**Lysobacter terricola** sp. nov., isolated from greenhouse soil

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Strain 5GH22-11\(^T\), which was isolated from greenhouse soil in the Yangpyeong region, Gyeonggi province, Republic of Korea, was characterized to be an aerobic, Gram-stain-negative, flagellated, rod-shaped bacterium. It could grow at temperatures from 10 to 33 °C (optimum of 28–30 °C), in the pH range of 6.0–10.0 (optimum of pH 7.0) and without NaCl. 16S rRNA gene sequence analysis showed that strain 5GH18-14\(^T\) showed the highest sequence similarities with *Lysobacter niabensis* GH34-4\(^T\) (98.6 %), *Lysobacter yangpyeongensis* GH19-3\(^T\) (98.1 %), *Lysobacter fragariae* THG-DN8.7 (97.9 %), *Lysobacter terrae* THG-A13\(^T\) (97.3 %), *Lysobacter rhizosphaerae* THG-DN8.3 (97.2 %), *Lysobacter tyrosinelyticus* THG-DN8.2 (97.2 %) and *Lysobacter oryzae* YC6269\(^T\) (97.2 %), revealing less than 95.5 % sequence similarities with all other species with validly published names. Phylogenetic trees also indicated that strain 5GH18-14\(^T\) formed a compact subcluster with *L. niabensis* GH34-4\(^T\), *L. yangpyeongensis* GH19-3\(^T\), *L. terrae* THG-A13\(^T\) and *L. oryzae* YC6269\(^T\) within the genus *Lysobacter*. The predominant quinone of strain 5GH18-14\(^T\) was Q-8. The polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidyl-N-methylethanolamine and unknown aminophospholipids. DNA–DNA hybridization values with closely related species were below 70 %. The DNA G + C content was 65.9 mol%. Based on the phylogenetic, physiological and chemotaxonomic data, it has been demonstrated that strain 5GH18-14\(^T\) represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter terricola* sp. nov. is proposed. The type strain is 5GH18-14\(^T\) (=KACC 16954\(^T\)=JCM 30862\(^T\)).

The genus *Lysobacter* was first proposed for non-fruiting, gliding bacterial strains with a high base ratio (Christensen & Cook, 1978), and later emended to encompass strains containing the polar lipids diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl-N-methylethanolamine and unknown aminogroup-containing lipids. At the time of writing, there are more than 30 species with validly published names listed (http://www.bacterio.net). Recently, three novel species, including ‘*Lysobacter tyrosinelyticus*’, ‘*Lysobacter fragariae*’ and ‘*Lysobacter rhizosphaerae*’ have been proposed but not yet validly published (Du et al., 2015; Singh et al., 2015). Species of the genus *Lysobacter* have been characterized as Gram-negative, aerobic bacteria with a high DNA G + C content and containing ubiquinone 8 (Q-8) as the major respiratory quinone, showing a predominance of iso-C\(_{15}:0\), iso-C\(_{16}:0\) and iso-C\(_{17}:1\omega9c\) (Christensen & Cook, 1978; Weon et al., 2006; Choi et al., 2014). They were isolated from soil, water, a sludge blanket reactor or a deep-sea sponge, and were known to produce various extracellular enzymes and antibiotics (Christensen & Cook, 1978; Nakayama et al., 1999; Ahmed et al., 2003; Park et al., 2008).

The soil from a greenhouse located in the Yangpyeong region, Gyeonggi province, Republic of Korea, was sampled. The soil samples were serially diluted in 0.85 % saline solution, and plated on R2A agar medium at 28 °C. Among the isolates, 5GH18-14\(^T\) was identified as the type strain of a putative novel species of the genus *Lysobacter* on the basis of 16S rRNA gene sequence analysis. The strain was lyophilized using 12 % (w/v) skimmed milk, and then preserved at 4 °C.

The 16S rRNA gene of strain 5GH18-14\(^T\) was amplified with the primers 9F and 1512R (Weisburg et al., 1991), and the purified PCR products were sequenced by Solgent (Daejeon, Republic of Korea). An almost complete sequence of 1478 bp was obtained. 16S rRNA gene
sequences of related taxa were obtained from the GenBank database and EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012). The sequence similarities among strain 5GH18-14\textsuperscript{T} and closely related species were calculated through the GenBank database and EzTaxon-e server. Sequence alignments were performed using the ARB software package (version December 2007; Ludwig et al., 2004). Trees were reconstructed in MEGA version 6 (Tamura et al., 2013) using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony algorithms (Fitch, 1971). A neighbour-joining tree was reconstructed with Kimura’s two-parameter model (Kimura, 1980). A maximum-likelihood tree was reconstructed using the nearest neighbour interchange as the maximum-likelihood heuristic search method. The maximum-parsimony tree was inferred using the min-mini heuristic algorithm (search level 3). The strength of each topology was checked using 1000 bootstrap replications.

