**Pedobacter ureilyticus** sp. nov., isolated from tomato rhizosphere soil

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The genus *Pedobacter* was first described by Steyn *et al.* (1998), and later emended by Vanparys *et al.* (2005), Gallego *et al.* (2006), Hwang *et al.* (2006) and Zhou *et al.* (2013). At the time of writing, the genus *Pedobacter* consists of 44 species with validly published names (http://www.bacterio.net/pedobacter.html). Species of the genus *Pedobacter* have mainly been isolated from soil samples, with the online Supplementary Material.

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

Abbreviations: CerPE, ceramide phosphorylethanolamine; CerPI, ceramide phosphoryl-myoinositol; CerPM, ceramide phosphoryl-1-β-mannoside.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain THG–T11T is KF532135.

A Gram-stain-negative, strictly aerobic, rod-shaped and pinkish-yellow bacterium, which was motile by gliding and designated strain THG–T11T, was isolated from tomato rhizosphere soil in Gyeonggi province, Republic of Korea. Based on 16S rRNA gene sequence comparisons, strain THG–T11T was found to be most closely related to ‘*Pedobacter zeaxanthinilaciens*’ TDMA-5 (95.9 % sequence similarity), *Pedobacter agri* PB92T (94.9 %), *Pedobacter rhizophaeae* 01–96T (94.6 %) and *Pedobacter alluvionis* NWER-II1T (94.5 %). The DNA G + C content was 38.4 mol\%. The only isoprenoid quinone detected in strain THG–T11T was menaquinone-7 (MK-7). The major component in the polyamine pattern was sym-homospermidine. The major polar lipids were phosphatidylethanolamine, an unidentifed phosphoglycolipid, an unidentified glycolipid, an unidentified lipid, unidentified aminophospholipids and unidentified aminolipids. The major ceramide was found to be ceramide phosphorylethanolamine. The major fatty acids were identified as iso-C\(_{15}:0\), summed feature 3 (C\(_{16}:1\)ω7c and/or C\(_{16}:1\)ω6c) and C\(_{18}:0\). These data support the affiliation of strain THG–T11T to the genus *Pedobacter*. Based on phenotypic, chemotaxonomic and phylogenetic analysis, it is proposed that strain THG–T11T represents a novel species of the genus *Pedobacter* for which the name *Pedobacter ureilyticus* sp. nov. is proposed, with THG–T11T as the type strain (＝KACC 17660T＝JCM 19461T).

†These authors contributed equally to this work.

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16S rRNA gene was amplified from the chromosomal DNA with the universal bacterial primer pair, 27F and 1492R (Weisburg et al., 1991), and the purified PCR products were sequenced by Solgent (Daejeon, Republic of Korea). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database and EzTaxon-e server (Kim et al., 2012). Multiple alignments were performed using the CLUSTAL X program (Thompson et al., 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed using the neighbour-joining method (Saitou & Nei, 1987) and the maximum-likelihood method in the MEGA5 program (Tamura et al., 2011), with bootstrap values based on 1000 replications (Felsenstein, 1985).

According to the EzTaxon-e server, 16S rRNA gene sequence analyses indicated that the closest relatives of strain THG-T11T were ‘Pedobacter zeaxanthinifaciens’ TDMA-5 (95.9 % sequence similarity), Pedobacter agri PB92 (94.9 %), Pedobacter rhizosphaerae 01-96 (94.6 %) and Pedobacter alluvianus NWER-III1 (94.5 %). The relationship between strain THG-T11T and members of the genus Pedobacter (including the recently reclassified Pedobacter antarcicus) was supported by the phylogenetic trees (Figs 1 and S1, available in the online Supplementary Material). The neighbour-joining tree (Fig. 1) showed that strain THG-T11T lay within the radiation of the genus Pedobacter. Strain THG-T11T and ‘Pedobacter zeaxanthinifaciens’ formed an independent cluster with a bootstrap value of 95 %. A similar tree topology was seen in the maximum-likelihood phylogenetic tree (Fig. S1). The generally accepted criterion for delineating a bacterial species states that strains with a 16S rRNA gene sequence dissimilarity above 3 % are considered to belong to separate species (Stackebrandt & Goebel, 1994; Tindall et al., 2010), hence our data suggest that strain THG-T11T represents a novel species of the genus Pedobacter.

