Lysobacter rhizophilus sp. nov., isolated from rhizosphere soil of mugunghwa, the national flower of South Korea

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A Gram-staining-negative, aerobic, motile, rod-shaped, catalase- and oxidase-negative strain with one polar flagellum, designated THG-YS3.6^T, was isolated from rhizosphere soil of a mugunghwa flower collected from Kyung Hee University, Yongin, South Korea. Growth occurred at 10–37 °C (optimum 25–30 °C), at pH 6–8 (optimum 7.0) and with 0–2.0 % NaCl (optimum 1 %). The isoprenoid quinone was ubiquinone-8 (Q–8). The major cellular fatty acids were iso-$C_{11:0}$, iso-$C_{11:0}$ 3-OH, iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{16:1}\omega7c$ alcohol, $C_{16:0}$, iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, one unknown phospholipid, one unknown lipid and three unknown aminolipids. The DNA G+C content of strain THG-YS3.6^T was 65.3 mol%. Based on 16S rRNA gene sequence analysis, the nearest phylogenetic neighbours of strain THG-YS3.6^T were identified as *Lysobacter yangpyeongensis* KACC 11407^T (98.7 %), *Lysobacter oryzae* KCTC 22249^T (98.0 %), *Lysobacter niabensis* KACC 11587^T (97.6 %) and *Lysobacter terrae* KACC 17646^T (97.1 %). The DNA–DNA relatedness values between strain THG-YS3.6^T and *L. yangpyeongensis* KACC 11407^T, *L. oryzae* KCTC 22249^T, *L. niabensis* KACC 11587^T and *L. terrae* KACC 17646^T were 53.8±1.0 %, 12.9±1.2 %, 10.9±0.6 % and 7.0±1.9 %, respectively. On the basis of the phylogenetic analysis, chemotaxonomic data, physiological characteristics and DNA–DNA hybridization data, strain THG-YS3.6^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter rhizophilus* sp. nov. is proposed. The type strain is THG-YS3.6^T (=KCTC 52082^T=CCTCC AB 2015358^T).

The genus *Lysobacter* was proposed by Christensen (Christensen *et al.*, 1978) with *Lysobacter enzymogenes* as the type species. The members of the genus *Lysobacter* have been isolated from various environments, such as air, soil, fresh water, oil, brine, airborne infections, tobacco leaves, human skin, mural paintings, sewage and activated sludge (Aslam *et al.*, 2009; Ngo *et al.*, 2015; Weon *et al.*, 2006, 2007; Yu *et al.*, 2013). At the time of writing, the genus *Lysobacter* contains 34 species with validly published names (http://www.bacterio.net/lysobacter.html). Members of the genus *Lysobacter* are rod-shaped and have DNA G+C contents of about 61.7–70.7 %. Their major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-*N*-methylethanolamine (Park *et al.*, 2008). Their predominant isoprenoid quinone is ubiquinone-8 (Q-8) (Lee *et al.*, 2006; Wei *et al.*, 2012). This paper reports on the taxonomic characterization of a novel species, *Lysobacter rhizophilus* sp. nov. using a polyphasic approach.

Strain THG-YS3.6^T was isolated from rhizosphere soil of a mugunghwa flower (*Hibiscus syriacus* L.) collected in Kyung

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain THG-YS3.6^T is KT962171.

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

Hee University, Yongin, South Korea (5 October 2015) (37° 16′ 33″ N 127° 10′ 40″ E). The soil sample was diluted to 10^{-3} and 10^{-4}, spread on nutrient agar (NA; Oxoid), and incubated at 30 °C in the dark. After 1 week, a single colony was picked and re-streaked repeatedly onto new NA plates until the purity was confirmed. The purified strain, THG-YS3.6^T, was maintained in glycerol (25 %, v/v) at −80 °C.

