Corynebacterium gottingense sp. nov., isolated from a clinical patient

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Abstract

A Gram-positive bacterial strain, 99221/2016T, was isolated from blood of a patient with bacteraemia at the Institute of Medical Microbiology, Göttingen, Germany. The strain was rod-shaped with a palisade arrangement of cells, non-spore-forming, non-lipophilic, catalase-positive and oxidase-negative. It grew well at 37 °C on Columbia blood agar and showed good growth under aerobic, microaerophilic and anaerobic conditions. The colonies were white-cream, circular and convex with a shiny, smooth surface. The predominant respiratory quinones were MK-8(H2) and MK-9(H2). The polar lipids profile contained phosphatidylethanolamine, diphasphatidylglycerol, phosphatidylglycerol and phosphatidylglycositol. Two unidentified phospholipids and several unidentified lipids were also detected. The prevalent cellular fatty acids comprised cis-9-octadecenoic acid (C18:1ω9c), hexadecanoic acid (C16:0) and pentadecanoic acid (C15:0). Corynemycosides with 28–36 carbons in length were present. The whole-cell hydrolysate contained meso-diaminopimelic acid and arabinose, glucose, galactose and ribose as major sugars. Analysis of the 16S rRNA gene sequence identities revealed that the strain is most closely related to Corynebacterium imitans DSM 44264T (98.0 %), Corynebacterium lipophiloflavum DSM 44291T (96.9 %), Corynebacterium afermentans subsp. afermentans DSM 44280T (96.9 %) and Corynebacterium afermentans subsp. lipophilum DSM 44282T (96.8 %). The identity with Corynebacterium diphtheriae DSM 44291T (98.0 %), Corynebacterium kutscheri DSM 44264T (96.9 %), Corynebacterium imitans DSM 44264T, Corynebacterium urealyticum DSM 44224T and Corynebacterium bovis DSM 44282T was below 98 %. The DNA G+C content was 69.2 mol%. DNA–DNA hybridization with Corynebacterium imitans DSM 44264T revealed a value of 36 %, confirming that the strain represents a novel species. The type strain 99221/2016T (DSM 103494T=JCM 31931T) is proposed to represent a novel species of the genus Corynebacterium with the name Corynebacterium gottingense.

The genus Corynebacterium, first described by Lehmann and Neumann [1] and later emended by Bernard et al. [2], currently comprises 121 validly published species (www. bacterio.net/corynebacterium.html) [3]. The type species of the genus is Corynebacterium diphtheriae. All species within the genus are rod-shaped, Gram-positive, non-spore-forming and catalase-positive. The range of DNA base compositions of the genus is wide, reaching from 46 mol% for Corynebacterium kutscheri [4] to 74 mol% for Corynebacterium auris [5]. The chemotaxonomic characteristics of the genus include the presence of dihydrogenated menaquinones with mostly eight and/or nine isoprene units, meso-diaminopimelic acid in the cell-wall peptidoglycan and usually the presence of mycolic acids (22–36 carbon atoms in length). The cellular fatty acids are mostly hexadecanoic, octadecanoic and cis-9-octadecenoic acids, but other fatty acids, including tuberculostearic acid, may be also present in moderate or small amounts. According to genus description, the phospholipid profile of Corynebacterium includes phosphatidylglycositol, phosphatidylglycositol mannoside, phosphatidylglycerol and other glycolipids [6]. Frischmann et al. [7] showed, however, that phosphatidylglycositol mannoside may be absent in some Corynebacterium species. Until now, phosphatidylethanolamine has been identified in only Corynebacterium bovis and Corynebacterium urealyticum [8]. Here we describe a Corynebacterium strain, 99221/2016T, that lacks phosphatidylglycositol mannoside, but in which phosphatidylethanolamine is present.

