Corynebacterium faecale sp. nov., isolated from the faeces of Assamese macaque

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A Gram-stain-positive, facultatively anaerobic, short rod-shaped, oxidase-negative and non-motile novel strain, designated YIM 101505T, was isolated from the faeces of a primate, Assamese macaque, and was studied to determine its taxonomic position. The cell wall contained meso-diaminopimelic acid and short-chain mycolic acids. Whole cell sugars were mannose, galactose and arabinose as major components. The major fatty acids (>10 %) were C16:1ω9c, C16:0 and C17:0ω8c and the major menaquinone was MK-9(H2). The polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, glycolipid and six unidentified lipids. The new isolate shared most of the typical chemotaxonomic characteristics of members of the genus Corynebacterium. The closest related species was Corynebacterium efficiens based on 16S rRNA gene (98.1 % similarity) and partial rpoB gene (91.4 % similarity) sequences. Similarities with other species of this genus were below 97 % based on the 16S rRNA gene. The DNA–DNA hybridization value between YIM 101505T and C. efficiens DSM 44549T was 47.7±3.6 %. Moreover, the physiological and biochemical characteristics of YIM 101505T and C. efficiens DSM 44549T were different. Thus, strain YIM 101505T is considered to represent a novel member of the genus Corynebacterium, for which the name Corynebacterium faecale sp. nov. is proposed. The type strain is YIM 101505T (=DSM 45971T=CCTCC AB 2013226T).

The genus Corynebacterium was first identified by Lehmann & Neumann (1896) and represented a large group of Gram-positive, asporogenous and rod-shaped bacteria. Typical chemotaxonomic characteristics of this genus are A1γ-type cell-wall peptidoglycan (meso-diaminopimelic acid with arabinose and galactose), short-chain mycolic acids (Schleifer & Kandler, 1972; Collins et al., 1982), usually MK-8(H2) and/or MK-9(H2) (Collins & Jones, 1981), and genomic DNA G+C content of 46–74 mol% (Bernard & Funke, 2012). The genus Corynebacterium currently comprises more than 100 species (http://www.bacterio.net/corynebacterium.html). Many strains of the genus (about 70 %) have been isolated from clinical materials or from other animal-related materials. Some species of the genus are medically relevant and can cause infections, while some have industrial uses (Bernard & Funke, 2012; Bernard, 2012). During investigations into cultivable actinomycetes from animal faeces, strain YIM 101505T was isolated from the faeces of a healthy primate, Assamese macaque, and its taxonomic position was studied.

Strain YIM 101505T was isolated from the faeces of Assamese macaque which was living semi-wild in Yunnan Wild Animal Park in Yunnan province, south-west China. After defecation (<5 min), the fresh faeces were immediately taken to the lab and then dried aseptically at 28 °C for 1 week. Before standard dilution, the sample was heated at 80 °C for 1 h and then suspended with 0.1 % Na2P2O7. The aqueous suspension was spread onto M6 agar (per litre distilled water: 1 % soluble starch, 0.03 % casein, 0.2 % KNO3, 0.2 % NaCl, 0.2 % KH2PO4, 0.002 % CaCO3, 0.005 % MgSO4·7H2O, 0.001 % FeSO4·7H2O and 1.2 % agar, pH 7.2). The clones were further purified using ISP2 (per litre distilled water: 0.4 % glucose, 0.4 % yeast extract, 1.0 % malt extract and 1.2 % agar, pH 7.2), then incubated at 28 °C and stored as aqueous glycerol suspensions (20 %, v/v) at −80 °C. Biomass of strain YIM 101505T and Corynebacterium efficiens DSM 44549T in this study was harvested.
from trypticase soy agar (TSA; Difco). The incubation temperature was set at 28 °C and the incubation time was 1 week (but 3 days for cellular fatty acid analysis and scanning electron microscopy observation).

Primer pair 27F/1523R (Cui et al., 2001) was used for amplification of the 16S rRNA gene according to the following procedure: denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 40 s, annealing at 55 °C for 40 s and extension at 72 °C for 90 s. After the circular reaction, the reaction system was kept at 72 °C for 10 min. The EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012) was used to identify related 16S rRNA gene sequences. The partial rpoB gene was amplified using the primer pair C2700F and C3130R (Khamis et al., 2004), and blasted on NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Toxigenicity of diphtheria toxin was detected using a PCR-based amplification based on the method of Pallen et al., 1994. Multiple alignments with corresponding 16S rRNA gene sequences of closely related species were carried out using the CLUSTAL X 1.83 program (Thompson et al., 1997).