Genomic DNA of strain THG-YS3.6^T was extracted and purified using a BioFact genomic DNA extraction kit. The 16S rRNA genes were amplified with the universal bacterial primers (Weisburg *et al.*, 1991). The 16S rRNA gene sequencing was performed by BioFact (South Korea). The 16S rRNA gene sequences of related strains were obtained from the GenBank database and EzTaxon server (Kim *et al.*, 2012). The multiple alignments were performed using the CLUSTAL X program (Larkin *et al.*, 2007) followed by gap editions in the BioEdit program (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1984). The phylogenetic trees were reconstructed using the neighbour-joining (Saitou *et al.*, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge *et al.*, 1969) methods in the MEGA 6 program, respectively (Tamura *et al.*, 2013). The bootstrap values were calculated based on 1000 replications (Felsenstein, 1985).

According to the EzTaxon server analysis, 16S rRNA gene sequence analysis indicated that the closest relatives of strain THG-YS3.6^T were *Lysobacter yangpyeongensis* KACC 11407^T (98.7 %), *Lysobacter oryzae* KCTC 22249^T (98.0 %), *Lysobacter niabensis* KACC 11587^T (97.6 %) and *Lysobacter terrae* KACC 17646^T (97.1 %). The 16S rRNA gene sequence of strain THG-YS3.6^T was a continuous stretch of 1496 bp. The relationship between strain THG-YS3.6^T and other members of the genus *Lysobacter* was also supported by the phylogenetic trees (Figs 1, S1 and S2, available in the online Supplementary Material).

The Gram staining was tested using a bioMérieux Gram stain kit according to the manufacturer's instructions (Buck, 1982). Cell morphology of strain THG-YS3.6^T was observed by transmission electron microscope (Model JEM1010; JEOL) at ×11 000 magnification, using cells grown for 4 days at 28 °C on NA. Capacity for growth was tested at different temperatures (4, 5, 10, 20, 25, 28, 30, 37, 40, 45 and 50 °C), salinities (0–10 %, at an intervals of 1 %, w/v, NaCl) and pH (pH 3, 4, 5, 6, 7, 8, 9 and 10) in 5 ml of nutrient broth (NB,Oxoid) at 30 °C for 15 days. For the pH experiments, two different buffers were used (final concentration, 100 mM): acetate buffer was used for pH 4.0–6.5, and phosphate buffer was used for pH 7.0–10.0. The motility test was performed by the hanging-drop technique using cells grown for 2 days at 28 °C in sulfide-indole-motility medium (SIM; Difco) (Skerman, 1967). Anaerobic growth was tested in serum bottles containing NB supplemented with thioglycolate (0.1 %) in which the air was substituted with nitrogen gas. Catalase activity was assessed by bubble production in 3 % (v/v) H_2O_2, and oxidase activity was assessed by using 1 % (w/v) *N,N,N′,N′*-tetramethyl-1,4-phenylenediamine reagent (Kovacs, 1956). Hydrolysis of starch, casein, cellulose, chitin, L-tyrosine, DNA, Tween 20 and Tween 80 was determined according to previously described methods (Chen *et al.*, 2012; Jorgensen *et al.*, 2009; Shigematsu *et al.*, 2003; Singh *et al.*, 2015; Yan *et al.*, 2016). Growth on R2A (Difco), tryptone soya agar (TSA; Oxoid), Luria-Bertani agar (LA; Oxoid), marine agar (MA; Oxoid) and MacConkey agar (MCA; Oxoid) was also tested. All the above tests, unless specifically indicated, were evaluated after 7 days of incubation at 28 °C. *L. yangpyeongensis* KACC 11407^T, *L. oryzae* KCTC 22249^T, *L. niabensis* KACC 11587^T and *L. terrae* KACC 17646^T were included as references for the investigation of the biochemical tests using the same laboratory conditions. In addition, tests of carbon-source utilization and enzyme activities were performed with API 20NE, API 32GN and API ZYM kits according to the instructions of the manufacturer (bioMérieux).