Strain 99221/2016T was isolated from blood of a patient with bacteraemia of unknown origin. It was isolated as a pure culture on Columbia blood agar (COS, Biomerieux) after 1 day of incubation at 37 °C, at the Institute of Medical Microbiology, University of Göttingen, Germany. It grew in the form of white-cream, circular and convex colonies with shiny, smooth surfaces. It grew aerobically, microaerophilically and as a facultative anaerobe on Columbia blood agar and showed good growth under aerobic, microaerophilic and anaerobic conditions. The colonies were white-cream, circular and convex with a shiny, smooth surface. The predominant respiratory quinones were MK-8(H2) and MK-9(H2). The polar lipids profile contained phosphatidylethanolamine, diphasphatidylglycerol, phosphatidylglycerol and phosphatidylglycositol. Two unidentified phospholipids and several unidentified lipids were also detected. The prevalent cellular fatty acids comprised cis-9-octadecenoic acid (C18:1ω9c), hexadecanoic acid (C16:0) and pentadecanoic acid (C15:0). Corynemycosides with 28–36 carbons in length were present. The whole-cell hydrolysate contained meso-diaminopimelic acid and arabinose, glucose, galactose and ribose as major sugars. Analysis of the 16S rRNA gene sequence identities revealed that the strain is most closely related to Corynebacterium imitans DSM 44264T (98.0 %), Corynebacterium lipophiloflavum DSM 44291T (96.9 %), Corynebacterium afermentans subsp. afermentans DSM 44280T (96.9 %) and Corynebacterium afermentans subsp. lipophilum DSM 44282T (96.8 %). The identity with Corynebacterium diphtheriae DSM 44291T (98.0 %), Corynebacterium kutscheri DSM 44264T (96.9 %), Corynebacterium imitans DSM 44264T, Corynebacterium urealyticum DSM 44224T and Corynebacterium bovis DSM 44282T was below 98 %. The DNA G+C content was 69.2 mol%. DNA–DNA hybridization with Corynebacterium imitans DSM 44264T revealed a value of 36 %, confirming that the strain represents a novel species. The type strain 99221/2016T (DSM 103494T=JCM 31931T) is proposed to represent a novel species of the genus Corynebacterium with the name Corynebacterium gottingense.
conditions was tested in GENboxCO2 jars (Biomerieux) for bacteria requiring a CO2 environment and in GENboxanaer (Biomerieux) jars for anaerobic bacteria to test the anaerobic growth. The strain was able to grow well at a temperature of 37°C on media without lipids, which indicated that the strain was non-lipophilic. Furthermore, tests for catalase and oxidase, CAMP testing and Gram-staining were performed. The presence of catalase was tested by smearing an overnight colony of the strain on a glass slide and adding a drop of 3% hydrogen peroxide solution. The presence of oxidase was tested using tetramethylphenylenediamine reagent according to the Kovacs method [9]. The CAMP test was performed with Staphylococcus aureus ATCC 25923 according to Gerhardt et al. [10]. The Gram-staining was done using commercially available test (Previ Colour C01250; Zeiss) test strips according to the manufacturer’s instructions. Strain 99221/2016T was catalase-positive, oxidase-negative, CAMP-negative and Gram-stain-positive. Under the microscope (×1250; Zeiss) the cells were observed as being non-sporo-forming and exhibited a rod shape with typical palisade arrangements. Further enzymatic activities and fermentation of different carbon sources were determined for 99221/2016T and five reference strains with the help of API Coryne (bioMérieuxSA) test strips according to the manufacturer’s instructions. In strain 99221/2016T, acid was produced from glucose, ribose and maltose, but not from xylose, mannitol, lactose, sucrose and glycogen. The strain was positive for pyrazinamidase, pyrolidonyl arylamidase, maltose. C. diphtheriae was the only species which was negative for catalase, alkaline phosphatase and α-glucosidase. Nitrate reduction was negative. The strain did not hydrolyse aesculin, urea or gelatin. The physiological properties of 99221/2016T resembled those of Corynebacterium imitans. What differentiated these two species was the pyrolidonyl arylamidase and α-glucosidase which were present in 99221/2016T. The physiological characteristics of Corynebacterium lipophiloflavum, Corynebacterium afermentans subsp. afermentans and Corynebacterium afermentans subsp. lipophilum were similar to each other, but different from these of 99221/2016T and C. imitans. In contrast to 99221/2016T and C. imitans, none of these strains utilized glucose, ribose or maltose. C. diphtheriae was the only species which was negative for alkaline phosphatase and reduced nitrate (Table 1).

Sensitivity or resistance toward common antibiotics was analysed using MHF agar with 5% horse blood and 20 mg l⁻¹ β-NAD (Biomerieux) according to European Committee on Antimicrobial Susceptibility Testing guidelines version 7.0. Isolate 99221/2016T was susceptible to linezolid, vancomycin, tetracycline and moxifloxacin, and resistant to penicillin G and oxacillin.

To determine the phylogenetic position of strain 99221/2016T, the 16S rRNA gene sequence (1492 bp) was compared with the 16S rRNA gene sequences of the type strains available on the EzTaxon server (www.ezbiocloud.net/) [11]. The nearest neighbours of 99221/2016T were C. imitans DSM 44264T (98.0% sequence identity), C. lipophiloflavum DSM 44291T (96.9%), C. afermentans DSM 44280T (96.9%) and C. afermentans subsp. lipophilum DSM 44282T (96.8%). These four strains, as well as the type species of the genus, C. diphtheriae DSM 44123T (94% pairwise sequence identity), were used as reference strains in subsequent studies.

