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Lysobacter terrae sp. nov. isolated from *Aglaia odorata* rhizosphere soil

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A Gram-stain negative, facultatively anaerobic, non-motile, rod-shaped bacterium, designated strain THG-A13^T, was isolated from *Aglaia odorata* rhizosphere soil in Gyeonggi-do, Republic of Korea. Based on 16S rRNA gene sequence comparisons, strain THG-A13^T had close similarity with *Lysobacter niabensis* GH34-4^T (98.5 %), *Lysobacter oryzae* YC6269^T (97.9 %) and *Lysobacter yangpyeongensis* GH19-3^T (97.3 %). Chemotaxonomic data revealed that strain THG-A13^T possesses ubiquinone-8 (Q8) as the predominant isoprenoid quinone and iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:1}\omega 9c$ as the major fatty acids. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol) and diphosphatidylglycerol. The G+C content was 66.3 mol%. The DNA–DNA relatedness values between strain THG-A13^T and its closest phylogenetic neighbours were below 18.0 %. These data corroborated the affiliation of strain THG-A13^T to the genus *Lysobacter*. These data suggest that the isolate represents a novel species for which the name *Lysobacter terrae* sp. nov. is proposed, with THG-A13^T as the type strain (=KACC 17646^T=JCM 19613^T).

The genus *Lysobacter* was first established by Christensen & Cook (1978), and later emended by Park *et al.* (2008). The species of the genus *Lysobacter* are characterized by a high G+C content (61.7–70.7 %) and a lack of flagella (Lee *et al.*, 2006; Wei *et al.*, 2012). At the time of writing, the genus *Lysobacter* contains 26 species with validly published names (http://www.bacterio.net/lysobacter.html), with *Lysobacter enzymogenes* as the type species. Species of the genus *Lysobacter* have been mostly isolated from soil and water samples. Some species of the genus *Lysobacter* are potential biocontrol agents for plant fungal pathogens due to their antifungal and antimicrobial activities (Hashizume *et al.*, 2004; Park *et al.*, 2008; Wei *et al.*, 2012). In this study, a novel species, *Lysobacter terrae* sp. nov. THG-A13^T, was characterized using a polyphasic taxonomic approach.

†These authors contributed equally to this paper.

Abbreviations: RP-HPLC, reversed phase HPLC; SIM, sulfide-indole-motility medium.

The NCBI GenBank accession number for the 16S rRNA gene sequence of strain THG-A13^T is KF483861.

Four supplementary figures are available with the online Supplementary Material.

Strain THG-A13^T was isolated from *Aglaia odorata* rhizosphere soil (in the spring of 2013) in Gyeonggi-do, South Korea (37° 30′ N 127° 15′ E). Approximately 1 g soil was suspended thoroughly in 10 ml sterile 0.85 % NaCl (w/v; saline solution). Serially diluted samples were spread on R2A agar (Difco) and incubated at 28 °C for a week. Single colonies were purified by transferring to new R2A plates. One isolate, THG-A13^T, was cultured routinely on R2A agar at 28 °C and stored in R2A broth suspension containing glycerol (25 %, w/v) at −70 °C.

For the sequencing of the 16S rRNA gene, genomic DNA was obtained using a Solgent genomic DNA extraction kit and the 16S rRNA gene was amplified according to the methods of Weisburg *et al.* (1991). The 16S rRNA gene sequencing was performed by Solgent; sequences of related taxa were obtained from the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim *et al.*, 2012) and GenBank database. Multiple alignments were performed using the program CLUSTAL X (Thompson *et al.*, 1997), followed by gap editions in the BioEdit program (Hall, 1999). The Kimura two-parameter model (Kimura, 1983) was used to calculate evolutionary distances. The neighbour-joining (Saitou & Nei, 1987) and the maximum-likelihood methods were used

to reconstruct phylogenetic trees, as implemented in MEGA 5.2 (Kumar *et al.*, 2008). The bootstrap values were calculated based on 1000 replications (Felsenstein, 1985).

The 16S rRNA gene sequence of strain THG-A13^T (1430 bp) was analysed. Sequence similarities indicated that the closest relatives of strain THG-A13^T were *Lysobacter niabensis* GH34-4^T (98.5 %), *Lysobacter oryzae* YC6269^T (97.9 %), *Lysobacter yangpyeongensis* GH19-3^T (97.3 %) and *L. enzymogenes* DSM 2043^T (95.5 %). Evidence of this relationship between strain THG-A13^T and other recognized species of the genus *Lysobacter* was derived from the phylogenetic tree (Figs 1 and S1, available in the online Supplementary Material).