Cells of strain THG-YS3.6^T were Gram-staining-negative, aerobic, motile rods with one polar flagellum. Morphological observation showed colonies of strain THG-YS3.6^T on NA to be yellow, round and sticky. Cells were 6.1–7.5 μm long and 1.0–1.3 μm wide (Fig. S3). Strain THG-YS3.6^T was found to grow well on R2A and NA, grow weakly on TSA and LA, but not grow on MA or MCA. Strain THG-YS3.6^T grew on NA at 10–37 °C (optimum 25–30 °C), at pH 6.0–8.0 (optimum 7.0) and in the presence of 0–2.0 % NaCl (optimum 1.0 %). The tests for oxidase and catalase activities were negative. Nitrate reduction and indole production were found to be negative. Strain THG-YS3.6^T was able to hydrolyse aesculin, Tween 20 and DNA but unable to hydrolyse starch, L-tyrosine, casein, Tween 80, cellulose and chitin. Physiological characteristics of strain THG-YS3.6^T are summarized in the species description, and a comparison of selective characteristics with related type strains is presented in Table 1.

For determination of the DNA G+C content, genomic DNA was extracted, purified by using a BioFact genomic DNA extraction kit and degraded into nucleosides. The nucleosides were analysed using a reverse-phase HPLC system (Alliance 2690 system; Waters) as described previously (Mesbah *et al.*, 1989). *Escherichia coli* DH5α was used as a reference strain. DNA–DNA hybridizations between strain THG-YS3.6^T and closely related strains were performed fluorometrically, according to the method developed by Ezaki *et al.* (1989) with modifications (Stabili *et al.*, 2008), using photobiotin-labelled DNA probes and micro-dilution wells with the hybridization temperature at 45 °C. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the means of the remaining three values were converted to percentage DNA–DNA hybridization values.

The DNA G+C content of strain THG-YS3.6^T was 65.3 mol %, which lies in the expected range of DNA G+C contents

Table 1. Physiological characteristics of strain THG-YS3.6^T and related type strains of species of the genus *Lysobacter*

Strains: 1, THG-YS3.6^T; 2, *L. yangpyeongensis* KACC 11407^T; 3, *L. oryzae* KCTC 22249^T; 4, *L. niabensis* KACC 11587^T; 5, *L. terrae* KACC 17646^T. Data for all strains are from this study, except as labelled. All strains are rod-shaped. All strains are negative for nitrate reduction and indole production. +, Positive; W, weakly positive; −, negative.

Characteristic	1	2	3	4	5
Gliding motility	+	−	−	−	+
Temperature range for growth(°C)	10–37	15–40	15–42	10–40	10–28
pH range for growth	6–8	5–8	5.5–11	4–9	6–8
NaCl tolerance range (%)	0–2	0–1	0–1	0–1	0–1
Catalase	−	−	+	+	+
Oxidase	−	+	+	+	+
Hydrolysis of:					
Esculin	+	−	−	−	+
Tween 20	+	−	+	−	−
Tween 80	−	+	+	−	−
L-Tyrosine	−	+	+	+	+
Starch	−	+	+	−	+
Cellulose	−	+	+	+	−
Casein	−	+	+	+	+
DNA	+	+	−	−	−
Growth on:					
D-Ribose	+	W	−	+	−
Sodium acetate	+	−	−	−	−
API ZYM					
Naphtol-AS-BI-phosphohydrolase	+	−	−	+	
α-Galactosidase	+	W	−	+	−
β-Galactosidase	+	−	−	−	+
α-Fucosidase	+	−	−	−	+
DNA G+C content (mol%)	65.3	67.3*	67.4†	66.6‡	66.3§

*Data from Weon *et al.* (2006).
†Data from Aslam et al. (2009).
‡Data from Weon *et al.* (2007).
§Data from Ngo *et al.* (2015).

of members of the genus *Lysobacter* (61.7–70.7 %) (Lee *et al.*, 2006; Wei *et al.*, 2012). DNA–DNA hybridization values between strain THG-YS3.6^T and its closest phylo genetic neighbours *L. yangpyeongensis* KACC 11407^T (53.8±1.0 %), *L. oryzae* KCTC 22249^T (12.9±1.2 %), *L. niabensis* KACC 11587^T (10.9±0.6 %) and *L. terrae* KACC 17646^T (7.0±1.9 %) gave values below the 70 % cut-off recommended for genomic species discrimination, clearly suggesting that strain THG-YS3.6^T represents a novel species of the genus *Lysobacter* (Wayne *et al.*, 1987).