Phylogenetic analyses were performed by three tree-making algorithms by using the software package MEGA version 6.0 (Tamura et al., 2013), namely the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. Kimura’s two parameter model was used to calculate evolutionary distance matrices (Kimura, 1980). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method described by Felsenstein (1985) with 1000 replications. DNA–DNA hybridization was performed between YIM 101505 T and C. efficiens DSM 44549 T using the fluorometric micro-well method (Ezaki et al., 1989; Christensen et al., 2000; He et al., 2005).

Morphological characteristics of the strain, which appeared after incubating on TSA at 28 °C for 3 days, was observed by scanning electron microscopy (QUANTA 200; FEI). Growth at various concentrations (w/v) of NaCl (0–5 % at intervals of 1 % and 7–15 % at intervals of 2 %) and MgCl₂·6H₂O (0, 1, 3, 7, 9, 11, 13 and 17 %), was tested on TSA basal medium (1.5 % tryptone, 0.5 % soytone, 1.5 % agar) at 28 °C. Growth temperature range (4, 10, 15, 20, 28, 30, 35, 40, 45 and 50 °C) was tested on TSA, and the pH range from 5.0 to 9.0 was tested on TSA supplemented with 0.5 % soytone. Growth at various concentrations (w/v) of glucose (0–25 % at intervals of 5 %), sucrose (0–25 % at intervals of 5 %), and yeast extract (0–25 % at intervals of 5 %) was tested on TSA supplemented with 0.5 % soytone at 28 °C.
Table 1. Differential characteristics between YIM 101505T and C. efficiens DSM 44549T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>YIM 101505T</th>
<th>C. efficiens DSM 44549T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Brilliant yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td>10–45</td>
<td>10–45</td>
</tr>
<tr>
<td>Growth pH</td>
<td>6–10</td>
<td>6–10</td>
</tr>
<tr>
<td>NaCl concentration for growth (w/v, %)</td>
<td>0–13</td>
<td>0–10</td>
</tr>
<tr>
<td>MgCl₂·6H₂O concentration for growth (w/v, %)</td>
<td>0–9</td>
<td>0–13</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 20</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tween 60</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Fructose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Gluconate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Malic acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activities:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase(C4)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Esterase lipase(C8)</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucoronidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>61.5</td>
<td>59.0–60.2*</td>
</tr>
</tbody>
</table>

*Data from Fudou et al., 2002.

Analysis of the isomer of diaminopimelic acid was performed on cellulose TLC with standard substances, L-, D-, and meso-diaminopimelic acid. Whole-cell sugars were obtained according to previous methods and then detected by using precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) by HPLC (Agilent 1100) (Hasegawa et al., 1983; Tang et al., 2009). Extraction and analysis of mycolic acids were mainly done according to the procedures described by Minnikin et al. (1975) and Frischmann et al. (2012). Polar lipids were extracted based on established procedures and analysed on two-dimensional TLC plates. Different chromogenic reagents were used to stain and verify the polar lipids on the TLC plates (Minnikin et al., 1984). Menaquinones were extracted using the method described by Minnikin et al. (1984) and analysed by HPLC (Kroppenstedt, 1982) using a Zorbax Eclipse XDB-C18 column (4.6×250 mm, 5 µm; Agilent). Cellular fatty acid analysis was performed by using the Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6) (Sasser, 1990). All the above experiments on strain YIM 101505T were run in parallel with C. efficiens DSM 44549T. The G+C content of the genomic DNA was determined according to the method established by Mesbah et al. (1989).

A BLAST search using the 16S rRNA gene sequence of strain YIM 101505T (1515 bp) showed that its closest relatives were the type strains of species of the genus Corynebacterium; strain YIM 101505T showed high similarities with C. efficiens DSM 44549T (98.1 %), Corynebacterium callunae DSM 20147T (96.9 %), Corynebacterium deserti GIMN1.010T (96.4 %) and Corynebacterium glutamicum ATCC 13032T (96.3 %), but similarities between strain YIM 101505T and other strains were all ≤96 %. Moreover, the level of similarity in the partial rpoB gene between strain YIM 101505T and C. efficiens was 91.4 %. Phylogenetic analysis also found YIM 101505T was affiliated to the genus Corynebacterium and formed a distinct clade with C. efficiens DSM 44549T (Fig. 1 and S1, available in the online Supplementary Material). Nevertheless, the level of similarity between YIM 101505T and C. efficiens DSM 44549T was >97 % based on 16S rRNA gene sequences, so DNA–DNA hybridization was performed between the two strains, giving a mean relatedness of 47.7 ±3.6 % (Table S1, available in the online Supplementary Material). All the above data supported that the two strains represented different genomic species.