Gram-staining was determined using a bioMérieux Gram stain kit. Cell morphology was observed with a transmission electron microscope (JEOL; model JEM1010) at 11 000 × magnification. Anaerobic growth was performed in R2A broth supplemented with thioglycollate [0.1 % (w/v), Sigma]. Motility was tested in sulfide-indole-motility medium (SIM; Difco). Growth at different temperatures (4, 10, 15, 20, 25, 28, 30, 37, 40 and 45 °C) was assessed after 7 days of incubation on R2A agar. Salt tolerance was tested in R2A broth containing 0–1 % (w/v) NaCl (at 0.2 % intervals), and 1–5 % (w/v) NaCl (at 1.0 % intervals). Growth at various pH values (pH 4–10, at intervals of 0.5 pH unit) was determined in R2A broth and evaluated by monitoring the OD_{600} after 5 days of incubation. For the experiments on pH, two different buffers were used (final concentration 100 mM): acetate buffer for pH 4.0–6.5 and phosphate buffer for pH 7.0–10.0. Production of flexirubin-type pigments was determined by the reversible colour shift to red, purple or brown when yellow or orange colonies are covered with aqueous 20 % (w/v) KOH solution (Fautz & Reichenbach, 1980). Oxidase and catalase activities were tested with 1 % (w/v) *N*, *N*, *N*′, *N*′-tetramethyl-1,4-phenylenediamine reagent and 3 % (v/v) H_2O_2, respectively. Methyl red and Voges–Proskauer reactions were tested in Clark and 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