The 16S rRNA gene sequence of strain 5GH18-14\textsuperscript{T} showed highest sequence similarities with Lysobacter niabensis GH34-4\textsuperscript{T} (98.6 %), Lysobacter yangpyeongensis GH19-3\textsuperscript{T} (98.1 %), ‘Lysobacter fragariae’ THG-DN8.7 (97.9 %), Lysobacter terrae THG-A13\textsuperscript{3} (97.3 %), ‘Lysobacter rhizosphaerae’ THG-DN8.3 (97.2 %), ‘Lysobacter tyrosinelyticus’ THG-DN8.2 (97.2 %) and Lysobacter oryzae YC6269\textsuperscript{T} (97.2 %), revealing less than 95.5 % sequence similarities with all the other species with validly published names. The neighbour-joining phylogenetic tree indicated that strain 5GH18-14\textsuperscript{T} formed a compact subcluster with ‘L. fragariae’ THG-DN8.7, ‘L. tyrosinelyticus’ THG-DN8.2, L. terrae THG-A13\textsuperscript{3}, L. niabensis GH34-4\textsuperscript{T}, ‘L. rhizosphaerae’ THG-DN8.3, L. oryzae YC6269\textsuperscript{T} and L. yangpyeongensis GH19-3\textsuperscript{T} within the cluster of the genus Lysobacter (Fig. 1). The maximum-likelihood and maximum-parsimony trees also showed a similar result (Fig. 1).

The cell morphology of strain 5GH18-14\textsuperscript{T} was observed by transmission electron microscopy (LEO; model 912AB). Gram-staining was tested with the Difco Gram staining kit according to the manufacturer’s instructions. Oxidase activity was tested by bubble production in 3 % (v/v) hydrogen peroxide solution and catalase was checked from the colour change in 1 % (w/v) tetramethyl-p-phenylenediamine (bioMérieux). Flexirubin-type pigment production was checked from the reversible colour shift from yellow or orange colonies to red, purple or brown using aqueous 20 % (w/v) KOH solution (Fautz & Reichenbach, 1980). The growth of strain 5GH18-14\textsuperscript{T} was tested on different media: R2A (Difco), trypticase soy agar (TSA; Difco), nutrient agar (NA; Difco) and MacConkey agar (Difco) at 28 °C. Casein (5 %, w/v), chitin (1 %, w/v), CM-cellulose (1 %, w/v), hypoxanthine (0.5 %, w/v), starch (1 %, w/v), Tween 80 (1 %, w/v), tyrosine (0.1 %, w/v) or xanthine (0.5 %, w/v) were added in R2A medium, and the hydrolysis of these substrates determined after 14 days of incubation at 28 °C. A DNase test was conducted with DNase test agar (Difco). The temperature range for growth was observed after the incubation period (up to 7 days) on R2A broth at temperatures of 4, 10, 15, 20, 25, 28, 30, 33, 35, 37 and 40 °C. The pH range for growth was tested in R2A broth at 28 °C by adjusting the final pH to 5.0, 6.0, 7.0, 8.0, 9.0 10.0 and 11.0 with appropriate buffers (Na\textsubscript{2}HPO\textsubscript{4}/NaH\textsubscript{2}PO\textsubscript{4} for pH 5.0–7.0 and Na\textsubscript{2}CO\textsubscript{3}/NaHCO\textsubscript{3} for pH 8.0–11.0). NaCl tolerance was checked in R2A broth containing 0, 1, 2, 3 and 5 % NaCl (w/v). Other phenotypic and enzymatic characterizations of strain 5GH18-14\textsuperscript{T} were determined using API 20NE, API ID 32GN and API ZYM kits (bioMérieux) according to the manufacturer’s instructions. The reference strains, ‘L. fragariae’ KACC 18545, L. niabensis KACC 11587\textsuperscript{T}, L. oryzae KACC 14553\textsuperscript{T}, ‘L. rhizosphaerae’ KACC 18544, L. terrae KACC 17646\textsuperscript{T}, ‘L. tyrosinelyticus’ KACC 18543 and L. yangpyeongensis KACC 11407\textsuperscript{T} were studied for comparisons.

