Luteipulveratus halotolerans sp. nov., an actinobacterium (Dermacoccaceae) from forest soil

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The taxonomic position of an actinobacterium strain, C296001T, isolated from a soil sample collected in Sarawak, Malaysia, was established using a polyphasic approach. Phylogenetically, strain C296001T was closely associated with the genus Luteipulveratus and formed a distinct monophyletic clade with the only described species, Luteipulveratus mongoliensis NBRC 105296T. The 16S rRNA gene sequence similarity between strain C296001T and L. mongoliensis was 98.7%. DNA–DNA hybridization results showed that the relatedness of strain C296001T to L. mongoliensis was only 21.5%. The DNA G+C content of strain C296001T was 71.7 mol%. Using a PacBio RS II system, whole genome sequences for strains C296001T and NBRC 105296T were obtained. The genome sizes of 4.5 Mbp and 5.4 Mbp determined were similar to those of other members of the family Dermacoccaceae. The cell-wall peptidoglycan contained lysine, alanine, aspartic acid, glutamic acid and serine, representing the peptidoglycan type A4γ L-Lys-L-Ser-D-Asp. The major menaquinones were MK-8(H4), MK-8 and MK-8(H6). Phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol and phosphoglycolipid were the polar lipids, while the whole-cell sugars were glucose, fucose and lesser amounts of ribose and galactose. The major fatty acids were iso-C16:0, anteiso-C17:0, iso-C16:1ω6c, anteiso-C17:1ω9c, iso-C18:0 and 10-methyl C17:0. Chemotaxonomic analyses showed that C296001T had typical characteristics of members of the genus Luteipulveratus, with the main differences occurring in phenotypic characteristics. On the basis of the phenotypic and chemotaxonomic evidence, it is proposed that strain C296001T be classified as a representative of a novel species in the genus Luteipulveratus, for which the name Luteipulveratus halotolerans sp. nov. is recommended. The type strain is C296001T (=ATCC TSD-4T = JCM 30660T).

The discovery of novel species of microbes from unique ecosystems with rich biodiversity contributes significantly to scientific knowledge of natural products. Capitalizing on the abundant and diverse biological resources available, the Sarawak Biodiversity Centre has been building a natural product library for the discovery of novel compounds (Yeo et al., 2014). A strategy undertaken is the exploration of unique ecosystems in Sarawak, the largest state in Malaysia, located on the island of Borneo. Here, actinomycetes are systematically isolated from soil, water, litter and plants found in a variety of tropical rain forest ecosystems.

During an isolation campaign, a novel actinomycete designated strain C296001T from the family Dermacoccaceae was identified. This family currently comprises 11 genera: Dermacoccus, Kytococcus (Stackebrandt et al., 1995), Demetria (Groth et al., 1997), Luteipulveratus (Ara et al., 2010), Yimella (Tang et al., 2010), Branchiibius (Sugimoto et al., 2011), Calidifontibacter (Ruckmani et al., 2011), Flexivirga (Anzai et al., 2011), Barrientosiimonas (Lee et al., 2013),
**Rudaeicoccus** (Kim et al., 2013) and **Tamlicoccus** (Lee, 2013). Members of this family are typically associated with terrestrial habitats, meat products, human blood and skin (De la Rosa et al., 1990; Cordero & Zumalacárregui, 2000; Becker et al., 2002; Papamanoli et al., 2002).

In the present study, we describe the characterization of strain C296001, a representative of the genus *Luteipulveratus* isolated from a soil sample collected from a secondary forest in Semengoh, Sarawak. The taxonomic position of this strain was established by a polyphasic approach. This isolate is closely associated with the genus *Luteipulveratus*, with the only described species, *Luteipulveratus mongoliensis* NBRC 105296, isolated in Mongolia (Ara et al. 2010). At the time of writing, no novel species of this genus had been reported.

A sample of top-soil was taken at the depth of 5–10 cm in a secondary forest in Semengoh, Sarawak in May 2008. The sampling point was adjacent to a *Syzygium polyanthum* tree, locally known as ‘Bungkang’, the leaves of which are traditionally used for flavouring when cooking fish or meat. The soil at the collection point was red–brown, acidic (pH 5.5) and had a very low moisture content (16.8%).