Gram-staining was determined using a bioMérieux Gram stain kit according to the manufacturer’s instructions. Growth was tested using several bacterial media, such as NA, tryptone soya agar (TSA; Oxoid), R2A agar (Difco), Luria–Bertani agar (LB agar; Oxoid), and MacConkey agar (Oxoid) at 28 °C. Growth at different temperatures (4, 10, 15, 20, 25, 28, 30, 37, 40 and 45 °C) and under various pH conditions (pH 4.0–11.0, at intervals of 0.5 pH unit) was assessed after 5 days of incubation in tryptone soya broth (TSB; Oxoid) at 28 °C. To achieve different pH values, three different buffers were used (final concentration, 100 mM): acetate buffer for pH 4.0–6.5, phosphate buffer for pH 7.0–9.0 and sodium bicarbonate buffer for pH 9.0–11.0. Salt tolerance was tested in NB supplemented with 0–7.0 % (w/v) NaCl (at 0.5 % intervals) after 5 days of incubation at 28 °C. Growth was estimated by monitoring the optical density at 600 nm. Anaerobic growth was tested in serum bottles containing TSB supplemented with thioglycolate (1 g l⁻¹), in which the air was substituted with nitrogen gas. The morphology of cells grown for 48 h at 28 °C on TSA was observed at ×11000 magnification with a transmission electron microscope (JEM1010; JEOL). Motility was assayed on sulfide-indole-motility medium (SIM; Difco). Production of flexirubin-type pigments was determined by the procedures outlined by Fautz & Reichenbach (1980). Catalase activity was determined from bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) N, N', N'-tetramethyl-1,4-phenylenediamine reagent. Methyl red and Voges–Proskauer reactions were tested in Clark–Lubs' medium (Scharlau). Tests for the degradation of starch [1 % (w/v); Difco], casein [2 % (w/v) skimmed milk; Oxoid], DNA (DNase agar; Oxoid), aesculin [0.1 % (w/v) aesculin and 0.05 % (w/v) ferric citrate; (Difco)], Tween 20 [1.0 % (w/v); Sigma], Tween 80 [1.0 % (w/v); Sigma], L-tyrosine [0.5 % (w/v); Sigma], CM-cellulose [0.1 % (w/v); Sigma] and chitin from crab shells [1.0 % (w/v); Sigma] were evaluated after 5 days of incubation at 28 °C. Carbon-source utilization and enzyme activities were tested by using API 20NE, API 50 CH and API ZYM test kits according to the instructions of the manufacturer (bioMérieux). ‘P. zeaxanthinifaciens’ KACC 14260, P. agri KACC 14024, P. rhizosphaerae KACC 14938 and P. alluvianus KACC 14286 were included in the phenotypic tests as reference strains and were investigated under the same laboratory conditions as THG-T11T.

Cells of strain THG-T11T were Gram-stain-negative rods (0.5–0.7 × 1.6–2.2 μm); Fig. S2 shows a transmission electron micrograph of the cells. Oxidase and catalase activities were negative. Flexirubin-type pigments were not produced. Colonies grown on TSA plates for 3–4 days were smooth, circular, pinkish-yellow and 0.5–1.5 mm in diameter. The isolate was found to be able to grow well on TSA, NA, R2A and LB agar, but not on MacConkey agar. Growth occurred at 4–40 °C (optimum 25–30 °C) and at pH 4.5–10.5 (optimum 7.5–8.0). In TSB medium, NaCl concentrations of up to 6.0 % (w/v) were tolerated. Cells were able to hydrolyse DNA and aesculin (weakly), but not casein, starch, CM-cellulose, chitin, L-tyrosine, Tween 20 or Tween 80. The methyl red and Voges–Proskauer test were negative. The physiological characteristics of strain THG-T11T are summarized in the species description and in Table 1. It is of note that a characteristic differentiating strain THG-T11T from other species of the genus Pedobacter is its strongly positive urease activity; most strains of species of the genus Pedobacter are negative for urease activity (Margesin & Shivaï, 2010b).

