Luteimonas notoginsengisoli sp. nov., isolated from rhizosphere

Juan Cheng,†1 Meng-Yue Zhang,†1 Wei-Xun Wang,1 Deene Manikprabhu,2 Nimaichand Salam,3 Tian-Yuan Zhang,1 Ying-Ying Wu,1 Wen-Jun Li3 and Yi-Xuan Zhang1

1School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang, 110016, PR China
2Department of Microbiology, Gulbarga University, Kalaburgi-585106, India
3State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, College of Ecology and Evolution, Sun Yat-Sen University, Guangzhou, 510275, PR China

A Gram-staining-negative, yellow-pigmented strain, designated SYP-B804T, was isolated from the rhizosphere of Panax notoginseng. The strain was rod-shaped with a single polar flagellum. The optimum temperature and pH required for growth of the strain were 28–32 °C and pH 7–8, respectively. 16S rRNA gene sequence analysis indicated that strain SYP-B804T showed highest 16S rRNA gene sequence similarity with Luteimonas mephitis DSM 12574T (98.0 %). However, the DNA–DNA relatedness value between them (38.1 ± 0.6 %) was less than the threshold value for the delineation of genomic species. Ubiquinone-8 (Q-8) was the predominant quinone. The major fatty acids were iso-C15 : 0 and iso-C17 : 1ω9c. The major polar lipids of the strain were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The G+C content of the genomic DNA was 71 %. On the basis of phenotypic, chemotaxonomic and molecular characteristics, strain SYP-B804T merits recognition as a representative of a novel species of the genus Luteimonas, for which the name Luteimonas notoginsengisoli sp. nov. is proposed, with SYP-B804T (=KCTC 42211T=JCM 30329T) as the type strain.

The genus Luteimonas was first proposed by Finkmann et al. (2000) for Gram-negative, yellow-coloured and rod-shaped bacteria to be included in the family Xanthomonadaceae of the class Gammaproteobacteria. Since then, members of this genus have been isolated from various environments, including soil (Zhang et al., 2010), fresh water (Chou et al., 2008), seawater (Baik et al., 2008), stratum water (Wu et al., 2013), crude-oil-contaminated seawater (Xin et al., 2014), seashore sediment (Romanenko et al., 2013), tidal flat sediment (Fan et al., 2014), deep-sea sediment (Roh et al., 2008), cucumber leaf (Sun et al., 2012), food waste (Young et al., 2007) and biofilters (Finkmann et al., 2000). They have been found to exhibit a wide range of physiological properties, e.g. from non-motile (Finkmann et al., 2000) to motile (bearing a single polar flagellum) (Roh et al., 2008), or from being psychrophilic in nature (Zhang et al., 2010) to moderately thermophilic (Young et al., 2007).

During a study of microbial diversity in rhizosphere samples of Panax notoginseng collected from Yunnan province (23° 48’ 3.64” N 103° 37’ 50.04” E), China, a strain designated SYP-B804T was isolated on lysogeny broth (LB) by serial dilution plating. Strain SYP-B804T showed 98.0 % 16S rRNA gene sequence similarity with Luteimonas mephitis DSM 12574T while the similarities were below 97 % with other members of the family Xanthomonadaceae. This result encouraged us to establish the taxonomic position of strain SYP-B804T through phenotypic, chemotaxonomic and molecular analyses.

Morphological characteristics of strain SYP-B804T were observed using light microscopy (BH-2; Olympus), scanning electron microscopy (QUANTA200; FEI) and transmission electron morphology (H-7650; Hitachi). Gram staining was carried out using the standard Gram reaction. Growth parameters under different conditions of temperature (4 to 50 °C), pH (4 to 12) and NaCl concentration (0 to 15 %, w/v) were observed using LB as the basal medium. The pH of the basal medium was maintained between pH 7–8, respectively.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SYP-B804T is KP076295.

Five supplementary figures and one supplementary table are available with the online Supplementary Material.

†These authors contributed equally to this paper.

The corresponding author

Correspondence

Wen-Jun Li
liwenjun3@mail.sysu.edu.cn
Yi-Xuan Zhang
zhangyxzsh@163.com

1School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang, 110016, PR China
2Department of Microbiology, Gulbarga University, Kalaburgi-585106, India
3State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, College of Ecology and Evolution, Sun Yat-Sen University, Guangzhou, 510275, PR China

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SYP-B804T is listed in Table S1. The presence of iso-C15 : 0 tidylglycerol, phosphatidylethanolamine and an unknown polar lipids observed were diphosphatidylglycerol, phosphatidylethanolamine and an unknown polar lipids consistent with members of the genus Luteimonas. The predominant respiratory quinone was Q-8, which is consistent with members of the genus Luteimonas. The polar lipids observed were diphosphatidylglycerol, phosphatidylethanolamine and an unknown polar lipid (Fig. S3). The cellular fatty acid profile of strain SYP-B804T is listed in Table S1. The presence of iso-C₁₅ : 0 and iso-C₁₇ : 0 3-OH as its major fatty acids indicate its affiliation to the genus Luteimonas. The data for reference strains are from this study unless indicated. +, Positive; −, negative; w, weakly positive.

