Corynebacterium mustelae sp. nov., isolated from a ferret with lethal sepsis

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A non-lipophilic coryneform bacterium, strain 3105T, was isolated from various tissues of a ferret with lethal sepsis. The strain was characterized by phenotypic and chemotaxonomic methods, which suggested an assignment of the isolate to the genus Corynebacterium. Strain 3105T exhibited the following peculiar features that made it possible to differentiate it phenotypically from all other corynebacteria: its distinctive ‘humid cellar’-like odour, strong adherence to agar and a greenish-beige pigment. Strain 3105T exhibited more than 2.8% 16S rRNA gene sequence divergence from its closest phylogenetic neighbour, Corynebacterium pseudotuberculosis NCTC 3450T (97.12% sequence similarity). Analysis of the highly variable region within the rpoB gene sequence showed that strain 3105T exhibited more than 14% divergence from its closest phylogenetic relative, again C. pseudotuberculosis. Based on the data presented, it is proposed that the ferret isolate should be classified within a novel species, Corynebacterium mustelae sp. nov. (type strain 3105T = CCUG 57279T = DSM 45274T).

During the 1990s, only four of the 25 newly defined Corynebacterium species were isolated from animals, whereas, in the last 10 years, nine of the 19 newly defined Corynebacterium species have been isolated from animals. It is generally agreed that the most frequently encountered Corynebacterium species in human clinical specimens are already defined (Funke & Bernard, 2007), whereas Corynebacterium species isolated from animals have not been systematically investigated. During our ongoing research project on the taxonomy of true Corynebacterium species, we have received a strain with unusual features that was isolated from an animal.

Strain 3105T was cultured from necropsy lung tissue as well as from the liver and kidneys of an approximately 3-year-old male ferret with lethal sepsis. Gram-staining of strain 3105T showed coryneform bacteria arranged in single cells with typical club-shaped elements, but filamentous forms were not observed. The isolate did not stain partially acid-fast. When grown aerobically on Columbia sheep blood agar plates (BD) at 35 °C, strain 3105T showed greenish-beige colonies with irregular margins, which are not seen in other true corynebacteria. Characteristically, strain 3105T had a ‘humid cellar’-like odour not observed in other coryneform bacteria. In addition, strain 3105T was strongly adherent to agar, a feature that is seen very rarely in true corynebacteria with the exceptions of Corynebacterium durum, C. freiburgense, C. sundsvallense and C. thomssenii (Funke & Bernard, 2007; Funke et al., 2009).

Strain 3105T was further screened for chemotaxonomic features and biochemical reactions, applying methods outlined previously (Funke et al., 1993). Chemotaxonomic investigations revealed the presence of meso-diaminopimelic acid as the diamino acid of the peptidoglycan and of mycolic acids, which, together with the negative reaction for partial acid-fastness, were compatible with the assignment of the strain to the genus Corynebacterium (Funke & Bernard, 2007). The main straight-chain saturated cellular fatty acids were palmitic (33%) and stearic (6%) acids. Oleic acid (24%) was the predominant unsaturated fatty acid and tuberculostearic acid was not detected.

Because of the severe disease of the animal from which strain 3105T had been isolated, we tested the isolate for the presence of the diphtheria toxin gene using the PCR primers Cdipht-1 (5’-ATCCACCTTTTAGCGGAGAACCTTGTTCA-3’) and Cdipht-2 (5’-GAAAACTTTTTCTTGCAGAACACGACTAA-3’), as outlined by Nakao & Popovic (1997). The strain did not harbour this virulence gene.