The sample was air-dried for 5 days at room temperature, ground into powder and sieved. The sample was processed under aseptic conditions using sterilized equipment. The soil dust was inoculated on seawater agar medium (SWA; 1 l seawater, 1.8 % agar, pH 7.2, containing 20 p.p.m. naldixic acid and 20 p.p.m. nystatin) using the streaking method (Mincer et al., 2002). Strain C296001 was isolated after a two-week incubation at 28 °C, transferred onto modified Bennett’s agar (0.5 % soluble starch, 0.5 % glucose, 0.1 % beef extract, 0.1 % yeast extract, 0.2 % N-Z-Case (casein enzymic hydrolysate), 0.2 % sodium chloride, 0.05 % calcium carbonate, 1.5 % agar; pH 7.2) and checked for purity. The strain was stored in 20 % (v/v) nutrient glycerol at −80 °C.

Morphological and cultural characteristics of strain C296001 were observed on Bennett’s agar (0.1 % yeast extract, 0.1 % beef extract, 0.2 % N-Z-Amine type A, 1.0 % glucose, 1.5 % agar; pH 7.3), yeast extract-soluble starch agar (YS; 0.2 % yeast extract, 1 % soluble starch, 1.5 % agar; pH 7.3) and International Streptomyces Project (ISP) media 2, 3, 4, 5, 6 and 7 (Shirling & Gottlieb, 1966) after incubation at 28 °C for 2 weeks. Hyphae and sporulation characteristics of the strain on soil extract agar (SEA; 250 ml soil extract, 0.1 % d-glucose, 0.05 % yeast extract, 0.05 % K2HPO4 and 1.5 % agar; pH 7.2) were observed directly under a light microscope using a long distance × 50 objective lens (LMPLFN50X; Olympus). In addition, a sterile glass coverslip was placed at a 45° angle on a SEA plate inoculated with strain C296001 and incubated at 28 °C for 7 days. The coverslip with growing culture on it was then placed onto a glass slide and stained with crystal violet for viewing under a light microscope at × 1000 magnification. Cell motility was tested on motility test medium (Défio, 1998) by stabbing the agar using a sterile needle used to pick the colony.

Samples for scanning electron microscopy (SEM) were prepared from 14-day-old culture on Bennett’s agar. Blocks of agar were cut into 1 cm² pieces and fixed using 4 % glutaraldehyde for 4 h at 4 °C. The samples were washed three times with 0.1 M sodium cacodylate for 10 min at room temperature followed by post-fixation with 1 % osmium tetroxide for 2 h at 4 °C. The samples were then dehydrated through a graded series of ethanol and acetone, followed by critical-point drying for 45 min and sputter gold coating before examination under the SEM (JSM 6400; JEOL). Sample preparation and subsequent examination was carried out at the Institute of Bioscience, Universiti Putra Malaysia.

The ability of strain C296001 to grow at different temperatures (tested at 4 °C up to 45 °C), NaCl concentrations (tested up to 10 %) and pH (tested at pH 2 to pH 10) was evaluated on Bennett’s agar media. Enzymic properties of this strain were determined with an API ZYM enzyme assay kit (bioMérieux). Hydrolytic activity of strain C296001 was tested on a variety of substrates, viz. starch, cellulose, casein, Tween 20, Tween 80, adenine, hypoxanthine, L-tyrosine, xanthine, uric acids and urea. Strain C296001 was also tested for nitrate reduction capability, gelatin liquefaction, and milk coagulation and peptonization properties. Production of melanoïd pigments was tested on ISP 6 and ISP 7 agar (Shirling & Gottlieb, 1966). The strain was tested for antibiotic resistance against kanamycin, erythromycin, chlorotetracycline hydrochloride, penicillin G sodium, ampicillin, bacitracin, chloramphenicol and streptomycin sulphate at concentrations of 10 mg ml⁻¹ and 30 mg ml⁻¹. For the antibiotic resistance tests, growth of strain C296001 was observed after inoculation in triplicate onto glucose asparagine agar (GAA; 0.05 % K2HPO4, 0.05 % asparagine, 1 % d-glucose, 1 % agar) containing the antibiotic tested and incubation at 28 °C for 7 days. Carbon utilization capability of the strain was tested on 37 carbon sources, according to the methods of Shirling & Gottlieb (1966).