To generate the phylogenetic tree, the 16S rRNA gene sequences of 26 nearest neighbours were retrieved from the All-Species Living Tree Project database version 123 (www.arb-silva.de/projects/living-tree/) [12]. This selection was completed with the 16S rRNA sequence of C. diphtheriae DSM 44123T. Phylogenies were inferred by using the Genome-to-Genome Distance Calculator web server (http://ggdc.dsmz.de/) [13] using the DSMZ phylogenomics pipeline [14] adapted to single genes. Basing on a multiple sequence alignment created with MUSCLE [15], maximum-likelihood (ML) and maximum-parsimony (MP) trees were reconstructed using RAxML [16] and TNT [17] respectively. For ML analysis, rapid bootstrapping in conjunction with the autoMRE bootstrapping criterion [18] and subsequent search for the best tree were used. For MP analysis, 1000 bootstrapping replicates were used in conjunction with tree-bisection-and-reconnection branch swapping and 10 random sequence addition replicates. The sequences were checked for a compositional bias using the χ² test as implemented in PAUP* [19].

The resulting ML tree (Fig. 1) shows that strain 99221/2016T clusters together with the next related type strain, C. imitans DSM 44264T, where the node shows 100% bootstrap support.

To determine the DNA relatedness of strain 99221/2016T to its most closely related species, C. imitans DSM 44264T, DNA–DNA hybridization was performed. The cells were disrupted using a Constant Systems TS 0.75 kW (IUL Instruments) apparatus, and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion et al. [20]. DNA–DNA hybridization was carried out as described by De Ley et al. [21] with the modifications described by Huss et al. [22] using a Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier thermostatted 6×6 multicell changer and a temperature controller with an in situ temperature probe (Varian). Measurements were done in duplicate. The resulting value of 34% (or 35.8% in the second measurement) was significantly below the aforementioned species threshold value of 70% [23]. This confirms that isolate 99221/2016T does not belong to the species C. imitans.

The genomic G+C content was determined by reversed-phase high-performance liquid chromatography of nucleosides [24] and was 69.2 mol%.

RiboPrint analysis was performed by digesting the chromosomal DNA of strain 99221/2016T and the five reference strains with PvuII, followed by hybridization of DNA fragments to a probe that encodes the rnu operon as described previously [25], using the DuPont Nutrition and Health RiboPrinter system. This system has been shown to have a
Table 1. Physiological and chemotaxonomic characteristics that differentiate Corynebacterium gottingense 99221/2016T from its closest phylogenetic relatives and from the type strain of the species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>Utilization of:</td>
<td></td>
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<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Ribose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>Reduction of nitrate</td>
<td>-</td>
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<td>Production of:</td>
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<tr>
<td>Pyrazinamidase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pyridoxyl arylamidase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>β-Glucuronidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>Urea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Activity of:</td>
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<tr>
<td>Oxidase</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Tuberculostearic acid</td>
<td>Trace amounts</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CAMP test</td>
<td>–</td>
<td>4*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lipophillism</td>
<td>No</td>
<td>No*</td>
<td>Yes*</td>
<td>No*</td>
<td>Yes*</td>
<td>Depends on biovar*</td>
</tr>
<tr>
<td>Dominant fatty acids (&gt;10% of total)</td>
<td>C16:0, C15:0, C18:1-9c</td>
<td>C16:0, C18:1-9c, C18:0, C15:0, C17:0, C18:1-9c, C18:0, C15:0</td>
<td>C16:0, C18:1-9c, C18:0, C15:0, C17:0, C18:1-9c, C18:0, C15:0</td>
<td>C16:0, C18:1-9c, C18:0, C15:0, C17:0, C18:1-9c, C18:0, C15:0</td>
<td>C16:0, C18:1-9c, C18:0, C15:0, C17:0, C18:1-9c, C18:0, C15:0</td>
<td></td>
</tr>
<tr>
<td>Polar lipids</td>
<td>PE, DPG, PG, PL, 2×PL, 5×UL</td>
<td>DPG, PG, PI, PIM, PL, 3×GL, 6×UL</td>
<td>DPG, PG, PI, PIM, PL, 3×GL, 3×UL</td>
<td>DPG, PG, PI, PIM, PL, 3×GL, 3×UL, 6×UL</td>
<td>DPG, PG, PI, PIM, PL, 3×GL, 3×UL, 6×UL</td>
<td></td>
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</table>

Discriminatory power for differentiation of bacteria at the level of species and even strains [25, 26]. The obtained ribotype pattern of strain 99221/2016T was different to those of type strains of related Corynebacterium species (Fig. S1, available in the online Supplementary Material).

Cellular fatty and mycolic acid compositions were determined from cells grown to stationary phase in trypticase soy broth agar at 37°C supplemented with 5% blood. Fatty acids and mycolic acids were prepared according to the protocol of Sassar [27] and Kämper and Kroppenstedt [28] and analysed by gas chromatography using the Microbial Identification System (MIDI) and the MIDI Sherlock software package.