For determination of the DNA G+C content, genomic DNA was extracted, purified and degraded enzymically into nucleosides (Moore & Dowhan, 1995). The nucleosides were analysed using a reverse-phase HPLC system (Alliance 2690 system; Waters) (Mesbah et al., 1989), with a reversed-phase column SunFire C18 (4.6 × 250 mm × 5 μm) and a solvent mixture of 200 mM (NH₄)₂HPO₄/acetonitrile (97:3, v/v) as the mobile phase. The DNA G+C content of strain THG-T11T was 38.4 mol%, which is close to the range expected
for members of the genus *Pedobacter* (36–45 %) (Steyn et al., 1998; Margesin & Shivaji, 2010b).

The polar lipids of strain THG-T11T were extracted from freeze-dried cells grown on TSA for 48 h at 28 °C (Minnikin et al., 1977; 1984). Polar lipids were examined by two-dimensional TLC using TLC Kieselgel 60F254 (Merck) plates (10 x 10 cm) with (1) chloroform/methanol/water (65:25:4, by vol.) in the first dimension and (2) chloroform/methanol/acetic acid/water (80:12:15:4, by vol.) in the second dimension.
by vol.) in the second. After being developed in the solvent system, the individual spots were visualized by charring. The total lipids were stained by spraying with 5% v/v molybdo-phosphoric acid and charred at 120°C for 10 min. Amino lipids were detected by spraying with 0.2% w/v ninhydrin and charred at 120°C for 5 min. Phospholipids were detected by spraying with molybdenum blue reagent at room temperature. The glycolipids were irreversibly stained with β-naphthol/sulfuric acid by charring at 120°C for 5 min. For detection of alkaline-stable sphingolipids, one portion of the crude polar lipids was treated with 0.5 M KOH and 2 M HCl (Choi & Lee, 2012). Alkaline-stable lipids were separated by TLC on silica gel with the acidic solvent system: chloroform/methanol/acetic acid/water (100:20:12:5, by vol.) (Yano et al. 1982; Naka et al. 2000; Minamino et al. 2003; Naka et al. 2003; Choi & Lee, 2012; Xiao et al., 2013). Sphingolipids were detected by spraying with ninhydrin and 10% ethanolic molybdophosphoric acid.

Table 1. Physiological characteristics of strain THG-T11T and related strains of species of the genus Pedobacter

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<td>+</td>
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<td>+</td>
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<td>+</td>
<td>–</td>
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<td>Colony colour</td>
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<td>Yellow</td>
<td>Light pink</td>
<td>Pinkish-yellow</td>
<td>Yellow</td>
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<td>–</td>
<td>w</td>
<td>+</td>
<td>+</td>
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<td>Temperature range for growth (°C)</td>
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<td>18–40</td>
<td>4–40</td>
<td>2–37</td>
<td>10–30</td>
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<tr>
<td>pH range for growth</td>
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<td>6.0–9.0</td>
<td>4.0–9.0</td>
<td>5.5–8.0</td>
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<tr>
<td>Growth in 2% (w/v) NaCl</td>
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<td>ND</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>Nitrate reduction</td>
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<td>–</td>
<td>+</td>
<td>+</td>
<td>w</td>
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<tr>
<td>Hydrolysis of: Casein</td>
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<td>–</td>
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<td>+</td>
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<tr>
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<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>+</td>
<td>w</td>
<td>–</td>
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<td>–</td>
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<td>+</td>
<td>w</td>
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<tr>
<td>d-Xylose</td>
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<td>–</td>
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<td>+</td>
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<td>+</td>
<td>–</td>
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<tr>
<td>d-Galactose</td>
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<td>–</td>
<td>w</td>
<td>+</td>
<td>+</td>
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<td>–</td>
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<td>–</td>
<td>w</td>
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<td>Sucrose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>w</td>
<td>+</td>
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<tr>
<td>Trehalose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>w</td>
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<tr>
<td>Melizitose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Raffinose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>+</td>
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<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>38.4</td>
<td>38.6*</td>
<td>38.0†</td>
<td>37.8‡</td>
<td>41.4§</td>
</tr>
</tbody>
</table>

