Luteipulveratus mongoliensis gen. nov., sp. nov., an actinobacterial taxon in the family Dermacoccaceae

Ismet Ara,1 Hideki Yamamura,2 Baljinova Tsetseg,3 Damdinsuren Daram3 and Katsuhiko Ando1

Correspondence
Ismet Ara
ara-ismet@nite.go.jp

1Biotechnology Development Center, Department of Biotechnology, National Institute of Technology and Evaluation (NITE), Kazusakamatari 2-5-8, Kisarazu, Chiba, 292-0818, Japan
2Division of Applied Biological Sciences, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Takeda-4, Kofu 400-8511, Japan
3Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar-51, Mongolia

A novel actinobacterial strain, MN07-A0370T, was isolated from Mongolian soil and its taxonomic status was determined using a polyphasic approach. Comparative 16S rRNA gene sequence studies revealed that strain MN07-A0370T represented a novel lineage within the actinobacteria. Strain MN07-A0370T formed a distinct clade in the family Dermacoccaceae and was most closely related to the members of the genera Dermacoccus (16S rRNA gene sequence similarity, 96.2 %–96.4 %), Demetria (94.1 %) and Kytococcus (93.7 %). The cell-wall peptidoglycan of the novel strain contained L-lysine, alanine, aspartic acid, glutamic acid and serine and represented peptidoglycan type A4a. The menaquinones were MK-8(H4) and MK-8(H6). The polar lipids were phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol and the whole-cell sugars were galactose, mannose, rhamnose, ribose and glucose. Mycolic acids were absent. The fatty acid profile was characterized by the presence of large amounts of saturated iso- and anteiso-branched-chain fatty acids as well as smaller amounts of saturated straight-chain and unsaturated acids. The major fatty acids were iso-C16 : 0, anteiso-C17 : 0, iso-C16 : 1H, C17 : 1ω8c and C17 : 0 10-methyl. The G+C content of the DNA was 68.2 mol%. On the basis of chemotaxonomic, physiological and biochemical differences from other genera of the family Dermacoccaceae, strain MN07-A0370T should be classified as representing a novel species in a new genus, for which we propose the name Luteipulveratus mongoliensis gen. nov., sp. nov. The type strain is MN07-A0370T (=NBRC 105296T=VTCC D9-09T).

Mongolia is one of the largest Asian countries and is unique in its environmental traits. Recently, these environments have attracted the attention of naturalists and other researchers looking for novel actinomycetes (Norovsuren et al., 2007). In a programme aimed at isolating novel rare actinomycetes from Mongolia as a potential source of bioactive secondary metabolites, strain MN07-A0370T, in the family Dermacoccaceae (Stackebrandt & Schumann, 2000), was studied to establish its taxonomic status. The family Dermacoccaceae currently contains three recognized genera, Dermacoccus and Kytococcus (Stackebrandt et al., 1995) and Demetria (Groth et al., 1997a). Isolates assigned to this family are typically associated with terrestrial habitats, notably cured meat products, skin, human blood and soil (De la Rosa et al., 1990; Cordero & Zumalacarregui, 2000; Becker et al., 2002; Papamanoli et al., 2002). Samples of grassland soil were collected in July 2007 from Terelj National Park, Töv Province, Ulaanbaatar, Mongolia. The samples were dried at room temperature for 5–7 days, rehydrated and centrifuged (Hayakawa et al., 2000) and used to inoculate humic acid-vitamin agar (Hayakawa & Nonomura, 1987) containing (l−1)20 mg trimethoprim and 10 mg nalidixic acid. Strain MN07-A0370T was isolated after incubation for about 2 weeks at room temperature. The strain was then incubated on Bennett agar (0.1 % yeast extract, 0.1 % beef extract; 0.2 % NZ amine type A; 1.0 % maltose monohydrate, 1.5 % agar; pH 7.3) (Jones, 1949) and yeast extract-soluble starch medium (YS medium; l−1: 2 g yeast extract, 10 g soluble

Abbreviation: ISP, International Streptomyces Project.
The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain MN07-A0370T is AB468971.
A supplementary table showing the cultural characteristics of strain MN07-A0370T is available with the online version of this paper.
starch, 15 g agar; pH 7.3) at 28 °C for 10–14 days and checked for purity.

