Corynebacterium ureicelerivorans sp. nov., a lipophilic bacterium isolated from blood culture

A. F. Yassin

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

A lipophilic coryneform bacterium isolated from a blood culture from a patient with signs of septicaemia was characterized by means of phenotypic and molecular taxonomic methods. Chemotaxonomic investigations revealed the presence of cell-wall chemotype IV and short-chain mycolic acids, which are consistent with the genus Corynebacterium. The isolate was characterized biochemically by the very rapid (approx. 60 s) positive result that was obtained in a urease test in the API Coryne system. Comparative 16S rRNA gene sequencing demonstrated that the isolate belonged phylogenetically to the genus Corynebacterium. The values for sequence divergence (> 1.4 %) with respect to known Corynebacterium species, together with phenotypic differences, show that the unidentified bacterium represents a novel member of this genus. On the basis of both the phenotypic and phylogenetic data, this isolate should be classified within a novel species of the genus Corynebacterium, for which the name Corynebacterium ureicelerivorans sp. nov. is proposed. The type strain is IMMIB RIV-2301T (= DSM 45051T = CCUG 53377T).

The application of chemotaxonomic methods and molecular-based approaches, especially 16S rRNA gene sequencing, has resulted in a much-improved taxonomy of the genus Corynebacterium. The genus currently comprises over 80 recognized species, many of which have been described during the last decade. Most of the newly described Corynebacterium species have been isolated from clinical human (e.g. Funke et al., 1997a, b, 1998b; Sjödén et al., 1998; Collins et al., 1999; Yassin et al., 2002a, b) or animal (e.g. Fernández-Garayzábal et al., 1998, 2004; Goyache et al., 2003a, b; Collins et al., 2001a, b, 2004) sources. During the course of the characterization of bacterial isolates encountered in clinical sources, a Gram-positive, rod-shaped organism was found that has chemotaxonomic characteristics consistent with its provisional assignment to the genus Corynebacterium. Further taxonomic and phylogenetic investigations indicated that it is different from previously described species of the genus Corynebacterium.

Strain IMMIB RIV-2301T was isolated from a blood culture from a patient suffering from a fever and exhibiting signs of septicaemia. The isolate was cultured on Columbia blood agar supplemented separately with 5% sheep blood, brain-heart infusion agar and trypticase soy agar (Oxoid) to determine its morphological properties. Lipophilic requirements were determined by using a standard procedure (Riegel et al., 1994). Fermentation and enzyme activity tests were performed using the API Coryne, API 20 Strep and the API ZYM systems according to the instructions of the manufacturer (bioMérieux), except for the period of incubation. The API Coryne and API Strep enzyme reactions and tests of acid production from carbohydrates were read after incubation at 37°C for 72 h. The isomeric form of the diaminopimelic acid was determined by using the methods of Becker et al. (1964), and the 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 purified using preparative TLC as described by Yassin (1988) before being separated, identified and quantified by GC as described by Yassin et al. (1993).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were carried out using procedures described previously (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 electrophoresis of the sequence reaction products. The 16S rRNA gene sequence of isolate IMMIB RIV-2301T along with those of Corynebacterium species with validly published names (retrieved from GenBank) were added to the ARB database (Ludwig et al., 2004) and aligned using the respective tool in the ARB package. The resulting sequence of strain IMMIB RIV-2301T is AM397636.

An extended neighbour-joining tree showing the position of strain IMMIB RIV-2301T is available as supplementary material with the online version of this paper.
alignment was corrected manually and evolutionary trees were then inferred using the maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix was calculated using the correction of Jukes & Cantor (1969). The topologies of the resulting trees were evaluated in bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method (based on 1000 resamplings).

