**Microbacterium mitrae** sp. nov., isolated from salted turban shell

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A novel bacterium (strain M4-8<sup>T</sup>) belonging to the genus *Microbacterium* was isolated from salted turban shell, which is a traditional fermented food in Korea. Its morphology, physiology, biochemical features and 16S rRNA gene sequence were characterized. Cells of this strain were Gram-positive, non-motile, non-spore-forming rods that formed yellow-pigmented colonies. It grew in 0–8 % (w/v) NaCl and at 15–37 °C, with optimal growth occurring in 1 % (w/v) NaCl and at 30 °C. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain M4-8<sup>T</sup> is associated with members of the genus *Microbacterium*. Within the phylogenetic tree, this novel strain shared a branching point with *Microbacterium hominis* IFO 15708<sup>T</sup> (97.8 % similarity). The DNA G+C content was 71.3 mol% and DNA–DNA hybridization experiments showed a low level (<29 %) of DNA–DNA relatedness between M4-8<sup>T</sup> and its closest relatives. The major fatty acids were iso-C<sub>15:0</sub> and anteiso-C<sub>15:0</sub> and the major cell-wall diamino acid was ornithine. Data obtained from DNA–DNA hybridization and chemotaxonomic phenotypic analysis support the conclusion that strain M4-8<sup>T</sup> represents a novel species within the genus *Microbacterium*. The name *Microbacterium mitrae* sp. nov. is proposed, with M4-8<sup>T</sup> (=KACC 21129<sup>T</sup> =JCM 16363<sup>T</sup>) as the type strain.

The genus *Microbacterium* was first described by Orla-Jensen (1919) and members of the genus can be isolated from a wide range of environments including soil, insects, human clinical specimens, marine environments and plants (Evtushenko & Takeuchi, 2006; Park *et al.*, 2008; Bakir *et al.*, 2008; Takeuchi & Hatano, 1998a, b; Lee *et al.*, 2006; Shivaji *et al.*, 2007; Collins & Bradbury, 1992). Members of the genus have also been found in the phyllospheres of *sweetcorn* and *cotton* (Thompson *et al.*, 1993; Legard *et al.*, 1994; McNulty & Kloeper, 1995).

In this study, a novel isolate (strain M4-8<sup>T</sup>) belonging to the genus *Microbacterium* was isolated from salted turban shell, which is a traditional fermented food from Korea. The cultured cell biomass for cellular composition analysis and DNA extraction was collected from marine agar (MA; BBL) plates incubated at 30 °C for 2 days. The Gram reaction was performed using the non-staining method described by Buck (1982). Motility was examined by the wet-mount method and spore formation was analysed using the staining method of Schaeffer & Fulton (1933). Cell morphology was observed under a Nikon phase-contrast microscope at ×1000 magnification using cells grown for 2 days at 30 °C on MA plates. Growth at different temperatures (4, 15, 25, 30, 37 and 45 °C) and pH (3.0–13.0 at intervals of 1.0 pH unit) was assessed on marine broth (MB; BBL). NaCl tolerance was determined at 30 °C in MB prepared without NaCl and then supplemented with 0–10 % (in increments of 1 %), 15 and 20 % (w/v) NaCl. Enzyme activity and utilization of various substrates as sole carbon sources were determined using API 20NE and API ZYM strips according to the manufacturer’s instructions (bioMérieux). Casein and starch hydrolysis was tested as described by Smibert & Krieg (1994). Catalase activity was determined by observing bubble production in 3 % (v/v) hydrogen peroxide solution and oxidase activity was determined using an oxidase reagent (bioMérieux). Indole and H<sub>2</sub>S formation were tested as described previously (Benson, 1994).

Strain M4-8<sup>T</sup> was non-spore-forming, Gram-positive, rod-shaped and non-motile. After 2 days of incubation on MA at 30 °C, colonies were circular (0.5–2.0 mm in diameter), smooth, yellow and convex. Strain M4-8<sup>T</sup> was able to grow at 15–37 °C, at pH 6.0–9.0 and in 0–8 % NaCl. Optimal growth conditions were 30 °C, pH 7.0 and 1 % NaCl. The strain was catalase-positive, oxidase-negative and able to hydrolyse casein but not starch. A detailed species description is presented below. Table 1 lists the characteristics that differentiate strain M4-8<sup>T</sup> from related members of the genus *Microbacterium*. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M4-8<sup>T</sup> is GQ351351.
Fatty acid methyl esters were obtained from cells grown in MB medium for 2 days at 30 °C and analysed by GC-MS (Kuykendall et al., 1988). For quantitative analysis of cellular fatty acid composition, a loop of cell mass was harvested after 2 days and cellular fatty acids were saponified, methylated and extracted according to the Sherlock Microbial Identification System (MIDI) as described by Sasser (1990). The predominant cellular fatty acids found in strain M4-8T were iso-C15 : 0 (55.0 %) and anteiso-C15 : 0 (26.5 %). Minor fatty acids were iso-C16 : 0 (7.5 %), iso-C17 : 0 (6.1 %), anteiso-C17 : 0 (2.4 %), iso-C14 : 0 (2.3 %) and C16 : 0 (0.3 %). No hydroxyl fatty acids were identified. The cellular fatty acid profile was consistent with those of other members of the genus Microbacterium. The cell-wall diamino acids were ornithine (major) and lysine.

