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# *Lysobacter novalis* sp. nov., isolated from fallow farmland soil

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A novel bacterial strain, designated THG-PC7$^T$, was isolated from fallow farmland soil in Yongin, South Korea. Cells of strain THG-PC7$^T$ were Gram-stain-negative, dark yellow, aerobic, rod-shaped and had gliding motility. Strain THG-PC7$^T$ grew optimally at 25–35 °C, at pH 7 and in the absence of NaCl. Comparative 16S rRNA gene sequence analysis identified strain THG-PC7$^T$ as belonging to the genus *Lysobacter*, exhibiting highest sequence similarity with *Lysobacter ximonensis* KCTC 22336$^T$ (98.7 %) followed by *Lysobacter niastensis* KACC 11588$^T$ (95.7 %). In DNA–DNA hybridization tests, DNA relatedness between strain THG-PC7$^T$ and its closest phylogenetic neighbour *L. ximonensis* was below 25 %. The DNA G+C content of the novel isolate was determined to be 62.5 mol%. Flexirubin-type pigments were found to be present. The major cellular fatty acids were determined to be iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and iso-$C_{17:1}\omega9c$. The major respiratory quinone was identified as ubiquonone-8 (Q8). The predominant polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unidentified aminophospolipid. On the basis of results from DNA–DNA hybridization and the polyphasic data, strain THG-PC7$^T$ represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter novalis* sp. nov. is proposed. The type strain is THG-PC7$^T$(=KACC 18276$^T$=CCTCC AB 2014319$^T$).

The genus *Lysobacter* was first proposed by Christensen & Cook (1978), and classified within the family *Xanthomonadaceae*. At the time of writing, following the reclassification of *Lysobacter thermophilus* (Yu *et al.* 2013), the genus *Lysobacter* comprises 26 species with validly published names (http://www.bacterio.net/lysobacter.html). Species of the genus *Lysobacter* are typically found in diverse environmental habitats, especially soil (Liu *et al.*, 2011; Srinivasan *et al.*, 2010; Ten *et al.*, 2009), and are closely related to members of the genera *Xanthomonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Thermomonas*, *Vulcaniibacterium* and *Xylella*. Members of the genus are Gram-negative, rod-shaped, have high G+C contents (61.7–70.7 mol%) and contain ubiquinone 8 (Q-8) as the major respiratory quinone (Christensen & Cook, 1978; Wei *et al.*, 2012; Weon *et al.*, 2006). Members of the genus are reported to lack flagella (Lee *et al.*, 2006; Wei *et al.*, 2012), except for *Lysobacter spongiicola* (Romanenko *et al.*, 2008), *Lysobacter arseniciresistens* (Luo *et al.*, 2012) and *Lysobacter mobilis* (Yang *et al.*, 2015). Typically species of the genus *Lysobacter* have a predominance of iso-branched fatty acids, and diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as the major polar lipids (Luo *et al.*, 2012; Park *et al.* 2008; Romanenko *et al.*, 2008; Wang *et al.*, 2011; Zhang *et al.*, 2011). All species of the genus *Lysobacter* with validly published names show negative results for urease activity and indole production (Ten *et al.*, 2009; Zhang *et al.*, 2011). In this study, we report on the characterization of a novel strain, THG-PC7$^T$, by using a polyphasic approach. On the basis of the results, we propose that it should be placed in the genus *Lysobacter* and named *Lysobacter novalis* sp. nov., with THG-PC7$^T$ as the type strain of this novel species.

†These authors contributed equally to this work.

The GenBank/EMBL/DDB/NCBI GenBank accession number for the 16S rRNA gene sequence of strain THG-PC7$^T$ is KM658501.

Three supplementary figures are available with the online Supplementary Material.

