**Microbacterium faecale** sp. nov., isolated from the faeces of *Columba livia*

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A novel, yellow, aerobic strain, YIM 101168T, isolated from the faeces of a dove (*Columba livia*), was studied to determine its taxonomic position. Cells were Gram-stain-positive, short rod-shaped, oxidase-negative, catalase-positive and non-motile. The strain could grow at 7–37 °C, at pH 6–10 and in the presence of 0–13 % (w/v) NaCl. The strain had a 16S rRNA gene sequence similarity and DNA–DNA hybridization relatedness value with *Microbacterium gubbeenense* NCIMB 30129 of 97.8 % and 41.5±8.7 %, respectively. Ornithine was detected as the diagnostic amino acid in the hydrolysate of the cell wall. Whole-cell sugars were found to be galactose, glucose, rhamnose, mannose and ribose. Major fatty acids (>10 %) were iso-C15 : 0, anteiso-C15 : 0 and anteiso-C17 : 0. Major menaquinones were identified as MK-10, MK-11 and MK-12. The polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, glycolipids and four unidentified lipids. The phylogenetic analyses as well as the chemotaxonomic and phenotypic characteristics indicate that strain YIM 101168T represents a novel species of the genus *Microbacterium*; the name *Microbacterium faecale* sp. nov. is proposed for the novel species and the type strain is YIM 101168T (=DSM 27232T=KCTC 39554T=CGMCC 1.15152T).

Bacteria of the genus *Microbacterium* are ubiquitous and are found in both terrestrial and aquatic habitats (Kook et al., 2014). They are Gram-stain-positive, asporogenous and always rod-shaped. The colonies of most type species of the genus are yellow, yellow-brown, light yellow, pale yellow or yellow-white with optimum growth temperatures of 20–30 °C (Suzuki & Hamada, 2012). The type species, *Microbacterium lacticum* ATCC 8180T, was proposed by Orla-jensen (1919). Then Collins et al. (1983) were the first to reclassify the genus *Microbacterium*, which described lysine as the diagnostic cell-wall amino acid in species of the genus. In addition, the genus *Aureobacterium*, of which species contain ornithine in the peptidoglycan, was merged into the genus *Microbacterium* by Takeuchi & Hatano (1998). Thus, the cell-wall peptidoglycan of species of the genus *Microbacterium* contains lysine or ornithine as the diagnostic amino acid. In this study, we present the taxonomic description of a novel strain, YIM 101168T, of the genus *Microbacterium*, which was isolated from the faeces of a healthy dove (*Columba livia*).

Fresh faeces were collected from Yunnan Wild Animal Park in Yunnan province, southwestern China. Fresh faeces (2 g collected about 5 min after defecation) were dried aseptically at 28 °C for one week and then suspended in 18 ml 0.85 % (w/v) NaCl solution. Then a suspension of the serially diluted sample was spread onto glycerol-asparagine agar (ISP 5, International Streptomyces Project Medium 5; Shirling & Gottlieb, 1966). The strain was then removed and applied to ISP 2 (International Streptomyces Project Medium 2; Shirling & Gottlieb, 1966) for further cultivation. Strain YIM 101168T was preserved at 5 °C on an ISP 2 slant and as aqueous glycerol suspensions (20 %, v/v) at –80 °C.

The genomic DNA was obtained by heating colonies at 99 °C within 100 µl 5% (w/v) Chelex 100 solution (Sigma). The 16S rRNA gene sequence was amplified using primers 27F and 1523R and was compared using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Multiple alignments with corresponding sequences were carried out using the CLUSTAL X 1.83 program (Thompson et al., 1997). Phylogenetic trees were generated based on

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**Abbreviations:** DPG, diphosphatidylglycerol; GL, Glycolipid; PG, Phosphatidyl glycerol; PI, Phosphatidylinositol; L, Lipids.

The GenBank accession number for the 16S rRNA gene sequence of strain YIM 101168T is K7756666.

Three supplementary figures and one supplementary table are available with the online Supplementary Material.
three algorithms: neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971), using the software package, MEGA 6.0 (Tamura et al., 2013). Kimura's two parameter model was chosen to calculate the evolutionary distance matrices of the trees (Kimura, 1980). The unrooted tree topology of the phylogenetic trees was assessed by bootstrap analysis based on 1000 resamplings (Felsenstein, 1985). DNA-DNA hybridization between strain YIM 101168\textsuperscript{T} and Microbacterium gubbeenense NCIMB 30129\textsuperscript{T} was carried out at a hybridization temperature of 47 °C using the fluorometric micro-well method (Ezaki et al., 2000; He et al., 2005).

