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

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A non-lipophilic coryneform bacterium, strain 3105^T, 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 3105^T 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 3105^T exhibited more than 2.8 % 16S rRNA gene sequence divergence from its closest phylogenetic neighbour, *Corynebacterium pseudotuberculosis* NCTC 3450^T (97.12 % sequence similarity). Analysis of the highly variable region within the *rpoB* gene sequence showed that strain 3105^T 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 3105^T = CCUG 57279^T = DSM 45274^T).

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 3105^T 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 3105^T 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 3105^T showed greenish-beige colonies with irregular margins, which are not seen in other true corynebacteria. Characteristically, strain 3105^T had a 'humid cellar'-like odour not observed in other coryneform bacteria. In addition, strain 3105^T 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 3105^T 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 3105^T had been isolated, we tested the isolate for the presence of the diphtheria toxin gene using the PCR primers Cdipt-1 (5'-ATCCACTTTTAGTGCGAGAACC-TTGGTCA-3') and Cdipt-2 (5'-GAAAACCTTTCTTCG-TACCACGGGACTAA-3'), as outlined by Nakao & Popovic (1997). The strain did not harbour this virulence gene.

Because strain 3105^T exhibited some phenotypic traits that are not seen in any other presently defined *Corynebacterium*, we decided to investigate the phylogenetic distinctiveness of strain 3105^T by sequencing almost entirely the 16S rRNA gene (>1470 bp) according to a

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and partial *rpoB* gene sequences of strain 3105^T are FJ374773 and FJ467330.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain 3105^T (*Corynebacterium mustelae* sp. nov.) and its closest phylogenetic neighbours. Percentages at nodes are levels of bootstrap support based on 1000 replications. *Rhodococcus equi* DSM 20307^T was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.

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 vitruerminis* (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. singulare* (95.73 %), *C. coyleae* (95.59 %) and *C. striatum* (95.58 %). As expected, the nearest other genera were *Dietzia*, *Rhodo-*

coccus 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

Taxa: 1, *Corynebacterium mustelae* sp. nov. 3105^T; 2, *C. diphtheriae*; 3, *C. felinum*; 4, *C. pseudotuberculosis*; 5, *C. ulcerans*; 6, *C. vitruerminis*. Data for reference species were taken from Collins *et al.* (2001) and Funke & Bernard (2007). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6
Odour	Humid cellar	–	ND	–	–	–
Colony colour*	GNB	WGR	ND	WGR	WGR	Y
Adherence to agar	+	–	ND	–	–	–
Enzyme activity						
Aesculinase	+	–	–	–	–	+
Nitrate reductase	–	+†	–	–	–	+
Pyrazinamidase	+	–	+	–	–	+
Pyrrolidonyl arylamidase	–	–	+	–	–	+
Urease	–	–	–	+	+	+
Acid production from sucrose	+	–‡	–	–	–	+

*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.

in the MEGA4 software (Tamura *et al.*, 2007), based on a comparison of approximately 1350 nucleotides, with bootstrap analysis (Fig. 1). From the phylogenetic analysis, it is evident that strain 3105^T represents a distinct *Corynebacterium* species. Table 1 outlines the phenotypic features that allow clear differentiation between the novel *Corynebacterium* strain and its nearest phylogenetic relatives.

Antimicrobial susceptibility testing was performed applying the microdilution method of the Clinical and Laboratory Standards Institute as well as the interpretation guidelines of this organization (CLSI, 2006). Strain 3105^T was susceptible to cefotaxime, ciprofloxacin, doxycycline, erythromycin, gentamicin, linezolid, meropenem, penicillin and vancomycin.

The present report provides further evidence that it is unlikely that the heterogeneity of true *Corynebacterium* species encountered in animals is fully understood. It is not known at present whether transmission of these corynebacteria to humans occurs.

Based on the results of this polyphasic taxonomic study, we propose that strain 3105^T is a representative of a novel species of the genus *Corynebacterium*, *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-ketoglucuronate, but not from glycerol, erythritol, L-arabinose, xylose, adonitol, methyl β -xyloside, galactose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, amygdalin, salicin, cellobiose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, lyxose, D-fucose, arabinol, gluconate or 2-ketogluconate. Activity of the following enzymes is detected: pyrazinamidase, α -glucosidase, aesculinase, esterase (C4), esterase lipase (C8), leucine and cystine arylamidases and phosphoamidase. Activity of the following enzymes is not detected: nitrate reductase,

pyrrolidonyl arylamidase, urease, alkaline and acid phosphatases, lipase, valine arylamidase, trypsin, chymotrypsin, α - 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, 3105^T (=CCUG 57279^T =DSM 45274^T), was isolated from necropsy lung tissue of a ferret with lethal sepsis.

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