Genomic DNA of the strain was extracted using the freezing and thawing method adapted from Muramatsu et al. (2003). PCR was performed for amplification of the 16S rRNA gene using the primers 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R (5’-TACGGRATCCAGTTCGACTT-3’). Near-complete 16S rRNA gene sequences were generated by the ABI Prism BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (ABI Prism 3130 XL Genetic Analyzer; Applied Biosystems). The BLAST tool was used to analyse the 16S rRNA sequence (1371 nt) for sequence similarity in the GenBank database. An alignment was then performed with corresponding sequences of representative genera in the family *Dermacoccaceae* retrieved from the NCBI GenBank database using the CLUSTAL W module of the MEGA 5.2 package (Tamura et al., 2011). Phylogenetic
trees were inferred using the neighbour-joining method (Saitou & Nei, 1987) and evolutionary distances were computed using the maximum composite likelihood method (Tamura et al., 2004) with bootstrap analysis (Felsenstein, 1985) of 1000 replicates. A sequence identity matrix for representative genera was calculated using the BioEdit program version 7.1 (Hall, 1999). DNA–DNA hybridization between strain C296001T and L. mongoliensis NBRC 105296T was carried out, based on the methods of Cashion et al. (1977), De Ley et al. (1970) and Huss et al. (1983), while the G+C content of the DNA was determined on the basis of the methods of Cashion et al. (1977), Mesbah et al. (1989) and Tamaoka & Komagata (1984); both analyses were performed by the identification services of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

Chemotaxonomic characteristics of strain C296001T were studied using cells grown on Bennett’s agar plates at 28 °C for 7 days. The biomass required was harvested by scraping and centrifuging soft colonies that were then washed twice with sterile reverse osmosis water. Cells of L. mongoliensis NBRC 105296T for parallel analysis of whole-cell sugars were cultivated and collected in the same manner. Analyses were carried out by the DSMZ identification services using standard methods. Cellular fatty acids (Kämpfer & Kroppenstedt, 1996; Kuykendall et al., 1988; Miller, 1982), respiratory quinones (Tindall, 1990a, b), phospholipids (Bligh & Dyer, 1959; Tindall, 1990a; Tindall, 1990b; Tindall et al., 2007) and whole-cell sugars (Schumann, 2011) were extracted and analysed from freeze-dried cells. Peptidoglycan structures (Rhuland et al., 1955; Schleifer, 1985; Schleifer & Kandler, 1972; Schumann, 2011; Tindall et al., 2010) and cell-wall sugars were analysed from biomass suspended in 2-propanol/water (1:1, v/v). The key chemical markers of the strain were evaluated to determine whether the isolate had a chemotaxonomic profile consistent with its classification in the family Dermacoccaceae.

Genomic DNA was extracted from cell pellets using enzymic digestion with subsequent protease treatment followed by chloroform extraction and alcoholic precipitation of the DNA. DNA was further cleaned up using a MoBio power clean column. SMARTbell libraries with an approximate insert size of 10–20 kbp were created and sequenced on the PacBio RS II system using P6-C4 chemistry. Read assembly was performed using HGAP 2.3 (Chin et al., 2013). For both assemblies, more than 95 % of the estimated genome size was comprised into one contig. The assembly graphs showed that there were no significant mis-assemblies (data not shown). Additionally, the genome sequence quality was assessed for single base deletions using low-coverage PCR-free paired-end Illumina reads. The estimated INDEL rate in both PacBio assemblies was less than 0.5 per 1 Mbp. Finally, Glimmer 3.02 (Delcher et al., 2007) was run to predict genes followed by anti-SMASH 2.07 (Blin et al., 2013) to predict natural product pathways.

Strain C296001T formed circular, flat to slightly convex colonies with glistening surface on Bennett’s and YS agar. Good growth was observed on ISP 2 to ISP 7 agar, Bennett’s agar and YS agar. Pale beige to pale yellow colonies were formed on Bennett’s agar, YS agar and ISP2 agar (see Fig. S1, available in the online Supplementary Material). Aerial mycelium was absent on all the agar media tested. Colonies became moist/wet after 3–5 days. Sporulation was not observed on any of the media tested but fragmentation of the hyphae was observed. A melanin test performed on ISP 6 and ISP 7 agar showed no production of melanoid pigments. The culture, however, produced light red coloration in ISP 7 broth medium, suggesting the production of soluble pigments in liquid culture.

