylogenetic tree was constructed using the neighbour-joining method included in the MEGA4 software suite (Tamura et al., 2007), based on a comparison of approximately 1350 nt. Bootstrap values, expressed as percentages of 1000 replications, are given at each branching point in Fig. 1. From the treeing analysis, it is evident that strain 1045T represents a distinct Corynebacterium species.

Antimicrobial susceptibility testing was performed using the microdilution method of the Clinical and Laboratory Standards Institute (CLSI), as well as the interpretation guidelines of this organization (CLSI, 2006). Strain 1045T was susceptible to cefotaxime, ciprofloxacin, doxycycline, erythromycin, gentamicin, linezolid, meropenem, penicillin, rifampicin and vancomycin. The strain was also susceptible to the viobiocidal compound O/129 (Funke et al., 1996).

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

Based on the results of the outlined polyphasic taxonomic study, it is proposed that strain 1045T represents a novel species of the genus Corynebacterium, Corynebacterium freiburgense sp. nov.

Table 1. Characteristics that differentiate strain 1045T from its nearest phylogenetic relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrazinamidase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Pyrroloidyl arylamidase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid production from lactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
and tagatose, but not from fructose, galactose, glucose, sorbose, starch, trehalose, turanose, xylitol or xylose. The following enzyme activities can be detected: nitrate reductase, β-galactosidase, β-glucosidase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, esterase (C4), leucine arylamidase, cystine arylamidase, α-glucosidase, α-acetylglucosidase, adonitol, α-glucuronidase, α-acetylgalactosidase, esterase (C4), leucine arylamidase, cystine arylamidase, α-glucosidase, α-acetylglucosidase, adonitol, α-glucuronidase, -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 is 1045T (=CCUG 56874T =DSM 45254T), isolated from a patient’s wound obtained from a dog bite.

**Acknowledgements**

We thank Leyla Kaya for expert technical assistance.

**References**