The morphological, physiological and biochemical characteristics of strain 5GH18-14\textsuperscript{T} are given in Table 1 and in the species description. Strain 5GH18-14\textsuperscript{T} can be differentiated from closely related species within the genus Lysobacter on the basis of its morphological, physiological and biochemical properties such as cell size, flagellum or motility, temperature range for growth, catalase activity, hydrolysis of some substrates, assimilation of carbon sources and enzyme activities (Table 1). Cells were 0.4–0.5 × 1.5–2.0 μm (Fig. S1, available in the online Supplementary Material).

For cellular fatty acid analysis, strain 5GH18-14\textsuperscript{T} and the reference strains were grown on R2A agar at 28 °C for 2 days, where the bacterial communities reached the late-exponential stage of growth, according to the four quadrants streak method (Sassar, 1990). Fatty acid methyl esters were prepared and analysed according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 5.0) and identified by the TSBA 50 database of the Microbial Identification System (Sassar, 1990). Cell biomass for polar lipids and respiratory quinones was obtained by centrifugation after shaking at 28 °C in R2A broth for 3 days. Quinones and polar lipids were extracted and analysed using the method of Minnikin et al. (1984). For the detection of polar lipids, molybdoophosphoric acid (for total lipids), phosphomolybdic acid (for phospholipids), ninhydrin (for aminolipids) and naphthol/sulfuric acid reagent (for glycolipids) were sprayed. The respiratory quinones were separated by TLC and identified by HPLC. The DNA G+C content was determined with the fluorometric method (Gonzalez & Saiz-Jimenez, 2002) using SYBR Green 1 and a real-time PCR thermocycler (Bio-Rad). Genomic DNA samples from Bacillus amyloliquefaciens subsp. plantarum DSM 23117\textsuperscript{T}, Pseudomonas stutzeri ATCC 17588\textsuperscript{T} and Micrococcus luteus ATCC 4698\textsuperscript{T} were calibration references.

The fatty acids of strain 5GH18-14\textsuperscript{T} consisted of predominantly iso-C\textsubscript{16}:0 (21.6 %), iso-C\textsubscript{15}:0 (19.1 %) and iso-C\textsubscript{17}:0 3-0H (11.6 %), with moderate amounts of iso-C\textsubscript{15}:0 3-0H (7.1 %), anteiso-C\textsubscript{15}:0 (6.8 %), C\textsubscript{16}:1ω7c alcohol.
The fatty acid compositions of strain 5GH18-14T and closely related species were quite similar, containing iso-C15:0, iso-C16:0 and iso-C17:1ω9c as the major fatty acids. The quantitative differences between the fatty acids among the species compared is shown in Table S1. The predominant quinone of strain 5GH18-14T was ubiquinone 8 (Q-8), which is also the major quinone of the genus Lysobacter. The polar lipids of strain 5GH18-14T were large amounts of phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylinositolmonophosphatidylglycerol (PME), and moderate or small amounts of three unknown phospholipids and two unknown amino-phospholipids (Fig. S2). Among closely related species, the polar lipids of strain 5GH18-14T were similar to those of L. terrae THG-A13T, L. oryzae KACC 11587T, which lacked PG and PME (Ngo et al., 2015).

DNA–DNA hybridization was conducted using the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted by using a non-radioactive DIG High Prime system and hybridized DNA was visualized using a DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

Strain 5GH18-14T revealed DNA–DNA hybridization values of 45±4% (reciprocal value of 45±3%), 45±5%, 45±3%, 46±4%, 38±6% and 32±5%, respectively, with L. niabensis KACC 11587T, L. yángpyeongensis KACC 11407T and L. yángpyeongensis KACC 11407T, which contained PE, DPG, PG and PME, but quite different from those of L. niabensis KACC 11587T, which lacked PG and PME (Ngo et al., 2015).