Colony morphology of strain C296001T grown on SEA plates for up to 21 days at 28 °C was observed under a light microscope. Cells of C296001T were also observed on days 2, 4 and 7. There were filaments growing with apical growth, becoming irregular (zig-zag) at maturation, followed by internal formation of coccoid elements and filaments of around 1 μm (see Figs S2 and S3). Scanning electron microscopy confirmed this observation (Fig. 1). Strain C296001T did not show the formation of a rudimentary short aerial mycelium-like structure on ISP4 and SEA, as has been observed for L. mongoliensis NBRC 105296T (Ara et al., 2010). Cells were non-motile.

The temperature range that supported the growth of strain C296001T on Bennett’s agar was 15–40 °C (optimum 20–28 °C) and no growth was observed at 4, 10 or 45 °C. Strain C296001T was able to grow with NaCl concentrations of up to 9 % (w/v). The strain grew well at pH 5–10, grew weakly at pH 4 and no growth was observed at pH 2 or pH 3. Strain C296001T was able to hydrolyse casein, Tween 20, Tween 80 and hypoxanthine. It tested positive for gelatin liquefaction, milk coagulation and peptonization, and nitrate reduction. It tested negative for hydrolysis of starch, cellulose, adonitol, α-L-rhamnose, xanthine, uric acids and urea. Results from the API ZYM enzyme assays were positive for alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, naphthol-AS-Bl-phosphohydrolase and α-glucosidase. Among the 37 carbon sources tested, strain C296001T was found to be capable of utilizing glucose, D-fructose, maltose, trehalose, galactose, sucrose and D-mannitol, weakly positive for utilization of dextrin, mannose, inositol, aesculin and cellobiose, and unable to utilize fucose, adonitol, succinic acid, D-sorbitol, D-arabitol, tartaric acid, raffinose, citric acid, glycogen, arbutin, D-ribose, L-arabinose, melibiose, D-xylene, dulcitol, α-lactose, inulin, L-rhamnose, melezitose, oxalic acid, pyruvic acid, malic acid, lactic acid, sorbose or propionic acid. The physiological and biochemical properties of strain C296001T that differentiated it from L. mongoliensis NBRC 105296T were the former’s inability to hydrolyse starch, growth at 40 °C, tolerance to sodium chloride (up to 9 %, w/v), enzyme activities and utilization of carbon sources (Table 1).
Strain C296001T also showed a different susceptibility profile to antibacterial compounds as compared with L. mongoliensis NBRC 105296T. The former was able to grow in the presence of 10 and 30 mg ml\(^{-1}\) kanamycin, chloramphenicol, ampicillin, chlortetracycline and erythromycin. While the strain was able to grow with low concentrations of penicillin G, it was not resistant to streptomycin and bacitracin at any of the concentrations tested. Unlike C296001T, L. mongoliensis NBRC 105296T was unable to grow on erythromycin and grew only with low concentrations of chlortetracycline and kanamycin. Strain C296001T was, therefore, more resistant compared with L. mongoliensis NBRC 105296T.

Phylogenetically, strain C296001T is closely associated with the genus Luteipulveratus, forming a distinct monophyletic clade with the type strain of the only described species, L. mongoliensis NBRC 105296T, which was isolated from grassland soil in the Terelj National Park, Mongolia (Ara et al. 2010). The phylogenetic tree reconstructed using 16S rRNA gene sequences of representative genera classified in the family Dermacoccaceae showed that strain C296001T formed a distinct monophyletic clade together with L. mongoliensis NBRC 105296T (Fig. 2). The 16S rRNA gene sequence similarity between strain C296001T and L. mongoliensis NBRC 105296T was 98.7 % and that between strain C296001T and other members of the Dermacoccaceae family ranged from 93.5 % to 96.3 %.