The strain formed moist, raised, yellow colonies on Bennett and yeast-starch agars. Aerial mycelium was absent. A powdery, white, aerial mycelium-like formation was observed on International Streptomyces Project (ISP) media 3, 4, 5 and 7 after incubation for 2 weeks at 28 °C. Spore motility was tested by observing cells suspended in phosphate buffer (1 mM; pH 7.0) under a light microscope. Morphological characteristics were observed using scanning electron microscopy as described by Tamura et al. (1994). The cultural characteristics of strain MN07-A0370T were observed on ISP 2–7 media (Shirling & Gottlieb, 1966), Bennett agar and YS medium after incubation at 28 °C for 3 weeks (see Supplementary Table S1, available in IJSEM online). Strain MN07-A0370T showed good growth on all agar media tested.

DNA was extracted as described by Marmur (1961) and Saito & Miura (1963) but with a slight modification: after DNA was extracted as described by Marmur (1961) and Minnikin et al. (1987). The required biomass was harvested by scraping and centrifuging soft colonies from 7-day-old Bennett agar plates grown at 28 °C. The harvested cell mass was washed twice with sterile distilled water and freeze dried. The whole-cell sugars, isoprenoid quinones, phospholipids and cellular fatty acids were analysed as described by Stanek & Roberts (1974), Minnikin et al. (1984) and Tamura et al. (1994). The diaminopimelic acid (A2pm) isomer in the cell-wall peptidoglycan was analysed as described by Nozawa et al. (2007). Cell-wall amino acids and mycolic acids were analysed as described by Tamura et al. (1994).

It is evident from Fig. 1 that strain MN07-A0370T formed a distinct clade in the 16S rRNA gene sequence tree for the family Dermacoccaceae and clustered with the genera Demetria, Dermacoccus and Kytococcus; this association was supported by all of the tree-making algorithms and by a bootstrap value of about 60% in the neighbour-joining analysis. The similarity values of the 16S rRNA gene sequences between strain MN07-A0370T and members of the genera Kytococcus, Demetria and Dermacoccus ranged from 93.7% to 96.4%.

Strain MN07-A0370T formed circular, convex, smooth colonies that were pale yellow to bright yellow on Bennett agar after 1 week of incubation at 28 °C (see Fig. 1).
The cells occurred singly or in pairs, short chains or irregular clusters when grown on Bennett agar (Fig. 2a, b). Cream-white to bright yellow smooth colonies with soft powdery surfaces were formed on ISP 3, 4, 5 and 7. Scanning electron microscopic observations revealed irregular coccoid- to short rod-shaped cells that varied in size (0.5 x 1.0–0.8 x 1.2 μm) on Bennett agar. Rudimentary short aerial mycelium-like structures were found on ISP 4 (Fig. 2c, d). The physiological and biochemical properties of strain MN07-A0370 T are listed in the genus and species descriptions and in Table 1. The temperature range for growth on Bennett agar was 10–30 °C (optimum 20–28 °C) and no growth was observed at 37 °C. The isolate grew well with up to 2.0 % (w/v) NaCl and poorly with 3.0 % NaCl on Bennett agar medium. The physiological and biochemical properties of strain MN07-A0370 T that differentiate it from the recognized species of the genus Dermacoccus are colony colour, morphology, urea decomposition, hydrolysis of gelatin and starch, degradation of Tween 80, nitrate reduction, enzyme activities such as z-fucosidase, β-glucosidase, lipase (C14) and trypsin, growth at 10, 37 and 40 °C, little growth with 3.0 % NaCl and no growth with 4.0, 10.0 and 12.5 % NaCl (Table 1).

The cell-wall peptidoglycan of strain MN07-A0370 T contained L-lysine, alanine, aspartic acid, glutamic acid and serine in a molar ratio of 0.8 : 2.7 : 0.6 : 1.0 : 0.9, respectively, which represented peptidoglycan type A4α. The predominant menaquinones were MK-8(H4) (49.0 %) and MK-8(H6) (48.8 %) and this differed from those of other genera in the family Dermacoccaceae (Stackebrandt et al., 1995; Groth et al., 1997a). Lysine as the diagnostic diamino acid is mainly associated with Coryneform members and nocardioform morphologies. In sporoactinomycetes, lysine diamino acid is mainly associated with coryneform and actinomycetes. In sporoactinomycetes, lysine diamino acid is mainly associated with coryneform and actinomycetes.

