Corynebacterium ulceribovis sp. nov., isolated from the skin of the udder of a cow with a profound ulceration

A. F. Yassin

Correspondence

A. F. Yassin

yassin@mibi03.meb.uni-bonn.de

Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, Sigmund-Freud-Straße 25, 53127 Bonn, Germany

A coryneform bacterium isolated from a cow with a profound ulceration was characterized by using phenotypic and molecular taxonomic methods. Chemotaxonomic investigations revealed the presence of cell-wall chemotype IV and short-chain mycolic acids consistent with the genus *Corynebacterium*. Comparative 16S rRNA gene sequencing showed that the organism formed a hitherto unknown subline within the genus *Corynebacterium*. Sequence divergence values of greater than 3.6 % from recognized *Corynebacterium* species, together with phenotypic differences, showed that the unidentified bacterium represents a new member of this genus. On the basis of phenotypic and phylogenetic considerations, it is proposed that the novel bacterium be classified as representing a novel species of the genus *Corynebacterium*, with the name *Corynebacterium ulceribovis* sp. nov. The type strain is IMMIB L-1395^T (=DSM 45146^T=CCUG 55727^T).

The genus *Corynebacterium* contains a large number of high-G+C content, Gram-positive bacteria and encompasses a diverse collection of aerobic to facultatively anaerobic, rod-shaped organisms. Currently the genus comprises more than 80 species, a high proportion of which have been described relatively recently, in the main because of the use of much-improved phenotypic and molecular identification methods. Most of the newly described *Corynebacterium* species have been isolated from human (e.g. Funke *et al.*, 1997, 1998; Renaud *et al.*, 2001; Sjöden *et al.*, 1998; Yassin *et al.*, 2002a, b) or animal (e.g. Fernández-Garayzábal *et al.*, 1998, 2004; Goyache *et al.*, 2003; Collins *et al.*, 2001, 2004) clinical sources. During the course of characterization of bacterial isolates encountered in clinical sources, a Gram-positive, rod-shaped organism was found that had chemotaxonomic characteristics consistent with a provisional assignment to the genus *Corynebacterium*. Findings from further taxonomic and phylogenetic investigations indicated that the isolate was different from recognized species of the genus *Corynebacterium*. Based on the evidence from the phylogenetic and phenotypic studies, it is proposed that this isolate, designated strain IMMIB L-1395^T, be classified as representing a novel species of the genus *Corynebacterium*.

Strain IMMIB L-1395^T was isolated from the skin of the udder of a cow with a profound ulceration. The strain was grown aerobically at 37 °C on Columbia agar (Oxoid) supplemented with 5 % sheep blood. It was characterized biochemically using the API Coryne, API 20 Strep and API ZYM systems, according to the manufacturer's instructions (bioMérieux), except for the time of incubation. The isomeric form of diaminopimelic acid was determined by using the methods of Becker *et al.* (1964) and whole-cell sugars were determined by using the method of Lechevalier (1968). Lipids were extracted using acid methanolysis and mycolic acids were detected with TLC as described by Minnikin *et al.* (1980). Fatty acids were analysed as described by Yassin *et al.* (2007).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR products were carried out using procedures described by Rainey *et al.* (1996). The purified PCR products were sequenced using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) as described in the manufacturer's protocol. An Applied Biosystems 310 DNA Genetic Analyzer was used for the electrophoresis of the sequence reaction products. The 16S rRNA gene sequence of strain IMMIB L-1395^T, as well as those of recognized species of the genus *Corynebacterium* retrieved from the GenBank, were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the respective tool of the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB L-1395^T is AM922112.

An extended neighbour-joining phylogenetic tree based on 16S rRNA gene sequences is available as supplementary material with the online version of this paper.

Fig. 1. Neighbour-joining tree showing the position of strain IMMIB L-1395^T within the radiation of species of the genus *Corynebacterium*. The tree was based on a comparison of 16S rRNA gene sequences that were at least 90 % complete (with regard to the *Escherichia coli* sequence). Numbers at nodes indicate the level of bootstrap support (%) based on neighbour-joining analyses of 1000 resampled datasets; solid circles indicate that the corresponding nodes (groupings) were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 10.0 % sequence divergence. An extended neighbour-joining tree is available as Supplementary Fig. S1 in IJSEM Online.

maximum-likelihood (Felsenstein, 1981) methods. The evolutionary distance matrix was calculated using the correction of Jukes & Cantor (1969). The topologies of the resultant tree were evaluated using bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings.

Cells of strain IMMIB L-1395^T stained Gram-positive and, on microscopic examination, appeared to be irregular short rods, non-motile and non-spore-forming. On Columbia blood agar, colonies were large (approx. 2–4 mm in diameter, after 48 h incubation at 37 °C), circular and non-haemolytic. The organism was facultatively anaerobic, catalase-positive and oxidase-negative. Using API Coryne, positive results were obtained for acid production from glucose and the production of pyrazinamidase. Using API ZYM, activity was detected for acid phosphatase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. All other enzyme tests were negative using this kit. An examination of cell-wall murein acid hydrolysates of strain IMMIB L-1395^T revealed the presence of *meso*-diaminopimelic acid as the dibasic amino acid. TLC analysis of wall sugars revealed the presence of galactose and arabinose, i.e. the organism had cell-wall chemotype IV *sensu* Lechevalier & Lechevalier (1970). Lipid analysis revealed the presence of corynemycolic acids. Examination of the non-hydroxylated, long-chain cellular fatty acids of strain IMMIB L-1395^T showed the presence of straight-chain saturated C_{14:0} (0.3 %), C_{15:0} (2.2 %), C_{16:0} (29.3 %), C_{17:0} (2.1 %),

C_{18:0} (4.3 %) and monounsaturated fatty acids C_{16:1ω9c} (2.2 %), C_{17:1} (0.05 %), C_{18:1} (0.2 %) and C_{18:1ω9c} (57.6 %). These chemotaxonomic characteristics together with the morphological and biochemical properties were strongly indicative that the organism belonged to the genus *Corynebacterium*.