Chromosomal DNA was extracted using a DNA extraction kit (iNtRON Biotechnology). The 16S rRNA gene sequence was PCR-amplified from chromosomal DNA using PCR Pre-Mix (Solgent) and two bacterial universal primers (Baker et al., 2003). The PCR product was purified using a PCR purification kit (Qiagen) and sequencing was performed as previously described (Roh et al., 2008). Almost full-length 16S rRNA gene sequences were assembled using SeqMan software (DNASTAR). The 16S rRNA gene sequence of the novel isolate was aligned with those of related taxa obtained from the NCBI database using the multiple sequence alignment program CLUSTAL X (1.8) (Thompson et al., 1997). The phylogenetic relationship between representatives of the genus Microbacterium was determined using the MEGA3 software program (Kumar et al., 2004). Distance matrices were determined using the method of Kimura (1980) and used to produce a dendrogram using the neighbour-joining (Saitou & Nei, 1987), minimum evolution (Rzhetsky & Nei, 1992) and maximum-parsimony (Kluge & Farris, 1969) methods. Bootstrap analysis evaluating the stability of the resulting trees was performed using a consensus tree based on 1000 randomly generated trees. DNA–DNA hybridization experiments were performed in triplicate using the fluorometric method of Ezaki et al. (1989). Hybridizations were determined at 37 °C, fluorescence values were quantified at 90 min using Synergy Mx (BioTek) and hybridization values were calculated as described previously (Chang et al., 2008). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain M4-8T is related to members of the genus Microbacterium. Comparison of the 16S rRNA gene sequence of strain M4-8T against those of known species of the genus Microbacterium using FASTA (EMBL/GenBank) showed that it had highest similarity with that of Microbacterium

### Table 1. Differential characteristics of strain M4-8T and the type strains of closely related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Colony morphology</td>
<td>Smooth, yellow</td>
<td>Smooth, yellowish-white</td>
<td>Smooth, yellow</td>
<td>Smooth, yellow</td>
<td>Smooth, yellow</td>
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<tr>
<td>NaCl range (%) for growth</td>
<td>0–8</td>
<td>0–3</td>
<td>1–3</td>
<td>0–4</td>
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<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>–</td>
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<td>Growth at 37 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Oxidase activity</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>H2S production</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Voges–Proskauer test</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>Methyl red test</td>
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<td>Nitrate reduction</td>
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<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Fermentation of d-glucose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>Casein</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>+</td>
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<td>Enzyme activity:</td>
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<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Acid phosphatase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>71.3</td>
<td>70.9*</td>
<td>69.7†</td>
<td>66.9‡</td>
<td>69.0‡</td>
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<tr>
<td>Major cell-wall diamino acid§</td>
<td>Orn</td>
<td>Lys*</td>
<td>Orn†</td>
<td>Orn‡</td>
<td>Orn‡</td>
</tr>
</tbody>
</table>

*Data from Takeuchi & Hatano (1998b).
†Data from Kim et al. (2005).
‡Data from Yokota et al. (1993).
§Orn, Ornithine; Lys, lysine.
hominis IFO 15708\(^T\) (97.8 %), followed by Microbacterium xylanilyticum S3-E\(^T\) (97.3 %), Microbacterium flavescens DSM 20643\(^T\) (97.1 %) and Microbacterium trichothecenolyticum IFO 15077\(^T\) (97.1 %). The clustering and phylogenetic tree drawn using the neighbour-joining, minimum-evolution and maximum-parsimony method is shown in Fig. 1. Phylogenetic trees based on 16S rRNA gene sequences showed a similar topology, regardless of the tree-making algorithm used (Fig. 1). DNA–DNA hybridization experiments were performed to determine the genomic relationship between the isolate and those strains with the greatest 16S rRNA gene sequence similarity (≥ 97.0 %). DNA–DNA
relatedness ranged from 3.2–28.8% with its closest relatives: *M. hominis* IFO 15708T (17.0%), *M. xylanilyticum* S3-E T (15.9%), *M. flavescens* DSM 20643T (3.2%) and *M. trichothecenolyticum* IFO 15077T (28.8%). The level of 16S rRNA gene sequence similarity coupled with low DNA–DNA relatedness values below the 70% threshold (Wayne et al., 1987) indicate that strain M4-8T represents a distinct genospecies.

Data for growth of strain M4-8T and its closely related phylogenetic neighbours at various NaCl concentrations show that strain M4-8T has more NaCl tolerance than the four most closely related species. Casein hydrolysis can also be used to differentiate between strain M4-8T and its closest relatives (Table 1). Data from 16S rRNA gene sequence analysis, DNA–DNA relatedness experiments, and physiological and biochemical tests highlighted genotypic and phenotypic differences between strain M4-8T and other species of the genus *Microbacterium*. Therefore, it is concluded that strain M4-8T (=KACC 21129T =JCM 16363T) represents a novel species of the genus *Microbacterium*, for which the name *Microbacterium mitrae* sp. nov. is proposed.

**Description of Microbacterium mitrae** sp. nov.

*Microbacterium mitrae* [mi’trae. L. n. mitra a headband, coif, turban of the Asians, also a zoological genus name (Mitra); L. gen. n. mitrae of Mitra sp.].

Cells are Gram-positive, non-motile, non-spor-forming and rod-shaped, forming yellow-pigmented, round colonies with a diameter of 0.5–2.0 mm after incubation for 2 days on MB agar plates at 30 °C. Growth occurs with 0–8% NaCl, at 15–37 °C and at pH 6.0–9.0, with optimal growth occurring in 1% (w/v) NaCl, at 30 °C and at pH 7.0. Does not reduce nitrate to nitrite and does not produce indole. Catalase-positive and oxidase-negative. Casein is hydrolysed, but starch is not. H2S is not formed. Produce indole. Catalase-positive and oxidase-negative. pH 7.0. Does not reduce nitrate to nitrite and does not produce nitrite. Catalase-positive and oxidase-negative.

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**References**