Polar lipids of strain THG-YS3.6^T were extracted and examined by two-dimensional TLC with solvent 1 chloroform/methanol/water (65 : 25 : 4, by vol.) and solvent 2 chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) as the developing solvents (Collins *et al.*, 1980; Minnikin, 1984). After being developed in the solvent system, lipids were visualized. For the presence of all lipids, TLC plates were developed by spraying with 5 % molybdophosphoric acid and charred at 120 °C for 5 min. Aminolipids were detected by spraying with 0.2 % ninhydrin at 120 °C for 2 min. Phospholipids were detected by spraying with molybdenum blue reagent (Sigma) at room temperature. Glycolipids were visualized with 2.5 % α-naphthol/sulfuric acid by charring at 120 °C for 2 min. Cellular fatty acids were extracted from cells at the exponential phase of growth and identified by using the Microbial Identification System (Sherlock version 6.1; database: TSSA6; MIDI) according to standard procedures (Sasser, 1990). The isoprenoid quinones of strain THG-YS3.6^T were extracted from freeze-dried cell material (Collins *et al.*, 1977; Minnikin, 1984) and then purified and analysed by HPLC (Hu *et al.*, 2001; Kroppenstedt, 1982).

The polar lipids profile of strain THG-YS3.6^T contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, one unknown phospholipid, one unknown lipid and three unknown aminolipids (Fig. S4). The cellular fatty acid profile of strain THG-YS3.6^T was found to contain iso-$C_{11:0}$,