known lysine-containing genera of actinomycetes have group-A peptidoglycan, with the exception of the genus Microbacterium, which possesses group-B peptidoglycan (Schleifer & Kandler, 1972). Diamino acids in combination with menaquinone types are extremely useful in differentiating genera of coryneform bacteria from each other. The combination of menaquinone MK-8(H4) plus MK-8(H6) and lysine in the cell wall is unique in the family Dermacoccaceae. However, the presence of the menaquinone type MK-8(H4) and lysine has been reported for Demetria terragena in the family Dermacoccaceae and Bogoriella caseilytica in the family Bogoriellaceae (Groth et al., 1997b). Phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol were detected as total phospholipids, whereas phosphatidylcholine, phosphatidylethanolamine and some other phospholipids containing glucosamine were not detected (phospholipid type PI sensu Lechevalier et al., 1977). The strain contained galactose, mannose, rhamnose, ribose and glucose as whole-cell sugars. Mycolic acids were not present. The fatty acid profile was characterized by the occurrence of large amounts of saturated iso- and anteiso-branched-chain fatty acids as well as smaller amounts of saturated straight-chain and unsaturated acids. The fatty acid composition of strain MN07-A0370 T was iso-C16:0 (45.8 %), anteiso-C17:0 (7.9 %), iso-C16:1 H (7.6 %), C17:1ω9c (7.3 %), C17:0 10-methyl (6.4 %), C17:0 (4.0 %), anteiso-C17:1 C (2.5 %), iso-C18:0 (2.4 %), C17:0 (2.3 %), C16:0 (2.1 %), C16:0 10-methyl (2.0 %), C18:0 (1.6 %), C16:0ω9c (1.5 %), C18:1ω9c (1.2 %), iso-C17:0 (1.1 %), iso-C14:0 (0.8 %), C15:0 (0.7 %), iso-C15:0 (0.4 %), anteiso-C15:0 (0.4 %), C18:0 10-methyl (0.4 %) and C15:1 B (0.4 %). The G+C content of the DNA of strain MN07-A0370 T was 68.2 mol%. Therefore, on the basis of differential chemotaxonomic characteristics (Table 1), especially for the peptidoglycan type with L-lysine in combination with the menaquinone components MK-8(H4) and MK-8(H6),
strain MN07-A0370<sup>T</sup> can be clearly separated from its closest phylogenetic neighbours and from all of the above-mentioned genera that contain lysine in the cell wall.

The closest phylogenetic neighbours of strain MN07-A0370<sup>T</sup> are *Dermacoccus barathri* MT2.1<sup>T</sup>, *Dermacoccus abyssi* MT1.1<sup>T</sup> and *Dermacoccus profundi* MT2.2<sup>T</sup> (16S rRNA gene sequence similarity 96.4%), all of which differed from the new isolate in the amino acids of the interpeptide bridge, the predominant menaquinones and the major fatty acids, as well as in physiological, cultural and biochemical characteristics (Table 1). Furthermore, morphological differences justify the separation of strain MN07-A0370<sup>T</sup> from the genus *Dermacoccus*. Pronounced

### Table 1. Characteristics that differentiate strain MN07-A0370<sup>T</sup> from the type strains of species of the closely related genera *Dermacoccus*, *Demetria* and *Kytococcus* in the family *Dermacoccaceae*