The almost-complete 16S rRNA gene sequence (1519 bp; GenBank accession number KT756666) confirmed that strain YIM 101168\textsuperscript{T} belonged to the genus Microbacterium and the closest relatives were Microbacterium gubbeenense DSM 15944\textsuperscript{T} (97.8 %) and Microbacterium amylolyticum N5\textsuperscript{T} (96.7 %). Phylogenetic analysis revealed that Microbacterium gubbeenense DSM 15944\textsuperscript{T}, Microbacterium amylolyticum N5\textsuperscript{T}, Microbacterium indicum BBH6\textsuperscript{T}, Microbacterium oryzae MB10\textsuperscript{T}, Microbacterium barkeri DSM 20145\textsuperscript{T} and Microbacterium luticocti DSM 19459\textsuperscript{T} were in a subclade (Fig. 1). The mean DNA–DNA hybridization relatedness value between strain YIM 101168\textsuperscript{T} and Microbacterium gubbeenense NCIMB 30129\textsuperscript{T} was 41.5±8.7 % (Table S1, available in the online Supplementary Material), which was below the 70 % threshold (Wayne et al., 1987). Thus, we propose that strain YIM 101168\textsuperscript{T} represents a novel genomic species within the genus Microbacterium.

Cell morphology was observed using transmission electron microscopy (JEM-2100) after 3 days of incubation on ISP2 at 28 °C. Catalase activity was evaluated by the production of bubbles in 3 % (v/v) H\textsubscript{2}O\textsubscript{2}. Oxidase activity was tested using oxidase reagent (bioMérieux). Gram-staining was carried out according to the steps of the standard Gram reaction (Murray et al., 1994). Growth at different temperatures (7, 10, 15, 20, 25, 30, 37, 40 and 45 °C), NaCl

![Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relatedness of strain Microbacterium faecale YIM 101168\textsuperscript{T} to close relatives of the genus Microbacterium. Bootstrap values (expressed as percentages of 1000 replications) of above 50 % are shown at the branching points. Asterisks denote topologies that were also recovered using the maximum-likelihood and maximum-parsimony methods. Bar, 0.01 substitutions per nucleotide position.](image-url)
concentrations [0, 1, 3, 5, 7, 9, 11, 13 and 15 % (w/v) at 28 °C] and pH values (4–13 at intervals of 1 unit at 28 °C) were tested with ISP 2 or ISP 2 broth as the basal medium. The buffer system described by Xu et al. (2005) was used in the media at the different pH values. Hydrolysis of gelatin, casein, Tween 20, Tween 80, starch and cellulose, milk reactions, nitrite reduction and anaerobic characteristics were investigated as described by Tindall et al. (2007). In addition, API 20NE, API 20E and API ZYM galleries (bio-Mérieux) were used to test some other physiological characteristics.

Cells of strain YIM 101168 T were Gram-stain-positive, catalase-positive, oxidase-negative, non-spore-forming and non-motile short rod-shaped (about 0.5×0.7 µm; Fig. S1). Colonies were aerobic, yellow and of diameter 0.5–3 mm with entire margins when incubated on ISP 2. Strain YIM 101168 T was capable of growing on ISP 2 containing 0–13 % (w/v) NaCl (optimum 0–1 %) and at 7–37 °C (optimum 25–28 °C), and in ISP2 broth (ISP2 without agar) at pH 6–10 (optimum 7–8). Cells were positive for the hydrolysis of urea, gelatin, esculin, L-arginine, L-lysine and L-ornithine, but not casein, Tween 20, Tween 80, starch and cellulose. Detailed morphological, physiological and biochemical characteristics are given in Table 1 and in the species description. In conclusion, strain YIM 101168 T and Microbacterium gubbeenense NCIMB 30129 T were mainly differentiated on the basis of temperature and pH ranges for growth, NaCl tolerance and some other physiological characteristics.

Menaquinones were extracted from freeze-dried cells according to the methods of Collins et al. (1977) and analysed using HPLC (Kroppenstedt, 1982). Polar lipid analysis was performed as described by Minnikin et al. (1984). The composition of cellular fatty acids was analysed using the Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6; Sasser, 1990). Hydrolysates of cell-wall peptidoglycan were obtained as described by Schleifer & Kandler (1977) and detected using HPLC under the conditions described by Tang et al. (2009). Hydrolysates of whole-cell sugars were prepared in parallel with the standards and then the derivatives were analysed according to the procedures described by Tang et al. (2009). The type of acyl in cell walls was tested using the method described by Uchida et al. (1999). The G+C content of the genomic DNA was determined using HPLC (Mesbah et al., 1989).