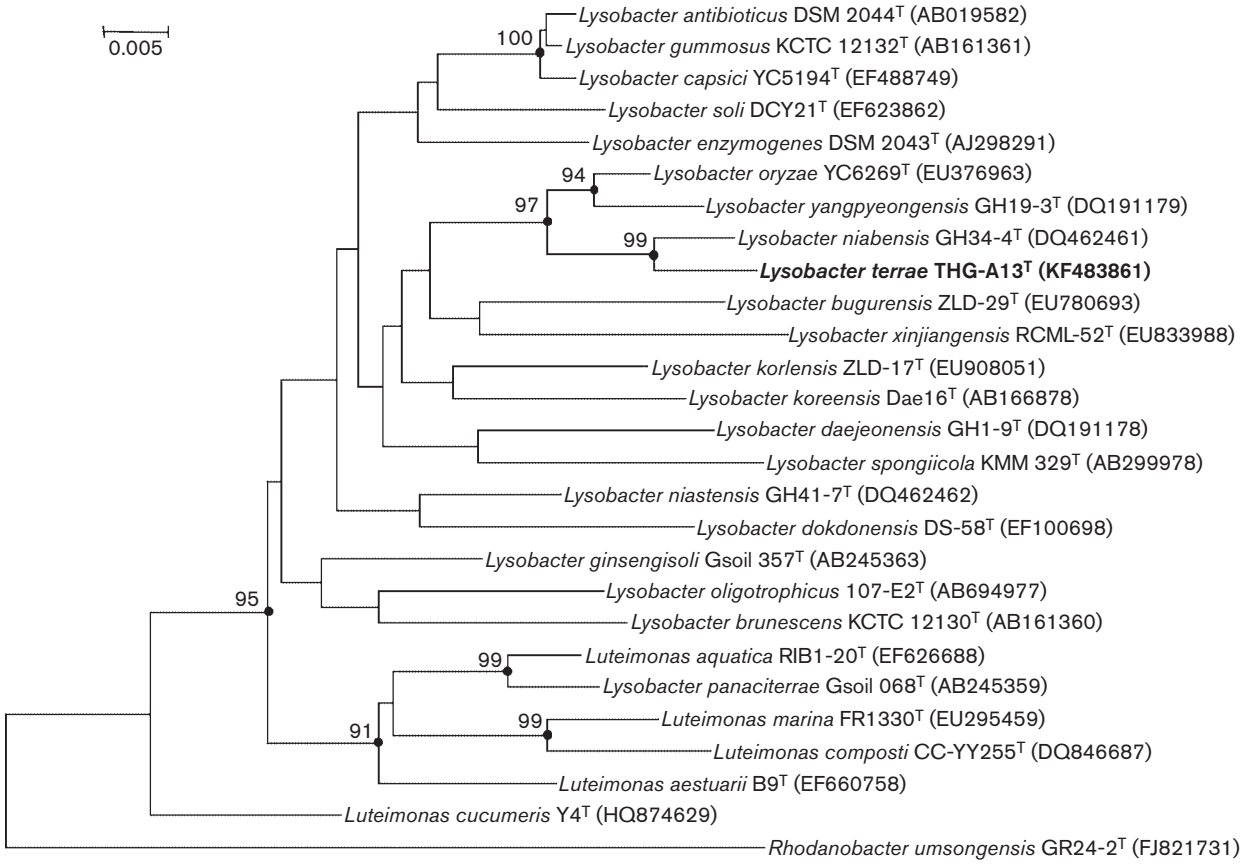

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of *Lysobacter terrae* sp. nov. THG-A13^T with related species of the genus *Lysobacter*. Filled circles at the nodes indicate branches that were also recovered using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) over 70 % are shown at the branching points. *Rhodanobacter umsongensis* GR24-2^T (FJ821731) was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.

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 determined after 5 days of incubation at 28 °C. Growth on MacConkey agar (Oxoid), nutrient agar (NA, Oxoid) and tryptone soya agar (TSA, Oxoid) was also tested. In addition, API 20NE, ID 32 GN and API ZYM test kits (bioMérieux) were used according to manufacturer instructions to evaluate carbon source utilization and enzyme activities. Strains of the species *L. niabensis* KACC 11587^T, *L. oryzae* KACC 14553^T, *L. yangpyeongensis* KACC 11407^T and *L. enzymogenes* KCTC 12131^T were included in experiments, under the same laboratory conditions, to act as references for the investigation of biochemical properties of strain THG-A13^T.

Strain THG-A13^T was a Gram-stain negative, facultative anaerobic and rod-shaped bacterium. No gliding or flagella-induced motility was observed, but numerous thin fimbriae were present around the cells, often connecting the cells to each other (Fig. S2). The occurrence of long cells (0.40–0.45 × 1.5–5.1 μm) are characteristic of species of the genus *Lysobacter*. Strain THG-A13^T was positive for the production of flexirubin-type pigments. Catalase activity was weakly positive, while oxidase activity was positive. The physiological characteristics of strain THG-A13^T are summarized in the species description and a comparison of selected characteristics of strain THG-A13^T and related type strains is given in Table 1.

In order to determine the G+C content and to perform DNA–DNA hybridization, genomic DNA of strain THG-A13^T was prepared as described previously (Moore & Dowhan, 1995). The G+C content was analysed as described by Mesbah *et al.* (1989) using a reverse phase HPLC system. DNA–DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), with photobiotin-labelled probes in microplate wells. DNA–DNA hybridization experiments were performed between strain THG-A13^T and closely related type strains of species of the genus *Lysobacter*. The optimum hybridization temperature was 51.5 °C.

The DNA G+C content of strain THG-A13^T was 66.3 mol%, which conforms to the expected range of G+C contents within the genus *Lysobacter*. The DNA–DNA relatedness values between strain THG-A13^T and the other species of the genus *Lysobacter* were in the range of 13–18 % (*L. niabensis* KACC 11587^T, 17.2 ± 0.5 %; *L. oryzae* KACC 14553^T, 16.9 ± 0.5 %; *L. yangpyeongensis* KACC 11407^T, 13.6 ± 0.9 %). These very low DNA relatedness values suggest that THG-A13^T represents a novel species of the genus *Lysobacter* (Wayne *et al.*, 1987).

For fatty acid analysis, cells of strain THG-A13^T and strains of the three most closely related species of the genus *Lysobacter* were harvested from R2A agar plates after incubation for 2 days at 28 °C. The cellular fatty acid profiles were prepared according to the protocol of the Sherlock Microbial Identification System (MIDI), detected by GC (Hewlett Packard 6890) and analysed with the Sherlock Aerobic Bacterial Database (TSBA60) (Sasser, 1990). For the extraction of isoprenoid quinones and polar lipids of strain THG-A13^T, cells were cultured on R2A broth for 3 days and freeze-dried after harvesting. The polar lipids were extracted (Minnikin *et al.*, 1977, 1984), and separated using two-dimensional TLC (Tindall, 1990). For the detection of polar lipids, TLC plates were sprayed with reagents: 5 % (v/v) molybdophosphoric acid was used for total polar lipids; 0.2 % (v/v) ninhydrin for amino lipids; molybdenum blue reagent for phospholipids and α-naphthol sulphuric acid reagent for glycolipids. Isoprenoid quinones were analysed by RP-HPLC (reversed phase HPLC; Waters 2690 Alliance system) [solvent, methanol/2-propanol (7 : 5, v/v); flow rate, 1.0 ml min^{-1}], as previously described (Collins & Jones, 1981;Hiraishi *et al.*, 1996; Tamaoka *et al.*, 1983).