To establish the phylogenetic position of strain IMMIB L-1395^T, its 16S rRNA gene sequence was determined in this study (1481 bases). Sequence database searches revealed that the unknown bacterium was most closely related to species of the genus *Corynebacterium* (data not shown). Treeing analysis confirmed the placement of strain IMMIB L-1395^T within the genus *Corynebacterium*, with the novel bacterium showing an affinity with *Corynebacterium hansenii*, *C. xerosis*, *C. amycolatum*, *C. sphenisci* and *C. freneyi*. A tree constructed using the neighbour-joining method based on a subset of *Corynebacterium* species, showing the nearest phylogenetic relatives of the unidentified bacterium, is shown in Fig. 1. Comparative 16S rRNA gene sequence analysis demonstrated that strain IMMIB L-1395^T represents a hitherto unknown corynebacterial species, but did not display a statistically significant association with any recognized species of the genus. The highest sequence similarity values were shown with *C. hansenii* (96.4 % similarity), *C. xerosis* (96.1 %), *C. amycolatum* (95.9 %), *C. sphenisci* (95.6 %) and *C. freneyi* (95.0 %). Significantly lower levels of relatedness were shown to other members of the genus *Corynebacterium* (data not shown). Although there is no precise correlation

Table 1. Characteristics that differentiate strain IMMIB L-1395^T (*Corynebacterium ulceribovis* sp. nov.) from its closest phylogenetic relatives

Strains: 1, strain IMMIB L-1359^T; 2, *C. hansenii* DSM 45109^T; 3, *C. xerosis* DSM 20743^T; 4, *C. amycolatum* DSM 6922^T; 5, *C. sphenisci* DSM 44792^T; 6, *C. freneyi* DSM 44506^T. All data are from this study. +, Positive reaction; –, negative reaction. All strains are positive for pyrazinamidase activity and acid production from glucose, but are negative for urease activity.

Characteristic	1	2	3	4	5	6
Nitrate reduction	–	–	+	–	+	+
Alkaline phosphatase	+	–	+	+	+	+
Acid production from:						
Maltose	–	+	+	+	+	+
Sucrose	–	+	+	–	–	+
D-Ribose	–	+	+	+	–	+
Trehalose	–	+	+	–	+	+

between percentage 16S rRNA gene sequence similarity and species delineation, it is generally recognized that divergence values of 3 % or more are significant (Stackebrandt & Goebel, 1994). Support for the distinctiveness of the unknown bacterium was also evident from phenotypic analyses. Tests that proved useful in distinguishing the novel species from some recognized *Corynebacterium* species are shown in Table 1. Based on the phylogenetic and phenotypic evidence presented, it is considered that strain IMMIB L-1395^T should be assigned to the genus *Corynebacterium* as representing a novel species, for which the name *Corynebacterium ulceribovis* sp. nov. is proposed.

Description of *Corynebacterium ulceribovis* sp. nov.

Corynebacterium ulceribovis (ul.ce.ri.bo'vis. L. n. *ulcus* -eris ulcer; L. n. *bos bovis* a cow, a bull; N.L. gen. n. *ulceribovis* of an ulcer of a cow).

Cells are Gram-positive, non-spore-forming, non-motile rods. Colonies are creamy, circular, smooth, entire and 2–4 mm in diameter after 48 h incubation at 37 °C on Columbia agar supplemented with 5 % sheep blood. Facultatively anaerobic, catalase-positive and oxidase-negative. Colonies are non-haemolytic. Acid is produced from D-glucose, but not from L-arabinose, glycerol, glycogen, inulin, D-lactose, maltose, D-mannitol, raffinose, D-ribose, D-sorbitol, sucrose, trehalose or D-xylose. Hippurate and Tween 80 are hydrolysed, but aesculin, gelatin and urea are not. Activity is detected for acid phosphatase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, pyrazinamidase and naphthol-AS-BI-phosphohydrolase. No activity is detected for arginine dihydrolase, chymotrypsin, cysteine arylamidase, esterase (C4), α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, lipase (C14), α -mannosidase, pyrrolidonyl arylamidase, trypsin or valine arylamidase. Nitrate is not reduced. Acetoin production is positive. Corynemycolic acids are present. Long-chain cellular acids are of the straight-chain saturated and monounsaturated

types, with C_{16:0} and C_{18:1 ω 9c} predominating; tuberculoostearic acid is not present.

The type strain, IMMIB L-1395^T (=DSM 45146^T=CCUG 55727^T), was isolated from the skin of the udder of a cow with a profound ulceration, in Schleswig Holstein, Germany.

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