<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>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Cream to bright yellow, smooth to matted colonies with white powdery surface on ISP 3, 4, 5 and 7 agars</td>
<td>Light yellow</td>
<td>Brilliant yellow</td>
<td>Cream to pale yellow</td>
<td>Bright orange</td>
<td>White to pale yellow</td>
<td>Deep buttercup yellow or cream–white</td>
<td>Muddy yellow</td>
</tr>
<tr>
<td>Spore morphology</td>
<td>Coccoid to short rod-shaped spores with rudimentary, short aerial mycelium-like formation</td>
<td>Coccoid</td>
<td>Coccoid</td>
<td>Coccoid</td>
<td>Coccoid</td>
<td>Irregular coccoid to short rod-shaped spores with filopodia</td>
<td>Coccoid</td>
<td>Coccoid</td>
</tr>
<tr>
<td>Decomposition of: Hypoxanthine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arbutin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>±</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tween 80</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>API ZYM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Fucosidase</td>
<td>±</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Growth at/on:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>37 °C</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>40 °C</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.0% NaCl</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.0% NaCl</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>10.0% NaCl</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12.5% NaCl</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, i-C&lt;sub&gt;16:1&lt;/sub&gt;H, C&lt;sub&gt;17:0&lt;/sub&gt;9c</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, i-C&lt;sub&gt;16:1&lt;/sub&gt;H, C&lt;sub&gt;17:0&lt;/sub&gt;9c</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, i-C&lt;sub&gt;16:1&lt;/sub&gt;H, C&lt;sub&gt;17:0&lt;/sub&gt;9c</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, i-C&lt;sub&gt;16:1&lt;/sub&gt;H, C&lt;sub&gt;17:0&lt;/sub&gt;9c</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, i-C&lt;sub&gt;16:1&lt;/sub&gt;H, C&lt;sub&gt;17:0&lt;/sub&gt;9c</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, i-C&lt;sub&gt;16:1&lt;/sub&gt;H, C&lt;sub&gt;17:0&lt;/sub&gt;9c</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, i-C&lt;sub&gt;16:1&lt;/sub&gt;H, C&lt;sub&gt;17:0&lt;/sub&gt;9c</td>
<td>i-C&lt;sub&gt;16:0&lt;/sub&gt;, ai-C&lt;sub&gt;17:0&lt;/sub&gt;, i-C&lt;sub&gt;16:1&lt;/sub&gt;H, C&lt;sub&gt;17:0&lt;/sub&gt;9c</td>
</tr>
<tr>
<td>Menaquinones</td>
<td>MK-8(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;, MK-8(H&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>MK-8(H&lt;sub&gt;2&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;, MK-8(H&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>MK-8(H&lt;sub&gt;2&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;, MK-8(H&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>MK-8(H&lt;sub&gt;2&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;, MK-8(H&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>MK-8(H&lt;sub&gt;2&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;, MK-8(H&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>MK-8(H&lt;sub&gt;2&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;, MK-8(H&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>MK-8(H&lt;sub&gt;2&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;, MK-8(H&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>MK-8(H&lt;sub&gt;2&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;, MK-8(H&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>68.2</td>
<td>66.8</td>
<td>69.1</td>
<td>65.2</td>
<td>66.0-71.0</td>
<td>66.0</td>
<td>68.0-69.0</td>
<td>ND</td>
</tr>
</tbody>
</table>
differences in chemotaxonomic, morphological and physiological properties were also found between strain MN07-A0370T and Demetria terrigena (Table 1). Therefore, we conclude that on the basis of our results and the remarkable differences in taxonomic characteristics between strain MN07-A0370T and all of the genera in the family Dermacoccaceae, strain MN07-A0370T should be assigned to a novel species in a new genus, for which the name Luteipulveratus mongoliensis gen. nov., sp. nov. is proposed.

Description of Luteipulveratus gen. nov.

Luteipulveratus (Lu.te.i.pul.ve.ra’tus. L. adj. luteus yellow; L. part. adj. pulverus scattered with dust; N.L. masc. n. Luteipulveratus a bacterium forming white powdery aerial mycelium on yellow colonies).

Cells are Gram-positive, irregular coccoid- to short rod-shaped (0.5 x 1.0–0.8 x 1.2 μm), and occur singly or in pairs, in short chains or small irregular clusters. Cells are non-motile and non-sporulating. Colonies are circular, convex, entire, smooth, 1.0–3.5 mm in diameter and pale yellow to bright yellow on Bennett agar. A rudimentary white and sterile powdery aerial mycelium-like structure is formed on ISP 3, 4, 5 and 7 agars after 1 week of incubation at 28 °C. Growth is aerobic and catalase-positive. The cell-wall peptidoglycan contains L-lysine, alanine, aspartic acid, glutamic acid and serine and represents peptidoglycan type A4z. Phospholipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylglycinol and the whole-cell sugars are galactose, mannose, rhamnose, ribose and glucose. Mycolic acids are absent. The fatty acid profile is characterized by the large amounts of saturated iso- and anteiso-branched-chain as well as smaller amounts of saturated straight-chain and unsaturated acids. The major fatty acids are iso-C16:0, anteiso-C17:0, iso-C16:1 H, C17:0 9c and C17:0 10-methyl, with small amounts of C17:1 8c anteiso-C17:1 c, iso-C18:0, C17:0, C16:0 10-methyl, C18:0, C16:1 9c, C16:1 9c antiiso-C17:0, iso-C14:0, C15:0 iso-C15:0, anteiso-C15:0, C18:0 10-methyl and C15:1 B.

The type strain is MN07-A0370T (=NBRC 105296T = VTCC D9-09T), which was isolated from grassland soil in Mongolia. The DNA G+C content of the type strain is 68.2 %.

Acknowledgements

This work was conducted under the Joint Research Project between the Department of Biotechnology, NITE, Japan and the Institute of Biology, Mongolian Academy of Sciences, Mongolia. We acknowledge Dr Tomohiko Tamura for scientific discussions and Ms Yayoi Sakiyama for technical assistance. We are grateful to Dr J. P. Euzéby for help with the nomenclature of the new genus.

References