for growth (4–13 at intervals of 1 pH unit) was tested in trypticase soy broth (TSB; Difco) at 28 °C using the buffer system described by Xu et al. (2005). Lipid requirement was tested by comparing cultures grown on brain heart infusion (BHI) agar with cultures grown on this medium supplemented with 1 % Tween 80 after 3 days at 28 °C (Riegel et al., 1994). Catalase activity was evaluated by the production of oxygen bubbles in 3 % (v/v) H₂O₂ and oxidase activity was determined by using oxidase reagent (bio-Mérieux). Gram-staining was performed using the standard Gram reaction. The CAMP test was performed on Columbia agar base with 5 % sheep blood in the presence of Staphylococcus aureus CCTCC AB 91113. Other phenotypic characteristics, such as the hydrolysis of gelatin, starch and cellulose, the coagulation and peptonization of milk, the reduction of nitrates and the relationship with oxygen, were determined according to the methods described by Tindall et al. (2007). API 20NE and API ZYM systems (bioMérieux) were also used to determine the utilization of carbon sources and enzyme activities.
When incubated on TSA at 28 °C for 3 days, strain YIM 101505<sup>T</sup> grew well aerobically, colonies were brilliant yellow, with entire margins and a diameter of 1–3 mm, and cells were short rods (about 1.0 µm in diameter and 1.0–2.3 µm in length; Fig. S2) and some of them were arranged in a 'V' formation at the later anaphase. Cells were Gram-stain-positive, catalase-positive and oxidase-negative. Optimal growth occurred at 28 °C (range 10–45 °C), with 1% (w/v) NaCl (range 0–13%) or 1% (w/v) MgCl<sub>2</sub>·6H<sub>2</sub>O (range 0–9%) and at pH 7 (range pH 6–10). Other characteristics of strain YIM 101505<sup>T</sup> are presented in the species description. Both strain YIM 101505<sup>T</sup> and <i>C. efficiens</i> DSM 44549<sup>T</sup> are non-lipophilic, and both were negative for the CAMP reaction. Detailed differential morphological, physiological and biochemical characteristics of strain YIM 101505<sup>T</sup> and <i>C. efficiens</i> DSM 44549<sup>T</sup> are given in Table 1.

Strain YIM 101505<sup>T</sup> contained meso-diaminopimelic acid and short-chain mycolic acids in its cell wall (Fig. S3), and mannosamine, galactose and arabinoamine as major whole-cell sugars. Major menaquinone assay showed that the major isoprenoid quinone of strain YIM 101505<sup>T</sup> was the same as in <i>C. efficiens</i> DSM 44549<sup>T</sup>. Thus, MK-9(H<sub>2</sub>)<sup>T</sup> could be concluded as the major isoprenoid quinone of strain YIM 101505<sup>T</sup>. However, strain YIM 101505<sup>T</sup> also contained another minor component quinone (4.1%) which has a lower polarity than MK-9(H<sub>2</sub>) and also appeared in the reference strain <i>C. efficiens</i> DSM 44549<sup>T</sup>. The above chemotaxonomic characteristics of YIM 101505<sup>T</sup> were almost uniform with those of <i>C. efficiens</i> DSM 44549<sup>T</sup> in the comparative experiments. The major fatty acids (>10%) of strain YIM 101505<sup>T</sup> were C<sub>18:1</sub>ω9c (54.2%), C<sub>16:0</sub> (27.8%) and C<sub>17:1</sub>ω8c (12.0%), and tuberculostearic acid was not found. The proportions of some fatty acids were different and there were fewer fatty acid types compared with <i>C. efficiens</i> DSM 44549<sup>T</sup> (Table 2). The polar lipid profile mainly contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, glycolipid and six unidentified lipids. The major polar lipids were similar to those of the closest genomic strain <i>C. efficiens</i> DSM 44549<sup>T</sup> (Fig. S4), and also similar to those of <i>C. glutamicum</i> DSM 2030<sup>T</sup> (Frischmann et al., 2012) which formed a cluster with strain YIM 101505<sup>T</sup> in the phylogenetic tree. The G+C content of the genomic DNA was determined to be 61.5 mol%.