Strain THG-PC7$^T$ was isolated from fallow farmland soil, in Yongin, South Korea by the plating of serial dilutions method on Reasoner's 2A agar (R2A, Difco). Soil (1 g) was suspended in 10 ml of sterile 0.85 % NaCl (w/v). Serial dilutions were prepared up to $10^{-4}$ using NaCl solution. Subsequently, 100 µl of each diluted sample was plated onto the R2A agar five times. The plates were incubated at 28 °C for 7 days. Single colonies were purified by transfer to new R2A agar plates. One isolate, THG-PC7$^T$, was selected for further study. The isolate was routinely cultured on R2A agar at 28 °C and preserved as a suspension in R2A broth (R2B; Difco) with glycerol (25 %, w/v) at −80 °C. Strain THG-PC7$^T$ has been deposited in the Korean Agriculture Culture Collection (KACC 18276$^T$) and China Centre for type Culture Collection (CCTCC AB 2014319$^T$). For comparative study, reference strains, *Lysobacter ximonensis* KCTC 22336$^T$, *Lysobacter niastensis* KACC 11588$^T$ and *Lysobacter enzymogenes* KACC 10127$^T$ (the type species of the genus), were obtained from the Korean Collection for type Cultures and the Korean Agricultural Culture Collection, respectively. These strains were cultured under the optimum conditions for strain THG-PC7$^T$.

The genomic DNA of strain THG-PC7$^T$ was extracted and purified using a commercial Genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified with the universal bacterial primer pair 27F and 1492R (Weisburg *et al.*, 1991) and the purified PCR products were sequenced by Solgent. The identification of phylogenetic neighbours was performed using the EzTaxon-e server (Kim *et al.*, 2012). Seq-Man software version 4.1 (DNASTAR) was used to compile the nearly complete (1453 bp) 16S rRNA gene sequence of strain THG-PC7$^T$. Multiple alignments were performed using the CLUSTAL X program (Thompson *et al.*, 1997) and gaps were edited using the BioEdit program (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura,