Strain IMMIB RIV-2301\textsuperscript{T} consisted of Gram-positive, non-motile, non-spore-forming, short-rod-shaped cells. On Columbia blood agar, colonies were very small (approx. 0.1–0.3 mm in diameter after 48 h incubation at 37 °C), circular, dry and non-haemolytic. Larger creamy colonies were observed on brain-heart infusion agar and on trypticase soy agar supplemented with 1 % Tween 80. The organism was facultatively anaerobic and was catalase- and urease-positive. The API Coryne numerical profile was 6101104, which corresponds to the numerical profile for Corynebacterium bovis. However, the latter species differs from strain IMMIB RIV-2301\textsuperscript{T} by its negative urease and positive \(\beta\)-galactosidase tests. Furthermore, the results of biochemical tests for strain IMMIB RIV-2301\textsuperscript{T} (i.e. it requires lipid for growth, is negative for nitrate reduction, is urease-positive, is negative for aesculin hydrolysis and produces acid from glucose) were consistent with the assignment of this strain to CDC coryneform group F-1 (Hollis & Weaver, 1981). However, the positive alkaline phosphatase test and the negative results for acid production from maltose and sucrose are not consistent with such an assignment. An examination of the cell-wall murein acid hydrolysates of the strain revealed the presence of meso-diaminopimelic acid as the dibasic amino acid. TLC analysis of the cell-wall sugars revealed the presence of galactose and arabinose, i.e. the organism possesses 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 this strain showed the presence of straight-chain saturated fatty acids \(C_{14:0}\) (0.02 %), \(C_{16:0}\) (10.2 %) and \(C_{18:0}\) (1.7 %), monounsaturated fatty acid \(C_{18:1}\)\textsuperscript{9c} (83.1 %) and tuberculostearic acid (4.98 %). These chemotaxonomic characteristics, together with the morphological and biochemical properties of isolate IMMIB RIV-2301\textsuperscript{T}, were strongly indicative that the organism belongs to the genus Corynebacterium.

To establish the phylogenetic position of strain IMMIB RIV-2301\textsuperscript{T}, its 16S rRNA gene sequence was determined in this study. A tree depicting the phylogenetic relationships of this unidentified bacterium within the genus Corynebacterium is shown in Fig. 1. Strain IMMIB RIV-2301\textsuperscript{T} formed a distinct subline with Corynebacterium mucifaciens DMMZ 2278\textsuperscript{T} as its nearest relative.

The clustering of the unknown bacterium with C. mucifaciens DMMZ 2278\textsuperscript{T} was supported by a bootstrap value of 77 % (Fig. 1). However, a sequence divergence of 1.4 % between the unknown organism and C. mucifaciens DMMZ 2278\textsuperscript{T} clearly demonstrated that they represent quite different species. It is now recognized that a 16S rRNA gene sequence similarity range above 98.7–99 % should be mandatory for establishing the genomic uniqueness of a novel isolate (Stackebrandt & Ebers, 2005). In addition, it is important to note that many genomically distinct species within the genus Corynebacterium, including Corynebacterium pseudodiphtheriticum and Corynebacterium propinquum (>99 % 16S rRNA gene sequence similarity), C. mucifaciens and Corynebacterium afermentans (98.5 % similarity) and Corynebacterium diphtheriae, Corynebacterium ulcerans and Corynebacterium pseudotuberculosis (>98 % similarity) (Pascual et al., 1995; Ruimy et al., 1995; Funke et al., 1997b), exhibit comparable, or even higher, levels of relatedness. Thus the 1.4 % 16S rRNA gene sequence divergence between strain IMMIB RIV-2301\textsuperscript{T} and its closest neighbour, C. mucifaciens DMMZ 2278\textsuperscript{T}, together with the distinctive phenotype of the unidentified strain (Table 1), clearly demonstrates that the latter represents a novel species. Furthermore, a sequence divergence of 6.7 % between strain IMMIB RIV-2301\textsuperscript{T} and the biochemically similar type strain of C. bovis clearly
Table 1. Characteristics that differentiate strain IMMIB RIV-2301T from biochemically similar members of Corynebacterium to which it is most closely related phylogenetically.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid requirement</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</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>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pyrazinamidase</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tuberculostearic acid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Test was positive in approx. 60 s using the API Coryne system.

The type strain, IMMIB RIV-2301T (=DSM 45051T=CCUG 53377T), was isolated from blood culture from a patient with signs of septicemia.

acknowledgements

I thank Professor Dr Hans-Georg Trüper for nomenclatural advice.

References


