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

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A novel actinobacterial strain, MN07-A0370^T, 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-A0370^T represented a novel lineage within the actinobacteria. Strain MN07-A0370^T 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 A4 α . 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-C_{16:0}, anteiso-C_{17:0}, iso-C_{16:1} H, C_{17:1} ω 9c and C_{17:0} 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-A0370^T 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-A0370^T (=NBRC 105296^T=VTCC D9-09^T).

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-A0370^T, 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 (1⁻¹) 20 mg trimethoprim and 10 mg nalidixic acid. Strain MN07-A0370^T 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; 1⁻¹: 2 g yeast extract, 10 g soluble

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MN07-A0370^T is AB468971.

A supplementary table showing the cultural characteristics of strain MN07-A0370^T 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-A0370^T 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-A0370^T 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 lysis, 20 % SDS (50 µl) and proteinase K (50 µl, 4 mg ml⁻¹, Nacalai Tesque) were used to denature and degrade proteins and phenol/chloroform/isoamyl alcohol (25:24:1, v/v) was used to remove denatured proteins. The 16S rRNA gene sequences were analysed as described by Tamura & Hatano (2001), using an ABI Prism BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (ABI Prism 3130 Genetic Analyzer; Applied Biosystems). The CLUSTAL_X program (Thompson *et al.*, 1997) was used to align the 16S rRNA gene sequence with corresponding sequences from public databases for all of the type strains in the family *Dermacoccaceae*. The phylogenetic trees were inferred using the neighbour-joining method (Saitou & Nei, 1987) in the CLUSTAL_X package and the neighbour-joining, minimum-evolution (Rzhetsky & Nei, 1992) and maximum-parsimony (Eck & Dayhoff, 1966) tree-making algorithms in the MEGA version 4 package

(Tamura *et al.*, 2007). The evolutionary distances were computed using the maximum composite likelihood method (Tamura *et al.*, 2004). The stability of the resultant tree topologies was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using the CLUSTAL_X and MEGA4 packages. The G+C content of the DNA was determined using the method of Mesbah *et al.* (1989).

Strain MN07-A0370^T was examined for key chemical markers to determine whether it had a chemotaxonomic profile consistent with its classification in the family *Dermacoccaceae*. 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-A0370^T 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-A0370^T and members of the genera *Kytococcus*, *Demetria* and *Dermacoccus* ranged from 93.7 % to 96.4 %.

Strain MN07-A0370^T 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. Phylogenetic tree based on 16S rRNA gene sequences constructed with the neighbour-joining algorithm showing the relationships between strain MN07-A0370^T and members of the family *Dermacoccaceae*. Asterisks indicate that the corresponding nodes were also recovered in trees generated with the minimum-evolution and maximum-parsimony tree-making algorithms. Values at nodes are levels of bootstrap support based on 1000 resamplings. *Dermatophilus congolensis* DSM 44180^T and *Dermatophilus chelonae* DSM 44178^T of the family *Dermatophilaceae* were used as the outgroups. Bar, 0.01 nucleotide substitutions per nucleotide position.

Supplementary Table S1 in IJSEM Online). 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 (0.5×1.0 – 0.8×1.2 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 α -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 A4 α . 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 and nocardioform morphologies. In sporoactinomycetes, lysine is found only in the genera *Catenuloplanes* (Yokota *et al.*, 1993) and *Couchioplanes* (Tamura *et al.*, 1994). To date, all

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 terrigena* 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-C_{16:0} (45.8 %), anteiso-C_{17:0} (7.9 %), iso-C_{16:1} H (7.6 %), C_{17:1} ω 9c (7.3 %), C_{17:0} 10-methyl (6.4 %), C_{17:0} (4.0 %), anteiso-C_{17:1} C (2.5 %), iso-C_{18:0} (2.4 %), C_{17:0} (2.3 %), C_{16:0} (2.1 %), C_{16:0} 10-methyl (2.0 %), C_{18:0} (1.6 %), C_{16:1} ω 9c (1.5 %), C_{18:1} ω 9c (1.2 %), iso-C_{17:0} (1.1 %), iso-C_{14:0} (0.8 %), C_{15:0} (0.7 %), iso-C_{15:0} (0.4 %), anteiso-C_{15:0} (0.4 %), C_{18:0} 10-methyl (0.4 %) and C_{15:1} 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),

Fig. 2. Scanning electron micrographs of irregular coccoid- to short rod-shaped cells from 1-week-old cultures of strain MN07-A0370^T grown on Bennett agar (a, b) and short, sterile and rudimentary aerial mycelium-like formation grown on ISP 4 agar (c, d) for 1 week at 28 °C. Bars, 2 μm (a, d) and 1 μm (b, c).