Because strain 3105T exhibited some phenotypic traits that are not seen in any other presently defined Corynebacterium, we decided to investigate the phylogenetic distinctiveness of strain 3105T by sequencing almost entirely the 16S rRNA gene (>1470 bp) according to a
published method (Beck et al., 2008). The BLAST software tool (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and the EzTaxon software (Chun et al., 2007) were applied to compare the determined 16S rRNA gene sequence with those of other bacteria. Strain 3105\(^{T}\) clustered within the genus Corynebacterium and 16S rRNA gene sequence similarities with the type strains of the 73 presently defined Corynebacterium species ranged from 92.13 % for Corynebacterium glucuronolyticum to 97.12 % for Corynebacterium pseudotuberculosis NCTC 3450\(^{T}\). According to the EzTaxon algorithm, the ten closest phylogenetic relatives (including C. pseudotuberculosis) of strain 3105\(^{T}\) were the type strains of Corynebacterium vitaeruminis (97.08 % 16S rRNA gene sequence similarity), C. felinum (96.85 %), C. ulcerans (96.78 %), C. diphtheriae (96.71 %), C. afermentans subsp. lipophilum (96.65 %), C. testudinoris (96.12 %), C. singularare (95.73 %), C. coyleae (95.59 %) and C. striatum (95.58 %). As expected, the nearest other genera were Dietzia, Rhodococcus and Tsukamurella, with 16S rRNA gene sequence similarities of approximately 92 %. If the new 16S rRNA gene sequence divergence criterion (98.7 % cut-off value for species differentiation; Stackebrandt & Ebers, 2006) is used, it is evident that strain 3105\(^{T}\) represents a novel Corynebacterium species.

In order to demonstrate further that strain 3105\(^{T}\) represents a hitherto-unknown species, we sequenced a highly variable part of the rpoB gene, as described by Khamis et al. (2004). When comparing this particular part of the rpoB gene sequence of strain 3105\(^{T}\) with those from other Corynebacterium species, the type strain of C. pseudotuberculosis was again the closest neighbour, with less than 86 % sequence similarity (59 mismatches within 406 bp), and C. felinum shared less than 85 % similarity. A 16S rRNA gene sequence phylogenetic tree was constructed using the neighbour-joining method included

### Table 1. Characteristics that differentiate strain 3105\(^{T}\) from its nearest phylogenetic relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>3</th>
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<td>Odour</td>
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<td>Colony colour*</td>
<td>GNB</td>
<td>WGR</td>
<td>ND</td>
<td>WGR</td>
<td>WGR</td>
<td>Y</td>
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<tr>
<td>Adherence to agar</td>
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<td>ND</td>
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<td>Enzyme activity</td>
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<tr>
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<td>Nitrate reductase</td>
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<td>+</td>
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<tr>
<td>Acid production from sucrose</td>
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<td>-</td>
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</tr>
</tbody>
</table>

*GNB, Greenish beige; WGR, whitish grey; Y, yellow.
†C. diphtheriae biotype Belfanti is negative.
‡Fewer than 1 % of isolates are able to produce acid from sucrose.
Corynebacterium mustelae sp. nov.

Description of Corynebacterium mustelae sp. nov.

Corynebacterium mustelae (mus.tel’ae. L. n. mustela a weasel, and also a scientific zoological genus name; L. gen. mustelae of a weasel, of Mustela, referring to the isolation of the type strain from a ferret, Mustela putorius furo).

Cells stain Gram-positive and are non-spore-forming, typically club-shaped rods that occur as single cells, in pairs or in small clusters. Colonies are greenish beige, with irregular edges, up to 1–2 mm in diameter after 48 h incubation and express a characteristic ‘humid cellar’-like odour. Non-lipophilic. Facultatively anaerobic. Catalase-positive. Acid is produced from ribose, glucose, fructose, mannose, N-acetylglucosamine, arbutin, maltose, sucrose, trehalose, gentiobiose, tagatose, l-fucose and 5-ketogluconate, but not from glycerol, erythritol, l-arabinose, xylose, adonitol, methyl β-xylulose, galactose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, salicin, celllobiose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, lyxose, D-fucose, arabinol, gluconate or 2-ketogluconate. Activity of the following enzymes is not detected: nitrate reductase, pyrrolidonyl arylamidase, urease, alkaline and acid phosphatases, lipase, valine arylamidase, trypsin, chymotrypsin, x- and β-galactosidases, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The Christie–Atkins–Munch–Petersen (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; the predominant unsaturated fatty acid is oleic acid.

The type strain, 3105T (=CCUG 57279T =DSM 45274T), was isolated from necropsy lung tissue of a ferret with lethal sepsis.

References