### Table 1. Physiological, biochemical and molecular characteristics of strain SYP-B804T and related type strains of the genus Luteimonas

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH range</strong></td>
<td>5–9</td>
<td>5–10</td>
<td>5–10</td>
</tr>
<tr>
<td><strong>NaCl range (w/v, %)</strong></td>
<td>0–2</td>
<td>0–6</td>
<td>0–9</td>
</tr>
<tr>
<td><strong>Oxidase activity</strong></td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><strong>H₂S production</strong></td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>DNA G + C content (mol%)</strong></td>
<td>70.8</td>
<td>67.0*</td>
<td>69.6†</td>
</tr>
<tr>
<td><strong>Assimilation of (API 20NE):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>N-Acetylgalactosamine</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Gluconate</td>
<td>w</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>w</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>Malic acid</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>Assimilation of (Biolog GENE III):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrin</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>−</td>
<td>w</td>
</tr>
<tr>
<td>D-Fructose 6-phosphate</td>
<td>+</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>D-Fucose</td>
<td>w</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>L-Fucose</td>
<td>w</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Gentibiose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Melibiose</td>
<td>w</td>
<td>−</td>
<td>w</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Pectin</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tween 40</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>L-Galactonic acid lactone</td>
<td>+</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucuronic acid</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Glucose-6-phosphate</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucuronic acid</td>
<td>w</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>α-Ketoglutaric acid</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>β-Hydroxy-D, L-butyric acid</td>
<td>w</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>N-Acetyl-D-glucosamine</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>N-Acetyl-D-mannosamine</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>−</td>
<td>−</td>
<td>w</td>
</tr>
<tr>
<td>L-Serine</td>
<td>w</td>
<td>+</td>
<td>w</td>
</tr>
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</table>

**Chemical susceptibility to (Biolog GENE III):**

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
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<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam</td>
<td>−</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Guanidine hydrochloride</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>−</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>D-Serine</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>+</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td><strong>Enzyme activity (API ZYM):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>w</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cysteine arylamidase</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*Data from Finkmann et al. (2000).†Data from Park et al. (2011).
The genomic DNA G+C content of strain SYP-B804<sup>T</sup> was analysed by reversed-phase HPLC (Mesbah et al., 1989) using *Escherichia coli* DH5α as the reference strain. The 16S rRNA gene sequencing was performed according to the method reported by Li *et al.* (2007). The sequences obtained were compared in the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon) (Kim *et al.*, 2012) and aligned with sequences of the most closely related taxa using CLUSTAL_X (Thompson *et al.*, 1997). The phylogenetic tree was reconstructed using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms, using MEGA version 5.0 (Tamura *et al.*, 2011). The evolutionary distance matrix was calculated using Kimura’s two-parameter model (Kimura, 1980), while the stability of the trees was evaluated with 1000 bootstrap replications (Felsenstein, 1985). DNA–DNA hybridization between strain SYP-B804<sup>T</sup> and closely related strains was carried out using the fluorometric micro-well method (Ezaki *et al.*, 1989; Christensen *et al.*, 2000; He *et al.*, 2005), with eight replications for each hybridization reaction.

The almost-complete 16S rRNA gene sequence of strain SYP-B804<sup>T</sup> (1506 bp) showed highest similarity to that of *Luteimonas mephitis* DSM 12574<sup>T</sup> while its similarities to other members of the genus were below 97 %. The neighbour-joining tree, supported by maximum-likelihood and maximum-parsimony methods, is shown in Fig. 1. The evolutionary distance matrix was calculated using Kimura’s two-parameter model (Kimura, 1980), while the stability of the trees was evaluated with 1000 bootstrap replications (Felsenstein, 1985). DNA–DNA hybridization between strain SYP-B804<sup>T</sup> and closely related strains was carried out using the fluorometric micro-well method (Ezaki *et al.*, 1989; Christensen *et al.*, 2000; He *et al.*, 2005), with eight replications for each hybridization reaction.