Table 1. Characteristics that differentiate strain MN07-A0370^T from the type strains of species of the closely related genera *Dermaococcus*, *Demetria* and *Kytococcus* in the family *Dermaococcaceae*

Taxa: 1, MN07-A0370^T; 2, *Dermaococcus barathri* MT2.1^T (data from Pathom-aree *et al.*, 2006b); 3, *Dermaococcus profundus* MT2.2^T (Pathom-aree *et al.*, 2006b); 4, *Dermaococcus abyssus* MT1.1^T (Pathom-aree *et al.*, 2006a); 5, *Dermaococcus nishinomiyaensis* DSM 20448^T (Stackebrandt *et al.*, 1995; Kocur *et al.*, 1975); 6, *Demetria terrigena* HK1 0089^T (Groth *et al.*, 1997a, b); 7, *Kytococcus sedentarius* DSM 20547^T (Becker *et al.*, 2002; Stackebrandt *et al.*, 1995); 8, *Kytococcus schroeteri* DSM 13884^T (Becker *et al.*, 2002). All taxa were positive for the presence of lysine in the cell wall and catalase activity. +, Positive; ±, weakly positive; –, negative; ND, no data available; ai, anteiso-methyl branched; i, iso-methyl branched; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid(s).

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Cream to bright yellow, smooth to matted colonies with white powdery surface on ISP 3, 4, 5 and 7 agars	Light yellow	Brilliant yellow	Cream to pale yellow	Bright orange	White to pale yellow	Deep buttercup yellow or cream–white	Muddy yellow
Spore morphology	Coccoid to short rod-shaped spores with rudimentary, short aerial mycelium-like formation	Coccoid	Coccoid	Coccoid	Coccoid	Irregular coccoid to short rod-shaped spores with filopodia	Coccoid	Coccoid
Decomposition of:								
Hypoxanthine	+	+	+	+	ND	–	ND	ND
Hydrolysis of:								
Arbutin	+	–	–	+	–	ND	ND	ND
Gelatin	+	–	–	–	+	–	+	+
Starch	±	–	–	+	–	+	–	–
Tween 80	–	–	–	+	+	+	–	+
Urea	+	+	+	–	–	–	–	–
API ZYM								
α-Fucosidase	±	+	–	+	+	–	ND	ND
β-Glucosidase	–	+	–	+	+	–	ND	ND
Lipase (C14)	–	+	–	+	+	±	ND	ND
Trypsin	+	+	+	–	+	±	ND	ND
Growth at/on:								
10 °C	+	+	+	+	–	ND	ND	ND
37 °C	–	+	+	+	+	–	+	+
40 °C	–	–	–	–	ND	–	+	+
3.0 % NaCl	±	+	+	+	+	+	+	+
4.0 % NaCl	–	+	+	+	+	+	+	+
10.0 % NaCl	–	+	+	+	–	±	+	+
12.5 % NaCl	–	–	+	–	–	–	ND	+
Fatty acids	i-C _{16:0} , ai-C _{17:0} , i-C _{16:1} H, C _{17:1} ω9c	i-C _{16:0} , ai-C _{17:0} , i-C _{17:0}	i-C _{16:0} , ai-C _{17:0} , i-C _{17:0}	i-C _{16:0} , ai-C _{17:0} , i-C _{17:0}	i-C _{15:0} , i-C _{17:0} , ai-C _{17:0}	C _{18:1} , C _{18:0} , C _{17:0} , C _{16:0} , i-C _{16:0}	ai-C _{17:0} , C _{17:0} , i-C _{17:1}	i-C _{17:1} , i-C _{17:0} , i-C _{15:0} , ai-C _{17:0}
Menaquinones	MK-8(H ₄), MK-8(H ₆)	MK-8(H ₂)	MK-8(H ₂)	MK-8(H ₂)	MK-8(H ₂)	MK-8(H ₄)	MK-8, MK-9, MK-10	MK-8, MK-7
Phospholipids	DPG, PG, PI	DPG, PG, PI	DPG, PG, PI	DPG, PG, PI	DPG, PG, PI	DPG, PG, PI, PE, PL	DPG, PG, PI	ND
DNA G + C content (mol%)	68.2	66.8	69.1	65.2	66.0–71.0	66.0	68.0–69.0	ND