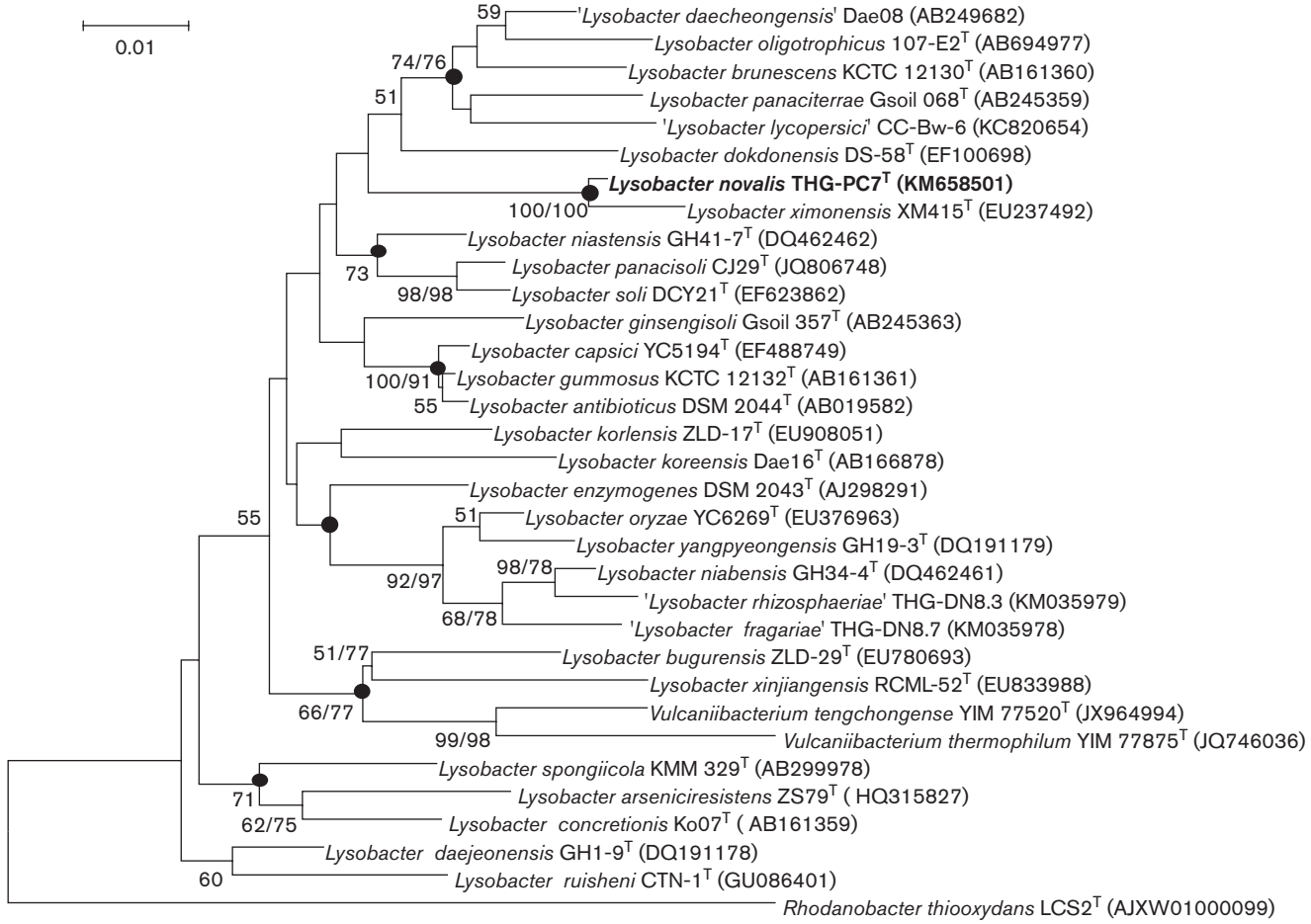


**Fig. 1.** Neighbour-joining tree showing the phylogenetic position of novel species based on 16 S rRNA gene sequences. Evolutionary distances were computed using the Kimura two-parameter distance model. Closed circles represent that the corresponding branches were recovered when the tree was reconstructed using the maximum-parsimony algorithm. Bootstrap values of more than 50 % based on 1000 replications are shown at the branching points. *Rhodanobacter thiooxydans* LCS2$^T$ was used as an out group. Bar, 0.01 substitutions per nucleotide position.

1983). Phylogenetic trees (Fig. 1 and Fig. S1, available in the online Supplementary Material) were reconstructed according to neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods using MEGA version 6 (Tamura *et al.*, 2013). To evaluate the phylogenetic trees, bootstrap analyses with 1000 sample replications were performed.

The 16S rRNA gene sequence of the novel strain determined in this study was a continuous stretch of 1453 bp. According to the EzTaxon-e server, strain THG-PC7$^T$ shared highest sequence similarity with *L. ximonensis* KCTC 22336$^T$ (98.7 %), followed by *L. niastensis* KACC 11588$^T$ (95.7 %). In addition, they also showed low sequence similarities (<96 %) with other species of the family *Xanthomonadaceae*. The phylogenetic tree showed that strain THG-PC7$^T$ is clustered within the genus *Lysobacter*. Strain THG-PC7$^T$ was located in a clade with *L. ximonensis* KCTC 22336$^T$. This clade was also recovered in the trees generated by the maximum-parsimony and maximum-likelihood algorithms with high bootstrap values. These results indicate that strain THG-PC7$^T$ is clearly grouped within the genus *Lysobacter*.