Strain SYP-B804<sup>T</sup> could be distinguished from the closely related strain *L. mephitis* DSM 12574<sup>T</sup> by various biochemical and physiological characteristics. For instance, strain SYP-B804<sup>T</sup> could only grow in the presence of 0–2 % NaCl, unlike strain DSM 12574<sup>T</sup> which could grow in the presence of up to 6 % NaCl. Strain SYP-B804<sup>T</sup> could assimilate dextrin, D-fructose and pectin but not aesculin, unlike strain DSM 12574<sup>T</sup>. On the basis of differences in biochemical, physiological and molecular characteristics, strain SYP-B804<sup>T</sup> could be considered to represent a novel species of the genus *Luteimonas* for which the name *Luteimonas notoginsengisoli* sp. nov. is proposed.

**Description of Luteimonas notoginsengisoli** sp. nov.

*Luteimonas notoginsengisoli* (no.to.gin.seng.i.so’li. N. L. neut. n. *notoginsengi*um *notoginseng*; L. n. *solum* soil; N.L. gen. n. *notoginsengi*oli of soil of a *notoginseng* root, the source of the organism).

Cells are Gram-staining-negative, motile rods (0.9–1.9 × 0.3–0.4 μm) with a single polar flagellum. Growth occurs within the range reported for members of the genus *Luteimonas*. Based on the results of the sequence analysis and phylogenetic tree, strain *L. mephitis* DSM 12574<sup>T</sup> was considered for studies of DNA–DNA hybridization. The DNA relatedness value between strains SYP-B804<sup>T</sup> and *L. mephitis* DSM 12574<sup>T</sup> was found to be 38.1 ± 0.6 %, which is much less than the cut-off point (70 %) for the delineation of genomic species (Wayne, 1987).

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain SYP-B804<sup>T</sup> (1506 bp), members of the genus *Luteimonas* and other members in the family Xanthomonadaceae. Bootstrap values (expressed as percentages of 1000 replications) of above 50 % are shown at branch points. Asterisks denote nodes that were also recovered using the maximum-parsimony and maximum-likelihood methods. Bar, 0.005 substitutions per nucleotide position.](image-url)
at the following conditions: temperature range (15–40 °C), pH 5–9 and 0–2 % (w/v) NaCl. Optimal growth occurs at 28–32 °C, pH 7–8 and in the presence of 1 % NaCl (w/v). Positive results for catalase, milk coagulation and peptidation and gelatin liquefaction but negative results for oxidase, nitrate reduction and H₂S production. Tweens 20, 40, 60 and 80 are hydrolysed, but not cellulose, urea or starch. Can assimilate acetic acid, acetoclastic acid, L-aspartic acid, dextrin, D-fructose, D-fructose 6-phosphate, D-galacturonic acid, L-galactonic acid lactone, gentiobiose, gelatin, glycyrl-L-proline, D-glucose 6-phosphate, glucuronamide, L-glutamic acid, pectin, propionic acid, L-serine and Tween 40 but not L-alanine, N-acetyl-D-galactosamine, N-acetyl neuraminic acid, γ-aminoobutyric acid, D-arabitol, D-aspartic acid, bromosuccinic acid, cellobiose, citric acid, formic acid, D-galactose, α-D-glucose, glycerol, L-histidine, α-hydroxybutyric acid, p-hydroxyphenylacetic acid, inosine, myo-inositol, α-ketobutyric acid, L-lactose, maltose, D-mannitol, methyl β-D-glucoside, 3-methyl glucose, methyl pyruvate, melibiose, raffinose, L-rhamnose, D-salicyl, D-serine, D-sorbitol, stachyose, sucrose, trehalose or turanose. Positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI phosphoric acid hydrolase. The predominant respiratory quinone is Q-8. The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and an unknown polar lipid. The major cellular fatty acids are iso-C₁₅ : 0 summed feature 9, iso-C₁₁ : 0 and iso-C₁₁ : 0 3-OH.

The type strain is SYP-B804ᵀ (=KCTC 42211ᵀ=JCM 30329ᵀ), which was isolated from the rhizosphere of Panax notoginseng. The DNA G+C content of strain SYP-B804ᵀ is 71 mol%.

Acknowledgements

The authors are grateful to Professor Takui Kudo (JCM, Japan) and Dr Rüdiger Pukall (DSMZ, Germany) for kindly providing the reference type strains. This research was supported by the Key Project of Yunnan Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2014). The authors are grateful to Professor Takuji Kudo (JCM, Japan) and Dr Rüdiger Pukall (DSMZ, Germany) for kindly providing the reference type strains. This research was supported by the Key Project of Yunnan Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2014).

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949

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