The menaquinones of freeze-dried cells of strain YIM 101168 T were MK-10 (34.0 %), MK-11 (36.8 %) and MK-12 (29.2 %), and of Microbacterium gubbeenense NCIMB 30129 T were MK-10 (14.4 %), MK-11 (66.0 %) and MK-12 (19.6 %). The polar lipid profiles of both strains comprised diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol (PI), glycolipid (GL) and unidentified lipids (Ls) (Fig. S2). The cell-wall hydrolysates of both strains were found to contain alanine, glycine, glutamic acid and ornithine (Fig. S3). Based on previous studies, both strains should have been consistent with glycolyl-type bacteria. However, the chemotaxonomic investigation into Microbacterium gubbeenense NCIMB 30129 T described above showed some differences to the study by Brennan et al. (2001). The latter study demonstrated that the menaquinones were MK-11 and MK-12, the polar lipids were DPG, PI and PG mannosides, and contained L-lysine as the diamino acid. The whole-cell hydrolysates of the two strains were found to contain galactose, glucose, rhamnose, mannoside and ribose. Both strains were found to contain anteiso-C₁₅:₀, iso-C₁₆:₀ and anteiso-C₁₇:₀ as the major fatty acids (>10 %), but their fatty acids profiles and the percentages of the various fatty acids present were different (Table 2). The G+C content of the genomic DNA of strain YIM 101168 T was determined to be 69.7 mol%. Thus, strain YIM 101168 T and Microbacterium gubbeenense NCIMB 30129 T were different based on the fatty acid profile as well as on the percentage of menaquinones and minor polar lipids. In addition, the polar lipid profiles could also differentiate strain YIM 101168 T from other close phylogenetic neighbours Microbacterium amylolyticum N5 T (absence of PI and Ls; Anand et al., 2012) and Microbacterium indicum BBH6 T (absence of DPG, GL and Ls; Shivaji et al., 2007).

In conclusion, some chemotaxonomic and physiological characteristics of strain YIM 101168 T are different from its closest phylogenetic relatives. Together with molecular analysis and the results of DNA–DNA hybridization, we suggest that strain YIM 101168 T represents a novel species of the genus Microbacterium. The name Microbacterium fae
cale sp. nov. is proposed.

**Description of Microbacterium faecale sp. nov.**


Cells are Gram-stain-positive, catalase-positive, oxidase-negative, non-spore-forming and non-motile short rods (about 0.5×0.7 µm). Colonies are aerobic, yellow and with a diameter of 0.5–3 mm and entire margins when incubated on ISP2. Capable of growing on ISP2 containing 0–13 % (w/v) NaCl and at 7–37 °C, and in ISP2 broth (ISP2 without agar) at pH 6–10. Positive for acetoin production and milk coagulation and peptonization. Negative for nitrate reduction, tryptophan deamination, H₂S and indole production. Acid is produced from the fermentation of D-glucose. Hydrolyzes gelatin, esculin, L-lysine and L-ornithine, but not urea, casein, Tween 20, Tween 80, L-arginine, starch and cellulose. Assimilates L-rhamnose, amygdalin, D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose and potassium gluconate, but not inositol, D-sucrose, adipic acid, citrate, D-sorbitol, D-melibiose, capric acid, malic acid and phenylacetic acid. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphtho-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase, but negative for lipase (C14), α-
### Table 1. Some characteristics of Microbacterium faecale YIM 101168<sup>T</sup> and its closest relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Colony colour</td>
<td>Yellow</td>
<td>Pale yellow</td>
<td>Yellow</td>
<td>Cream</td>
<td>Yellow</td>
<td>Yellow</td>
<td>White</td>
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<tr>
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<td>6.0–9.0</td>
<td>7.0–10.0</td>
<td>5.0–9.0</td>
<td>5.0–11.0</td>
<td>6.0–9.0</td>
<td>5.5–9.7</td>
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<td>Tolerance to NaCl (% w/v)</td>
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<td>0–11</td>
<td>0–11</td>
<td>0–7</td>
<td>0–6</td>
<td>0–8</td>
<td>0–10</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>H₂S production</td>
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<td>–</td>
<td>ND</td>
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<td>Hydrolysis of:</td>
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<td>Glucosyl</td>
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<td>+</td>
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<td>Assimilation of citrate</td>
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<td>–</td>
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<td>+</td>
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<td>β-D-Glucosidase</td>
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<td>2-glucose</td>
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<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Polar lipids</td>
<td>DPG, PG, GL, PI, Ls</td>
<td>DPG, PG, GL, PI, Ls&lt;sup&gt;†&lt;/sup&gt;</td>
<td>DPG, PG, GL</td>
<td>PG, PI</td>
<td>DPG, PG, PI, GLs</td>
<td>DPG, PG, PI, GLs</td>
<td>DPG, PG, PI, GLs</td>
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<tr>
<td>Major menaquinones</td>
<td>MK-10, MK-11, MK-12</td>
<td>MK-10, MK-11, MK-12&lt;sup&gt;†&lt;/sup&gt;</td>
<td>MK-10, MK-11</td>
<td>MK-11, MK-12</td>
<td>MK-9, MK-10, MK-11</td>
<td>MK-11, MK-12, MK-13</td>
<td>MK-11, MK-12</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>69.7</td>
<td>68.0*</td>
<td>68.0</td>
<td>68.0</td>
<td>68.0</td>
<td>68.0</td>
<td>68.7</td>
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</table>

*Data from Brennan et al. (2001).
†Data exhibited some differences in comparison to Brennan et al. (2001).
Microbacterium gubbeenense