After culturing for 2 days on R2A at 28 °C, the morphology of cells of the novel strain was examined. Suspended cells were placed on carbon- and Formvar-coated nickel grids for 30 s and grids were floated on one drop of 0.1 % (w/v) aqueous uranyl acetate, blotted dry and then viewed (11 000×) with a transmission electron microscope (JEM1010; JEOL) under standard operating conditions (Fig. S2). Gram-staining was determined using a Gram stain kit (bioMérieux) according to the manufacturer's instructions. Cells were grown in R2A broth for 24 h at 28 °C and then tested for gliding motility by the hanging-drop technique (Skerman, 1967). Growth at different temperatures (4, 10, 15, 18, 25, 28, 30, 35, 37, 40 and 42 °C) was assessed after 7 days of incubation on R2A agar. Growth in different media such as nutrient agar (NA; Difco), tryptone soya agar (TSA, Oxoid), Luria–Bertani agar (LB; Oxoid), R2A agar, marine agar (MB; Difco) and MacConkey Agar (Oxoid) was tested at 28 °C for 7 days. Growth under different pH conditions (pH 4.0–10.0, at intervals of 0.5 pH unit) was determined after 4 days of incubation at 28 °C in R2A broth. The following buffers were used to adjust pH values: citric acid/sodium citrate (pH 4.0–6.0), $Na_2HPO_4/NaH_2PO_4$ (pH 6.0–8.0), $Na_2CO_3/NaHCO_3$ (pH 8.0–10.0) and $Na_2HPO_4$/NaOH (pH 10.0) (Gomori, 1955). The pH of the medium was confirmed after autoclaving. Tolerance to salinity was evaluated in R2A broth supplemented with 0–5.0 % (w/v) NaCl (at 0.5 % intervals) after 4 days of incubation at 28 °C. Growth conditions such as pH and salinity were estimated by monitoring the optical density at 600 nm. Anaerobic growth was tested in serum bottles containing R2A broth supplemented with thioglycolate (0.1 %, w/v) in which the air was substituted with $N_2$ gas. Production of flexirubin-type pigments was determined by the reversible colour shift to red, purple or brown when yellow or orange colonies were covered with aqueous 20 % (w/v) KOH solution (Fautz & Reichenbach, 1980). Catalase activity was determined by the production of bubbles from 3 % (v/v) $H_2O_2$ solution mixed with freshly grown cells. Oxidase activity was checked by using 1 % (w/v) *N,N,N,N*-tetramethyl-*p*-phenylenediamine reagent (Sigma) according to the manufacturer's instructions. Nitrate reduction was tested in nitrate broth containing 0.2 % (w/v) $KNO_3$ (Skerman, 1967). Indole production was analysed using Kovacs's reagent in 1 % tryptone broth (Skerman, 1967). Urease activity was evaluated in Christensen's medium (Christensen, 1946). Hydrolysis of the following substrates was tested using R2A agar as the basal medium and was evaluated after 4 days of incubation at 28 °C: casein [2 % (w/v) skimmed milk, Oxoid], 1 % (w/v) starch (Difco), 0.1 % (w/v) aesculin [0.02 % (w/v) ferric citrate, Difco], Tween 80 [0.01 % (w/v) $CaCl_2.2H_2O$] and 1 % (v/v) Tween 80, (Sigma), Tween 20 [0.01 % (w/v) $CaCl_2.2H_2O$ and 1 % (v/v) Tween 20 (Sigma), 1 % (w/v) chitin (Sigma), 0.5 % (w/v) L-tyrosine (Sigma), 12 % (w/v) gelatin (Sigma), 0.1 % (w/v) carboxymethyl-cellulose (CMC,Sigma) and DNA [DNase agar (Scharlau) DNase activity revealed by flooding the plates with 1M HCl]. The assimilation of carbon sources and enzyme activities were determined by using commercial kits according to the manufacturer's instructions. The differential phenotypic characteristics of strain THG-PC7$^T$ and its closest neighbours are shown in Table 1.