The DNA G+C content of strain C296001T was 71.7 %. The mean DNA–DNA relatedness between strain C296001T and L. mongoliensis NBRC 105296T was 21.5 %, calculated from two hybridization experiments. Based on the ‘same species’ threshold criterion of a 70 % DNA–DNA relatedness established by Wayne et al. (1987) and the DNA–DNA hybridization requirement threshold of 98.7 % rRNA gene sequence similarity recommended by Stackebrandt & Ebers (2006), strain C296001T can be clearly separated from L. mongoliensis NBRC 105296T and is proposed to represent a novel taxonomical unit.

The whole genome sequences of strain C296001T and the single other member of the genus, L. mongoliensis NBRC 105296T, were determined by PacBio sequencing. Actinomycetes typically have DNA G+C-rich genomes and contain repetitive regions (>1 kbp) that are accurately resolved by long-read sequencing on the PacBio RS II system.

The genome sizes of strains C296001T and L. mongoliensis NBRC 105296T were 4.5 and 5.4 Mbp and the whole-genome G+C content was 70 and 68 % respectively. The genome sizes determined were in line with those of other actinomycetes from the family Dermacoccaceae. Dermacoccus nishinomiyaensis strain M25 is reported to have a genome size of 3.03 Mbp (ASM72540v1) and the human commensal Kytococcus sedentarius DSM 20547 has a slightly smaller genome of 2.79 Mbp (Sims et al., 2009). ORFs were identified using Glimmer (Delcher et al., 2007) and fed into AntiSMASH (Blin et al., 2013) for the prediction of secondary metabolite pathways. The software predicts 23 biosynthetic gene clusters (BGCs) for strain C296001T and 30 BGCs for strain NBRC 105296T. Alongside a high number of putative BGCs, strain C296001T contains clusters for two bacteriocins, a siderophore, a terpene, ectoine and two other categories. Strain NBRC 105296T contains 20 putative BGCs as well as clusters for one lantipeptide, one type II polyketide, a terpene, two siderophores, ectoine, two bacteriocins and two other categories. The higher number of predicted BGCs in the genome of strain NBRC 105296T corresponds to the observed genome size of 5.4 Mbp, which is 20 % larger than that of strain C296001T. Interestingly, in both strains no biosynthetic genes encoding non-ribosomal peptide synthases or type 1 polyketide synthases could be identified.

The cell-wall peptidoglycan of strain C296001T classified in the family Dermacoccaceae showed that strain C296001T contained lysine, alanine, aspartic acid, glutamic acid and serine in a molar ratio of 1.0 : 1.3 : 0.8 : 1.0 : 0.5, respectively. This corresponds to the peptidoglycan type A4\(\text{L-}_{\text{Lys-L-Ser-D-Asp}}\).
The major menaquinones were MK-8(H4) (76 %), MK-8 (9 %) and MK-8(H2) (3 %) whereas the polar lipids were phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol and phosphoglycolipid. The whole-cell sugars were glucose, fucose and a less amount of ribose and galactose. A whole-cell sugars test performed in parallel for L. mongoliensis NBRC 105296T detected galactose, fucose and lesser amounts of ribose and glucose. Mannose and rhamnose as reported by Ara et al. (2010) were not detected in our study. Analysis of purified cell-wall sugars