Freeze-dried cells were used for diaminopimelic acid (DAP), whole-cell sugar, polar lipid and menaquinone analyses. DAP isomers in the whole-cell hydrolysates (6 N HCl, 100°C, 18 h) were detected by thin-layer chromatography (TLC) on cellulose plates by using the solvent system of Rhuland et al. [29].

The sugars in the whole-cell hydrolysates (1 N H2SO4, 95°C, 2 h) were analysed by TLC on cellulose plates according to Staneck and Roberts [30].

The polar lipids were extracted based on the modified procedure of Bligh and Dyer [31]. Total lipid material was detected using molybdophosphoric acid spray reagent and specific functional groups were detected with the help of molybdenum blue (for phospholipids), ninhydrin (for aminolipids), -anisaldehyde (for phosphatidylinositol mannosides) and Dragendorff reagent (for phosphatidylylcholine) [32].

The respiratory quinones were extracted by using the method of Kröger [33] and analysed by high-performance liquid chromatography (HPLC) [34, 35].

The fatty acid pattern of strain 99221/2016T was dominated by cis-9-octadecenoic acid C18:1-9c (43%), hexadecanoic acid C16:0 (32%), pentadecanoic acid C15:0 (12%) and octadecanoic acid C18:0 (9% of total fatty acids). Very similar fatty acid profiles appeared in the reference strains and they conformed with the genus description (Tables 1 and S1).
Mycolic acids (corynemycolates) of 28–36 carbon atoms in length were detected.

In 99221/2016<sup>T</sup>, MK-8(H<sub>2</sub>) (56%) and MK-9(H<sub>2</sub>) (33%) were identified as the predominant menaquinones, but small amounts of MK-7(H<sub>2</sub>) and MK-10(H<sub>2</sub>) were also found (Tables 1 and S2). The polar lipids of 99221/2016<sup>T</sup> contained significant amount of phosphatidylethanolamine as well as diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. Two unidentified phospholipids and several unidentified lipids were also present (Fig. S2). The presence of phosphatidylethanolamine in Corynebacterium has, until now, only been reported in <i>C. bovis</i> and <i>C. urealyticum</i> [8]. In the five reference strains analysed here, phosphatidylethanolamine was absent. Phosphatidylinositol mannoside is normally present in Corynebacterium species, and it was found in the cells of the five reference strains analysed. However, it was not detected in the cells of strain 99221/2016<sup>T</sup> (Table 1, Fig. S2). Absence of phosphatidylinositol mannoside has only been reported in <i>Corynebacterium lubricantis</i> [36] and <i>Corynebacterium epidermidicans</i> [7]. The cell wall of strain 99221/2016<sup>T</sup> was based on meso-DAP. The major whole-cell sugars were arabinose, galactose, glucose and ribose. This chemotaxonomic profile is in agreement with the Corynebacterium genus description.

In conclusion, the morphological, chemotaxonomic and biochemical features presented here, together with the 16S rRNA based phylogeny, DNA–DNA hybridization analysis and Riboprint patterns indicate that 99221/2016<sup>T</sup> represent a new species within the genus <i>Corynebacterium</i> for which the name <i>Corynebacterium gottingense</i> is proposed.

**DESCRIPTION OF CORYNEBACTERIUM GOTTINGENSE SP. NOV.**

<i>Corynebacterium gottingense</i> (got.tin.gen‘se N.L. neut. adj. gottingense pertaining to Gottinga, the latinized name of
Göttingen, a city in Germany where the strain was first isolated).

Gram-stain-positive, rod-shaped with typical palisade arrangements of the cells. Non-spor-forming, non-lipo-
philic, catalase-positive and oxidase-negative. Colonies were white-cream, circular and convex with shiny, smooth surfa-
ces. Grows at 37 °C aerobically and microaerophilically on Columbia blood agar after 1 day of incubation and as facul-
tative anaerobe after 2 days of incubation. Results of the CAMP test are negative. Acid is produced from glucose, ribose and maltose, but not from xylose, mannitol, lactose, sucrose and glycogen. Positive for pyrazinamidase, pyroli-
donyl arylamidase, alkaline phosphatase and \( \alpha \)-glucosidase. Nitrate reduction is negative. Aesculin, urea and gelatin are not hydrolysed. The predominant respiratory quinones are MK-8(H2) and MK-9(H2). The polar lipid profile contains phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol, as well as two phosphatidylethanolamine, diphosphatidylglycerol, phos-
phatidyglycerol and phosphatidylglycerol, as well as two

The type strain, 22991/2016T (=DSM 303494T=JCM 31931T), was isolated from blood of a clinical patient with bacteraemia of unknown origin at the Institute of Medical Microbiology, University of Göttingen, Germany.

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Conflicts of interest
The authors declare that there are no conflicts of interests.

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