For fatty acid analysis, strains were cultured on R2A agar at 28 °C for 48 h. Cells in the exponential growth phase were used. Fatty acids were extracted, methylated and saponified, as described by the Sherlock Microbial Identification system (MIDI) and were analysed by capillary GC (Hewlet Packard 6890) using the Microbial Identification software package with the Sherlock system MIDI 6.1 and the Sherlock Aerobic Bacterial Database (TSBA 6.1, Sasser 1990) (Table 2). For quinone and polar lipid analysis, freeze-dried cells of strain THG-PC7$^T$ and *L. ximonensis* KCTC 22336$^T$ were used. Respiratory quinones were extracted from 300 mg freeze-dried cells with chloroform/methanol (2 : 1, v/v), separated using hexane and eluted with hexane/diethyl ether (90 : 10, v/v), then eluent was evaporated using a rotatory evaporator and dissolved in acetone, according to the method of Collins (1985). Ubiquinone purification was determined by using a reversed-phase HPLC system (Alliance 2690 system, Waters) [wavelength 270 nm; solvent, MeOH/2-propanol (7 : 5, v/v); flow rate, 1.0 ml min$^{-1}$]. The polar lipids of strain THG-PC7$^T$ and the reference strain *L. ximonensis* KCTC 22336$^T$ were extracted and analysed by two-dimensional TLC (Fig. S3). Separately, each sample was spotted on the corner of a two-dimensional thin layer chromatograph using TLC Kiesel gel 60 $F_{254}$ plates (10×10 cm, Merck), and developed in the first dimension with chloroform/methanol/water (65 : 25 : 4, by vol.), and in the second dimension by chloroform/acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.). TLC plates were sprayed with

**Table 1.** Differential characteristics of strain THG-PC7$^T$ and phylogenetically related species of the genus *Lysobacter*

Strains: 1, THG-PC7$^T$; 2. *L. ximonensis* KCTC 22336$^T$; 3. *L. niastensis* KACC 11588$^T$; 4. *L. enzymogenes* KACC 10127$^T$ (type species of the genus *Lysobacter*). All taxa were positive for the following activities: catalase, flexirubin pigments, hydrolysis of L-tyrosine and casein, assimilation of *N*-acetylglucosamine and maltose. All taxa were negative for the following activities: indole production, glucose acidification, hydrolysis of urease, α-mannosidase and for the assimilation of L-arabinose, D-mannitol, gluconate, caprate, adipate and phenylacetate. All taxa were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α- glucosidase. All the data were obtained from this study, except where indicated otherwise. CMC, Carboxymethyl-cellulose; DY, dark yellow; Y, yellow; LB, light beige; +, positive result; −, negative result.

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Colony colour | DY | Y | LB | DY |
| Nitrate reduction | − | − | + | − |
| Arginine dihydrolase | − | − | − | + |
| Oxidase | − | − | + | + |
| Hydrolysis of: | | | | |
| DNA | − | − | − | + |
| Tween 80 | + | + | + | + |
| Tween 20 | + | + | − | − |
| Starch | − | + | + | + |
| CMC | + | − | − | + |
| Chitin | − | − | − | + |
| Gelatin | − | + | + | + |
| Aesculin | − | + | − | + |
| Urea | − | − | − | + |
| Assimilation of: | | | | |
| β-Galactosidase | − | + | + | + |
| D-Glucose | + | + | − | + |
| D-Mannose | + | + | − | + |
| Citrate | − | − | − | + |
| Enzyme activities | | | | |
| Cystine arylamidase | + | + | − | + |
| α-Galactosidase | − | + | − | − |
| β-Galactosidase | + | + | + | − |
| β-Glucuronidase | − | + | − | − |
| β-Glucosidase | − | + | + | − |
| *N*-Acetyl-β-glucosaminidase | + | + | − | + |
| α-Fucosidase | − | + | − | − |
| DNA G+C content (mol%) | 62.5 | 63.5* | 66.6† | 69.0‡ |

*Data from Wang *et al.* (2009).
†Data from Weon *et al.* (2007).
‡Data from Christensen & Cook (1978).

**Table 2.** Cellular fatty acid profile of strain THG-PC7$^T$ and its closest phylogenetic neighbours

Strains: 1, THG-PC7$^T$; 2, *L. ximonensis* KCTC 22336$^T$; 3, *L. niastensis* KACC 11588$^T$; 4, *L. enzymogenes* KACC 10127$^T$ (the type species of the genus *Lysobacter*). For fatty acid analysis all strains were cultured on R2A agar at 28 °C for 48 h. Cells at the exponential growth phase were used for analysis. All the data are from this study. Fatty acids amounting to less than 0.5 % in all strains are not listed. TR, Trace amounts (<1.0 %); ND, not detected.