*Data from Asker et al. (2008) and Kim et al. (2013).
†Data from Roh et al. (2008) and Lee et al. (2012).
‡Data from Kwon et al. (2011).
§Data from Gordon et al. (2009).
The polar lipid profile of strain THG-T11<sup>T</sup> is shown in Fig. S3. The polar lipids observed were phosphatidylethanolamine, an unidentified phosphoglycolipid, an unidentified glycolipid, unidentified aminophospholipids, unidentified aminolipids and an unidentified lipid. The sphingolipids of strain THG-T11<sup>T</sup> were identified by comparing the TLC R<sub>f</sub> values with those of S. mizutaii KACC 12159<sup>T</sup>. The TLC of the alkaline-stable sphingolipids showed that strain THG-T11<sup>T</sup> displayed a ceramide pattern nearly identical to that of S. mizutaii KACC 12159<sup>T</sup>, which is in line with the profile reported by Choi & Lee (2012) and Xiao et al. (2013). CerPM-1 was not detected in strain THG-T11<sup>T</sup>, but the other four ceramides were present (CerPE-1, CerPE-2, CerPI-1 and CerPI-2). The major ceramide in strain THG-T11<sup>T</sup> was identified as a ceramide phosphorylethanolamine (CerPE-2).

For fatty acid methyl ester analysis, cells of strain THG-T11<sup>T</sup> and reference strains were harvested from TSA plates after incubation for 2 days at 28 °C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MDI) and analysed by a gas chromatograph (model 6890, Hewlett Packard) and the Sherlock Aerobic Bacterial Database (TSBA60) (Sasser, 1990). The isoprenoid quinones of strain THG-T11<sup>T</sup> were extracted from freeze-dried cellular material. Menaquinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum, and extracted again in hexane only. The crude hexane/quinone solution was purified using Sep-pak Vac silica cartridges (Waters) and subsequently analysed using a Waters reversed-phase HPLC system (Alliance 2690) [solvent: methanol/2-propanol (7:5, v/v), flow rate: 1.0 ml min<sup>−1</sup>] (Collins & Jones, 1981; Tamaoka et al., 1983; Hiraishi et al., 1996). The polycoumarins of strain THG-T11<sup>T</sup> were extracted as described by Busse & Auling (1988) and Taibi et al. (2000). Polycoumarins were extracted from 100 mg freeze-dried sample with 2 ml of 0.2 M HClO<sub>4</sub>. Experiments were carried out at 100 °C for 30 min with occasional shaking. Each mixture contained 1,8-diamino-octane (10 µmol per 100 mg cells) as the internal standard. Samples were analysed using HPLC (Alliance 2690; Waters) fitted with a reverse-phase column (120 ODS-P 4.6 × 250 mm × 5 µm; Watchers) at 1.0 ml min<sup>−1</sup>, a wavelength of 234 nm and 60 % (v/v) methanol as the mobile phase.