The major cellular fatty acids (>10.0 %) of strain THG-A13^T were iso-$C_{15:0}$ (18.6 %), iso-$C_{16:0}$ (16.4 %) and iso-$C_{17:1}\omega 9c$ (11.0 %), which was consistent with other species of the genus *Lysobacter*. A comparison of the fatty acid profiles of strain THG-A13^T and selected closely related strains is shown in Table 2. The polar lipid profile of strain THG-A13^T included phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, unidentified phosphoglycolipids, an unidentified glycolipid, unidentified aminophospholipids, unidentified aminolipids, an unidentified phospholipid and an unidentified lipid (Fig. S3). Although the major polar lipids were similar to those found in other members of the genus *Lysobacter*, differences were found between selected closely related strains of species of the genus *Lysobacter* and the novel strain (Table 1, Fig. S4). Strain THG-A13^T contained Q-8 as the major respiratory quinone, which is typical of the genus *Lysobacter* (Luo *et al.*, 2012; Wei *et al.*, 2012), with small amounts of Q-7 also present.

On the basis of 16S rRNA gene sequence analysis, fatty acid composition, G+C content and DNA–DNA relatedness values, it has been demonstrated that strain THG-A13^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter terrae* sp. nov. is proposed.

Description of *Lysobacter terrae* sp. nov.

Lysobacter terrae (L. gen. n. *terrae* of the earth).

Cells are Gram-stain negative, facultatively anaerobic, non-motile, rod-shaped and long (0.40–0.45 × 1.5–5.1 μm). After culture on R2A agar for 3 days, colonies are circular, yellow, smooth and 1.0–1.5 mm in diameter. Growth occurs at 4–37 °C (optimum 28–30 °C), at pH 5.0–9.0 (optimum 7.0–7.5), and in 0–0.2 % (w/v) NaCl. Grows well on R2A agar, grows weakly on NA, but not on TSA and MacConkey agar. Catalase and oxidase activities are weakly positive and positive, respectively. Aesculin, casein, Tween 20, Tween 80, CM-cellulose and L-tyrosine are hydrolysed,

Table 1. Physiological characteristics and major polar lipids of *Lysobacter terrae* THG-A13^T and related type strains of species of the genus *Lysobacter*

Strains: 1. THG-A13^T; 2. *L. niabensis* KACC 11587^T; 3. *L. oryzae* KACC 14553^T; 4. *L. yangpyeongensis* KACC 11407^T; 5. *L. enzymogenes* KACC 10127^T (the type species of the genus *Lysobacter*). All data (except for the DNA G+C contents, which are taken from Christensen & Cook, 1978; Weon *et al.*, 2006, 2007; Aslam *et al.*, 2009) are from this study. All strains are non-motile and did not grow on MacConkey agar. All strains are negative for indole production, glucose acidification, arginine dihydrolase and the Voges–Prokauer test. All strains are positive for the hydrolysis of gelatin, β-galactosidase, aesculin and for the methyl red test. In API ZYM tests, all strains are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, but negative for α-fucosidase. In API 20NE tests, all strains are positive for β-galactosidase, β-glucosidase, and protease and negative for D-glucose, L-arabinose, D-mannitol, potassium gluconate, adipic acid and phenylacetic acid. +, Positive; −, negative; W, weakly positive; ND, not detected; PE, phosphatidylethanolamine; PME phosphatidyl-*N*-methylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol.