DNA–DNA hybridization was conducted using the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted by using a non-radioactive DIG High Prime system and hybridized DNA was visualized using a DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).
Table 1. Differential characteristics between strain 5GH18-14T and closely related species of the genus Lysobacter

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 5GH18-14T</th>
<th>Strain 1, 2</th>
<th>Strain 3, 4</th>
<th>Strain 5, 6</th>
<th>Strain 7, 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolation source</strong></td>
<td>Greenhouse soil</td>
<td>Rhizosphere of strawberry plant</td>
<td>Greenhouse Soil</td>
<td>Rhizosphere of rice</td>
<td>Rhizosphere of strawberry plant</td>
</tr>
<tr>
<td><strong>Cell size</strong></td>
<td>0.4–0.5 × 1.5–2.0</td>
<td>0.7–0.9 × 3.4–4.7*</td>
<td>0.5 × 2.0–5.0</td>
<td>0.3–0.5 × 1.8–2.0</td>
<td>0.5–0.9 × 3.0–4.0</td>
</tr>
<tr>
<td><strong>Flagellum or motility</strong></td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Temperature range for growth (°C)</strong></td>
<td>10–33</td>
<td>10–28a</td>
<td>5–37b</td>
<td>15–42c</td>
<td>18–28d</td>
</tr>
<tr>
<td><strong>Growth at 1% (w/v) NaCl</strong></td>
<td>–</td>
<td>+a</td>
<td>+ b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Catalse/oxidase</strong></td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Hydrolysis of</strong></td>
<td>Aesculin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Starch</strong></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Assimilation of</strong></td>
<td>D-Glucose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>Propionic acid</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>Valeric acid</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>Enzyme activity</strong></td>
<td>Esterase (C4)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>N-Acetylglucosamine</strong></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>β-Galactosidase</strong></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>β-Glucosidase</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>N-Acetyl-β-glucosaminidase</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Major polar lipids</strong></td>
<td>DPG, PE, PG, PME</td>
<td>DPG, PE, PG, PME</td>
<td>DPG, PE</td>
<td>PG, PE, PME</td>
<td>DPG, PE, PG, PME</td>
</tr>
<tr>
<td><strong>DNA G + C content (mol%)</strong></td>
<td>65.9</td>
<td>66.9a</td>
<td>62.5b</td>
<td>67.4c</td>
<td>67.8a</td>
</tr>
</tbody>
</table>

*Data from a, Singh et al. (2015); b, Weon et al. (2007); c, Aslam et al. (2009); d, Ngo et al. (2015); e, Du et al. (2015); f, Weon et al. (2006).

On the basis of the phylogenetic analysis, phenotypic characteristics and DNA–DNA hybridization values, strain 5GH18-14T is considered to represent a novel species of the genus Lysobacter, for which the name Lysobacter terricola sp. nov. is proposed.

**Description of Lysobacter terricola sp. nov.**

*Lysobacter terricola* (ter’ri’co.la. L. n. terra earth, soil; L. suff. -cola inhabitant, dweller; N.L. n. *terricola* a dweller upon earth, soil-dweller, referring to the isolation of the type strain from soil).

Cells are aerobic, non-spore-forming, flagellated, Gram-negative rod (0.4–0.5 × 1.5–2.0 μm). Grows at temperatures from 10–33°C with optimum growth at 28–30°C, and in the pH range of 6.0–10.0, with optimum growth at pH 7.0. Grows only at 0 % NaCl (w/v) with no growth at even 1 % NaCl (w/v). Grows on R2A and NA, but does not grow on TSA or MacConkey agar. Colonies on R2A agar plates are irregular and yellow. Catalase-negative and oxidase-positive. Flexirubin-type pigments are absent. Hydrolyses casein and tyrosine, but does not hydrolyse starch, DNA, xanthine, hypoxanthine, Tween 80, chitin or cellulose. Positive for aesculin hydrolysis and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. Assimilates sodium acetate, glycogen, L-arabinose, D-mannose, D-mannitol, L-histidine, potassium 2-ketogluconate, 4-hydroxybenzoic acid, L-rhamnose, D-ribose, inositol, sucrose, itaconic acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-sorbose, L-fucose, D-sorbitol, valeric acid, L-glutine, potassium 2-ketogluconate, 4-hydroxybenzoic acid or L-proline. Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, x-glucosidase and N-acetyl-β-glucosaminidase, but negative for esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, x-chymotrypsin, x-galactosidase, β-glucuronidase, β-glucosidase, x-mannosidase and x-fucosidase. The major fatty acids are iso-C15:0 3-OH, anteiso-C15:0 and iso-C17:0 3-OH. The predominant quinone is Q-8. The polar lipids are large amounts of phosphorylcholine, diphasphatidylglycerol, phosphatidylglycerol and phosphatidylmonomethylethanolamine, and moderate or small amounts of three unknown phospholipids and two unknown aminophospholipids.

The type strain, 5GH18-14T (= KACC 16954T = JCM 30862T), was isolated from greenhouse soil, from the Yangpyeong region, Gyeonggi province in the Republic of Korea. The DNA G+C content of the type strain is 65.9 mol%.

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**References**


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