In conclusion, morphological, physiological and biochemical characteristics, together with molecular analysis and chemotaxonomic characteristics, suggest that strain YIM 101505<sup>T</sup> is a strain of the genus <i>Corynebacterium</i> but also different from the closest related species. Thus, the name <i>Corynebacterium faecale</i> sp. nov. is proposed for the novel strain YIM 101505<sup>T</sup>.

### Description of <i>Corynebacterium faecale</i> sp. nov.

<i>Corynebacterium faecale</i> (fae.ca’le. L.n. <i>faex</i> dregs; L. neut. adj. <i>faecale</i> reflecting that the type strain was isolated from faeces).

Cells are Gram-stain-positive, non-sporing form, non-motile, short rods (about 1.0 µm in diameter and 1.0–2.3 µm in length) and facultatively anaerobic. Colonies are brilliant yellow with a diameter of 1–3 mm and entire margins when incubated on TSA. Catalase-positive and oxidase-negative. Optimal growth occurs at 28 °C (range 10–45 °C), with 1% (w/v) NaCl (range 0–13%) or 1% (w/v) MgCl<sub>2</sub>·6H<sub>2</sub>O (range 0–9%) and at pH 7 (range pH 6–10). Non-lipo-philic, CAMP-negative with <i>Staphylococcus aureus</i> and no toxigenicity of diphtheria toxin is detected based on PCR amplification. Positive for nitrate reduction and acid is produced from fermentation of glucose and sucrose; negative for H<sub>2</sub>S production, indole production, and milk coagulation and peptonization. Cells can hydrolyse arginine, urea, casein, Tween 20 and Tween 40, and can assimilate glucose, mannosamine, mannitol, maltose, malic acid, citrate and phenylacetic acid. Negative for hydrolysis of aesculin, gelatin, starch, Tween 60 and Tween 80, and negative for assimilation of arabinose, N-acetyl-glucosamine, gluconate, capric acid and adipic acid. For enzyme activities obtained from API ZYM, positive for alkaline phosphatase, leucine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-galactosidase, β-glucuronidase and α-

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**Table 2. Cellular fatty acid composition of strain YIM 101505<sup>T</sup> and the type strain of its closest relative, <i>C. efficiens</i> DSM 44549<sup>T</sup>**

Both strains were cultivated in the same medium and grown under the same conditions. Values are percentages of the total fatty acids.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>YIM 101505&lt;sup&gt;T&lt;/sup&gt;</th>
<th>&lt;i&gt;C. efficiens&lt;/i&gt; DSM 44549&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branched</td>
<td>0.9</td>
<td>–</td>
</tr>
<tr>
<td>Hydroxy</td>
<td>2.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt; 3-OH</td>
<td>–</td>
<td>0.4</td>
</tr>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>–</td>
<td>0.4</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>27.8</td>
<td>29.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>2.4</td>
<td>2.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;20:0&lt;/sub&gt;</td>
<td>–</td>
<td>0.1</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1&lt;/sub&gt;ω9c</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:1&lt;/sub&gt;ω8c</td>
<td>12.0</td>
<td>6.3</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;ω9c</td>
<td>54.2</td>
<td>56.8</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;18:1&lt;/sub&gt;H</td>
<td>–</td>
<td>0.4</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;19:1&lt;/sub&gt;I</td>
<td>–</td>
<td>1.2</td>
</tr>
<tr>
<td>Summed feature*</td>
<td>3</td>
<td>–</td>
</tr>
</tbody>
</table>

*Summed feature 3 comprised C<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω6c.

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glucosidase, but negative for the others. The cell wall contains meso-diaminopimelic acid and short-chain mycolic acids. Whole cells contain mannose, galactose and arabinose as major sugars. Major menaquinone is MK-9(H2). Major fatty acids (>10%) are C16:1ω9c, C16:0 and C17:1ω8c. Polar lipid profile mainly comprises dihydroxyphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, glycolipid and six unidentified lipids.

The type strain, YIM 101505T (=DSM 45971T=CCTCC AB 2013226T), was isolated from the faeces of a primate, Assamese macaque, in Yunnan Wild Animal Park in Yunnan province, south-west China. The G+C content of the genomic DNA of the type strain is 61.5 mol%.

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References