Characteristic	1	2	3	4	5
Growth temperature (°C)	4–37	5–37	15–42	15–40	10–40
Growth at pH	5.0–9.0	5.0–8.0	5.5–11.0	5.0–8.0	6.0–10.0
Salt tolerance at 1 % (w/v)	−	+	−	−	+
Oxidase	+	+	+	+	+
Catalase	W	+	+	−	+
Nitrate reduction	−	−	−	+	−
Facultative anaerobic growth	+	−	+	−	+
Assimilation of					
Capric acid	+	−	+	−	+
Malic acid	−	−	−	−	+
Trisodium citrate	−	−	−	−	+
N–Acetyl–glucosamine	−	−	−	−	+
D-Maltose	−	−	−	−	+
D-Mannose	−	−	−	−	W
Hydrolysis of					
DNA	−	W	−	−	−
Casein	+	−	+	+	+
CM-cellulose	+	−	−	+	+
L–tyrosine	+	−	+	+	+
Starch	−	−	−	−	+
Chitin	−	−	−	−	+
Tween 20	+	+	+	+	−
Tween 80	+	+	+	−	+
Urea	−	+	−	−	−
Enzyme activities					
α-Galactosidase	+	−	−	−	−
β-Galactosidase	W	W	−	−	−
β-Glucuronidase	−	W	−	−	−
β-Glucosidase	W	+	−	W	−
N-Acetyl-β-glucosaminidase	+	−	−	+	+
α-Mannosidase	W	−	−	W	−
DNA G+C content (mol%)	66.3	62.5	67.4	67.3	69.0
Major Polar lipids	DPG, PG, PE	DPG, PE	PG, PE, PME	DPG, PG, PE, PME	ND

but starch, chitin and DNA are not. The methyl red test is weakly positive, but the Voges–Proskauer test is negative. Positive for the flexirubin-type pigments test (the reversible colour shift from yellow to red). In API ZYM tests, positive results are obtained from alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase and *N*-acetyl-β-glucosaminidase; weakly positive results are obtained from lipase (C14), β-galactosidase, β-glucosidase and α-mannosidase; negative results are obtained from β-glucuronidase and α-fucosidase. In API 20NE tests, nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate, and phenylacetic acid is negative; β-galactosidase, β-glucosidase

Table 2. Cellular fatty acid profiles of strain THG-A13^T and phylogenetically related species of the genus *Lysobacter*

Strains: 1. THG-A13^T; 2. *L. niabensis* KACC 11587^T; 3. *L. oryzae* KACC 14553^T ; 4. *L. yangpyeongensis* KACC 11407^T and 5. *L. enzymogenes* DSM 2043^T. Data for strains 1–4 are from this study, and data for strain 5 were taken from Aslam *et al.* (2009). Cells of all strains were cultured on R2A agar for 3 days at 28 °C. Fatty acids comprising less than 0.5 % are not listed. ND, Not detected; tr, trace (<1.0 %).

Fatty acid	1	2	3	4	5
Saturated					
$C_{16:0}$	6.0	4.5	9.5	10.5	8.6
$C_{18:0}$	1.2	1.0	1.7	2.2	ND
Unsaturated					
iso-$C_{15:1}\omega9c$	3.8	ND	2.6	2.6	ND
$C_{16:1}\omega7c$ alcohol	7.7	7.8	2.8	6.8	tr
$C_{16:1}\omega11c$	2.0	1.0	1.6	2.1	tr
iso-$C_{17:1}\omega9c$	11.0	10.0	15.7	5.6	4.7
Branched-chain					
iso-$C_{11:0}$	4.7	6.4	1.0	3.7	3.4
iso-$C_{11:0}$ 3OH	7.7	9.3	7.8	5.8	6.6
iso-$C_{12:0}$	ND	1.3	tr	1.7	tr
iso-$C_{14:0}$	3.2	8.7	2.6	4.6	1.4
iso-$C_{15:0}$	18.6	12.7	19.6	12.9	20.5
anteiso-$C_{15:0}$	5.9	5.9	4.2	4.6	3.8
iso-$C_{16:0}$	16.4	23.7	16.9	23.9	13.8
iso-$C_{17:0}$	3.8	1.6	12.3	1.9	2.9
*Summed feature 3	1.4	2.0	tr	2.9	15.8

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\ \omega6c$ in all strains except 5, where it contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2OH.

and capric acid are positive; gelatin hydrolysis (protease) is weakly positive. The major fatty acids (>10 %) are iso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:1}\omega9c$. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The predominant isoprenoid quinone is Q-8.

The type strain, THG-A13^T (=KACC 17646^T=JCM 19613^T), was isolated from *Aglaia odorata* rhizosphere soil in Gyeonggi-do, Republic of Korea. The G+C content of the genomic DNA of the type strain is 66.3 mol%.

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