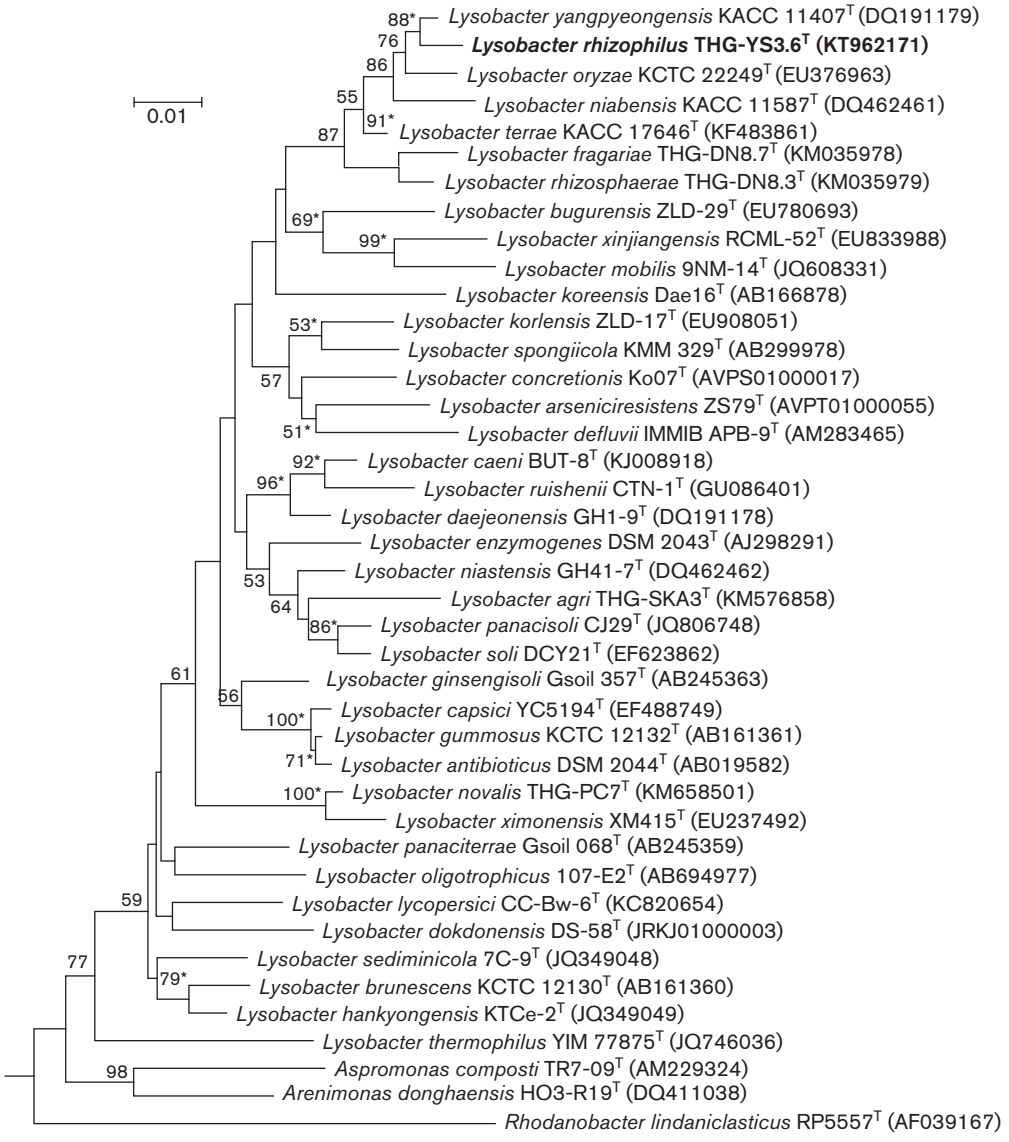

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain THG-YS3.6^T within the genus *Lysobacter*. Asterisks indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony and maximum-likelihood methods. *Rhodanobacter lindaniclasticus* RP5557^T was used as an outgroup. Bootstrap values are shown as percentages of 1000 replicates, only values above 50 % are shown. Bar, 0.01 substitutions per nucleotide position.

iso-$C_{11:0}$ 3-OH, iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{16:1}\omega7c$ alcohol, $C_{16:0}$, iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl) (Table S1). The isoprenoid quinone detected in strain THG-YS3.6^T was ubiquinone-8 (Q-8), one of the typical characteristics of the genus *Lysobacter*.

In summary, the characteristics of strain THG-YS3.6^T were consistent with descriptions of the genus *Lysobacter* with regard to morphological, biochemical and chemotaxonomic properties. The results of the polyphasic approach to determine the taxonomic position of strain THG-YS3.6^T among its closest phylogenetic neighbours indicated that strain THG-YS3.6^T should be assigned to the genus *Lysobacter* as a representative of a novel species, for which the name *Lysobacter rhizophilus* sp. nov. is proposed.

Description of *Lysobacter rhizophilus* sp. nov.

Lysobacter rhizophilus [rhi.zo′phi.lus. Gr. n. *rhiza* root; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos* loving); N.L. masc. adj. *rhizophilus* root-loving].

Cells are Gram-staining-negative, aerobic, motile rods with one polar flagellum (6.1–7.5×1.0–1.3 μm). Growth occurs at 10–37 °C (optimum 25–30 °C), at pH 6.0–8.0 (optimum pH 7.0) and with 0–2.0 % NaCl (optimum 1.0 %). Catalase and oxidase activities are negative. Aesculin, Tween 20 and DNA are hydrolysed but starch, L-tyrosine, casein, Tween 80, cellulose and chitin are not. In API 20NE tests, β-galactosidase, β-glucosidase and gelatin hydrolysis (protease) are positive. In API ZYM tests, positive results are obtained for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, cystine arylamidase, trypsin, α-chymotrypsin, α-fucosidase, α-galactosidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, *N*-acetyl-β-glucosaminidase, β-galactosidase and β-glucosidase. In API GN32 tests, the following compounds are able to be utilized: D-ribose and sodium acetate. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, one unknown phospholipid, one unknown lipid and three unknown aminolipids. The isoprenoid quinone is ubiquinone-8 (Q-8). The major fatty acids are iso-$C_{11:0}$, iso-$C_{11:0}$ 3-OH, iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{16:1}\omega7c$ alcohol, $C_{16:0}$, iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl).

The type strain is THG-YS3.6^T (=KCTC 52082^T=CCTCC AB 2015358^T), isolated from rhizosphere soil of a mugunghwa flower collected from Kyung Hee University, Yongin, South Korea. The DNA G+C content of the type strain is 65.3 mol%.

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