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C296001T</th>
<th>NBRC 105296T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour and appearance</td>
<td>Pale beige to pale yellow, waxy appearance</td>
<td>Cream to bright yellow, convoluted/wrinkly appearance</td>
</tr>
<tr>
<td>Spore morphology</td>
<td>Coccoid to short rod-shaped</td>
<td>Coccoid to short rod-shaped with rudimentary, short aerial mycelium-like formation*</td>
</tr>
<tr>
<td>Decomposition/ hydrolysis of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Temperature tolerance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 °C</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>40 °C</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Tolerance of 6–9 % NaCl</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Tolerance of pH 10</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>API ZYM test</td>
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<td></td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>α-Mannosidase</td>
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<tr>
<td>Carbon utilization test</td>
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<tr>
<td>Fucose</td>
<td>−</td>
<td>+</td>
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<tr>
<td>D-Arabitol</td>
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<td>+</td>
</tr>
<tr>
<td>Glycogen</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Trehalose</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Galactose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cellular fatty acids (major components, &gt;4 %)</td>
<td>iso-C16 : 0 (56.96 %), anteiso-C17 : 0 (14.06 %), iso-C18 : 1 H (8.84 %), 10-methyl C17 : 0 (4.25 %), anteiso-C17 : 09c (5.82 %), iso-C18 : 0 (4.95 %)</td>
<td>iso-C16 : 0 (45.8 %), anteiso-C17 : 0 (7.9 %), iso-C18 : 1 H (7.6 %), 10-methyl C17 : 0 (6.4 %), C17 : 109c (7.3 %), C17 : 0 (4.0 %)*</td>
</tr>
<tr>
<td>Menaquinones (respiratory quinones)</td>
<td>MK-8 (9 %), MK-8(H2) (3 %), MK-8(H4) (76 %)</td>
<td>MK-8(H4) (49 %), MK-8(H6) (48.8 %)*</td>
</tr>
<tr>
<td>Peptidoglycan structure</td>
<td>Type A4z 1-Lys-1-Ser-D-Asp (alanine, 1.3; serine, 0.5; aspartic acid, 0.8; glutamic acid, 1.0; lysine, 1.0)</td>
<td>Type A4z (alanine, 2.7; serine, 0.9; aspartic acid, 0.6; glutamic acid, 1.0; l-lysine, 0.8)*</td>
</tr>
<tr>
<td>Whole-cell sugars</td>
<td>Glucose, fucose, ribose, galactose</td>
<td>Galactose, fucose, ribose, glucose</td>
</tr>
<tr>
<td>Cell-wall sugars</td>
<td>Galactose, fucose</td>
<td>ND</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>Phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol, phosphoglycolipid</td>
<td>Phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol*</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>71.7</td>
<td>68.2*</td>
</tr>
</tbody>
</table>

*Data from Ara et al. (2010).
of strain C296001T detected the presence of galactose and fucose. The fatty acids comprised iso-C16:0 (56.96 %), anteiso-C17:0 (14.06 %), iso-C16:1 H (8.84 %), anteiso-C17:1ω9c (5.82 %), iso-C18:0 (4.95 %), 10-methyl C17:0 (4.25 %), C17:1ω8c (1.19 %), iso-C17:0 (0.91 %), iso-C18:1 H (0.7 %), C16:1 2-OH (0.56 %) and iso-C14:0 (0.25 %).

On the basis of morphological, physiological, biochemical and chemotaxonomic characteristics (Table 1), strain C296001T can be clearly distinguished from L. mongoliensis NBRC 105296T and is hence a representative of a novel species of the genus Luteipulveratus, for which the name Luteipulveratus halotolerans sp. nov. is proposed.

Description of Luteipulveratus halotolerans sp. nov.

Luteipulveratus halotolerans (ha.lo.to’le.rans. Gr. n. hals halos salt; L. part. adj. tolerans tolerating; N.L. part. adj. halotolerans salt-tolerating, referring to the ability of the organism to tolerate high salt concentrations).

Aerobic, Gram-stain-positive and non-motile actinomycete. Forms circular, flat to slightly convex, glistening colonies on Bennett’s agar and YS agar. Aerial mycelium absent. Good growth observed on ISP 2 to 7 agar, Bennett’s agar and YS agar, with no diffusible pigment produced on these media. Good growth at temperatures between 15 and 40 °C; optimum temperature is between 20 and 28 °C. Grows well in the presence of up to 9 % (w/v) NaCl and at pH 5–10. Substrate hyphae apical growth regular, but becoming irregular (zig-zag) at maturation; subsequent internal formation of cocoid elements and filaments of around 1 μm. Positive for hydrolysis of casein, Tween 20, Tween 80 and hypoxanthine. Positive for gelatin liquefaction, milk coagulation and peptonization, and nitrate reduction. API ZYM enzyme assay: positive for alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and β-glucosidase. Utilizes glucose, D-fructose, maltose, trehalose, galactose, sucrose and D-mannitol as sole carbon sources. Predominant fatty acids are iso-C16:0, anteiso-C17:0, iso-C16:1 H, anteiso-C17:1ω9c, iso-C18:0 and 10-methyl C17:0. In addition, C17:1ω8c, iso-C17:0, iso-C18:1 H, C16:1 2-OH and iso-C14:0 are detected.

The type strain is C296001T (=ATCC TSD-4T=JCM 30660T). The DNA G+C content of the type strain is 71.7 %.

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