strain MN07-A0370^T 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^T are *Dermaococcus barathri* MT2.1^T, *Dermaococcus abyssus* MT1.1^T and *Dermaococcus profundus* MT2.2^T (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^T from the genus *Dermaococcus*. Pronounced

differences in chemotaxonomic, morphological and physiological properties were also found between strain MN07-A0370^T 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-A0370^T and all of the genera in the family *Dermacoccaceae*, strain MN07-A0370^T 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. *pulveratus* 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 × 1.0–0.8 × 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 A4 α . Phospholipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol 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-C_{16:0}, anteiso-C_{17:0}, iso-C_{16:1} H, C_{17:1} ω 9c and C_{17:0} 10-methyl. The menaquinones are MK-8(H₄) and MK-8(H₆). Phylogenetically, this genus is a member of the family *Dermacoccaceae*. The type species is *Luteipulveratus mongoliensis*.

Description of *Luteipulveratus mongoliensis* sp. nov.

L. mongoliensis (mon.go.li.en'sis. N.L. masc. adj. *mongoliensis* pertaining to soil from Mongolia).

Morphological, chemotaxonomic and general characteristics are as given above for the genus description. Good growth occurs on Bennett agar, yeast-starch agar and ISP 2, 3, 4, 5, 6 and 7 agars. Production of bright yellow, water-soluble pigments is positive on Bennett and yeast-starch agar after 1 week of incubation. The optimal growth temperature is 20–28 °C; no growth at 37 °C. Grows well with up to 2.0 % (w/v) NaCl; no growth with >3.0 % NaCl. Catalase is produced; nitrates are not reduced; gelatin is hydrolysed; starch is poorly hydrolysed; Tween 20, urea and arbutin are hydrolysed, but not Tween 80. Casein,

calcium malate, tyrosine and hypoxanthine are decomposed, but not xanthine or adenine. According to the API ZYM enzyme assay, positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, α -glucosidase, *N*-acetyl- β -glucosaminidase and α -mannosidase. Weakly positive for α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, β -galactosidase and α -fucosidase; negative for esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, α -galactosidase, β -glucuronidase and β -glucosidase. According to the API Coryne enzyme assay, positive for catalase, alkaline phosphatase, pyrazinamidase, β -galactosidase, α -glucosidase and *N*-acetyl- β -glucosaminidase; negative for nitrate reduction, pyrrolidonyl arylamidase and β -glucuronidase. According to the API 50CH test, positive for acid from glucose, maltose and sucrose; negative for acid from ribose, xylose, mannitol, lactose and glycogen. The predominant fatty acids are iso-C_{16:0}, anteiso-C_{17:0}, iso-C_{16:1} H, C_{17:1} ω 9c and C_{17:0} 10-methyl, with small amounts of C_{17:0}, anteiso-C_{17:1} C, iso-C_{18:0}, C_{17:0}, C_{16:0}, C_{16:0} 10-methyl, C_{18:0}, C_{16:1} ω 9c, C_{18:1} ω 9c, iso-C_{17:0}, iso-C_{14:0}, C_{15:0}, iso-C_{15:0}, anteiso-C_{15:0}, C_{18:0} 10-methyl and C_{15:1} B.

The type strain is MN07-A0370^T (=NBRC 105296^T =VTCC D9-09^T), which was isolated from grassland soil in Mongolia. The DNA G + C content of the type strain is 68.2 %.

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