The fatty acid profiles of this strain and related type strains of species of the genus *Pedobacter* are shown in Table S1. The major cellular fatty acids were identified as iso-C<sub>15:0</sub> (31.0 %), summed feature 3 as defined by MIDI (C<sub>16:1ω7c</sub> and/or C<sub>16:1ω6c</sub> 26.2 %) and C<sub>16:0</sub> (10.1 %). The presence of large amounts of iso-C<sub>15:0</sub> 3-OB, C<sub>16:0</sub>, C<sub>16:1ω7c</sub>, iso-C<sub>17:0</sub> 3-OH and iso-C<sub>17:0</sub> 3-OB, in particular, is typical of members of the genus *Pedobacter* (Steyn et al., 1998; Margesin & Shivaji, 2010b). The predominant respiratory quinone detected in strain THG-T11<sup>T</sup> was menaquinone-7 (MK-7) and the major polyamine was *sym*-homospermidine (Fig. S4); these are both characteristic of species within the family *Sphingobacteriaceae* (Margesin & Shivaji, 2010a).

In summary, the characteristics of strain THG-T11<sup>T</sup> are consistent with descriptions of other species of the genus *Pedobacter* with respect to its morphological, biochemical and chemotaxonomic properties. On the basis of the phylogenetic distances between strain THG-T11<sup>T</sup> and species of the genus *Pedobacter*, strain THG-T11<sup>T</sup> should be assigned to the genus *Pedobacter* as a representative of a novel species, for which the name *Pedobacter ureilyticus* sp. nov. is proposed.

**Description of *Pedobacter ureilyticus* sp. nov.**

*Pedobacter ureilyticus* [ure.i.ly’ti.cus. N.L. n. urea -ae urea; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lytikos*) able to loosen, able to dissolve; N.L. masc. adj. *ureilyticus* ureadissolving].

Gram-stain-negative rods, strictly aerobic, 0.5–0.7 × 1.6–2.2 µm and motile by gliding. Catalase and oxidase activities are negative. Grows on TSA at 4–40 °C, with optimum growth at 25–30 °C. Growth occurs on TSA, NA, R2A and LB agar, but not on MacConkey agar. Grows in TSB at pH 4.5–10.5 and at 0–6 % (w/v) NaCl. Colonies are smooth, circular, pinkish-yellow and 0.5–1.5 mm in diameter. Cells are able to hydrolyse DNA and aesculin (weakly), but not casein, starch, CM-cellulose, chitin, L-tyrosine, Tween 20 or Tween 80. The methyl red and Voges-Proskauer tests are negative. In API ZYM tests, results are positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and N-acetyl-β-glucosaminidase; weakly positive for esterase (C4) and α-galactosidase; negative for lipase (C14), β-glucuronidase, β-glucosidase, x-mannosidase and x-fucosidase. According to API 20NE tests, tests for arginine dihydrolase, urease, β-glucosidase (asculin hydrolysis), protease (gelatin hydrolysis) and β-galactosidase (PNPG) and the assimilation of D-glucose, L-arabinose, D-mannose and N-acetylglucosamine are positive; the maltose test shows a weak reaction; negative for nitrate reduction, indole production, glucose acidification and the assimilation of D-mannitol, gluconate, caprate, adipate, malate, citrate and phenylacetate. In API 50 CHB tests: positive for acid production from D-xylose, D-galactose, N-acetylglucosamine, aesculin and gluconate; weakly positive for glycerol, erythritol, mannose, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, β-gentiobiose, L-fucose and 5-ketogluconate; negative for D-arabinose, L-arabinose, ribose, glucose, L-xylose, adonitol, methyl β-D-xylopyranoside, fructose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucoside, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose,
The predominant menaquinone is MK-7. The major polar lipids are phosphatidylethanolamine, an unidentified phosphoglycolipid, an unidentified glycolipid, unidentified aminophospholipids, unidentified aminolipids and an unidentified lipid. Contains CerPE-2 as the major sphingophospholipid. Minor amounts of the following ceramides are also present: CerPE-1, CerPl-1 and CerPl-2. The major polyamine is sym-homospermidine.

The type strain, THG-T11^T (=KACC 17660^T=JCM 19461^T), was isolated from tomato rhizosphere soil in Gyeonggi province, Republic of Korea. The G+C content of the genomic DNA of the type strain is 38.4 mol%.

Acknowledgements

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References