| Fatty acid | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Straight chain | | | | |
| $C_{10:0}$ | 2.0 | 1.0 | TR | 3.9 |
| $C_{14:0}$ | 1.3 | 2.6 | ND | 1.0 |
| $C_{16:0}$ | 5.9 | 8.4 | 5.5 | 8.6 |
| $C_{18:0}$ | 2.2 | 2.0 | ND | 1.9 |
| Branched chain | | | | |
| iso-$C_{10:0}$ | 1.4 | 1.5 | TR | ND |
| iso-$C_{11:0}$ | 4.8 | 4.2 | 5.2 | 5.1 |
| iso-$C_{14:0}$ | 3.4 | 2.8 | 4.1 | TR |
| anteiso-$C_{11:0}$ | 2.2 | 2.5 | 3.9 | 1.2 |
| iso-$C_{15:0}$ | 13.4 | 13.6 | 19.8 | 15.2 |
| iso-$C_{15:1}$ F | 1.3 | 1.6 | 1.2 | ND |
| anteiso-$C_{15:0}$ | 9.5 | 12.0 | 8.2 | 9.9 |
| iso-$C_{16:0}$ | 18.4 | 16.9 | 15.2 | 20.6 |
| iso-$C_{17:0}$ | 2.3 | 1.5 | 4.8 | 2.7 |
| Unsaturated | | | | |
| iso-$C_{17:1}\omega 9c$ | 11.2 | 7.8 | 10.3 | 9.9 |
| Hydroxyl | | | | |
| $C_{11:0}$2-OH | 3.2 | 5.1 | TR | 1.5 |
| iso-$C_{11:0}$3-OH | 8.6 | 4.9 | 8.7 | 5.5 |
| $C_{16:0}$3-OH | 1.1 | 1.5 | TR | 2.0 |
| Summed feature 3* | 3.3 | 5.6 | 8.9 | 6.2 |

*Summed feature 3 comprises $C_{16:1}\omega 6c$ and/or $C_{16:1}$ ω7*c*.

the following reagents: 5 % (v/v) molybdatophosphoric acid (for total lipids, Sigma), 0.2 % (w/v) ninhydrin (for aminolipids, Sigma) and 2.5 % (v/v) α-naphthol-sulphuric acid (for glycolipids, Sigma). Spraying was followed by heating at 120 °C for 10 min. TLC plates were also sprayed with Molybdenum blue reagent (Sigma) for the detection of phospholipids. No heating step was needed for this reagent (Minnikin *et al.*, 1984).

For determination of the DNA G+C content, genomic DNA was extracted, purified by the method of Moore & Dowhan (1995) and degraded enzymically into nucleosides (nuclease P1 and alkaline phosphatase; Sigma). The mixture of nucleosides obtained was separated using a reversed-phase HPLC system (Alliance 2690 system, Waters) as described previously (Mesbah *et al.*, 1989) with a reversed-phase column SunFireTM C18 (4.6 × 250 mm × 5 μm), at a flow rate of 1.0 ml min$^{-1}$, and a solvent mixture of 200 mM $(NH_4)H_2PO_4$/acetonitrile (97 : 3, v/v) as the mobile phase, and detection at a wavelength of 270 nm. The genomic DNA of *Escherichia coli* strain B (Sigma-Aldrich D4889) was used as standard.

DNA–DNA hybridization was 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. DNA–DNA hybridization experiments were performed for strain THG-PC7$^T$ and its closely related reference strain *L. ximonensis* KCTC 22336$^T$. The optimum renaturation temperature (43 °C) was calculated as [(0.51x G+C content) + 47] – 36 (Gillis *et al.*, 1970), where 36 °C is the correction for the presence of 50 % formamide (McConaughy *et al.*, 1969). 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 relatedness values.

The DNA G+C content of strain THG-PC7$^T$ was 62.5 mol%. The DNA–DNA relatedness value between strain THG-PC7$^T$ and *L. ximonensis* KCTC 22336$^T$ was 22.5±0.5 %, respectively. The DNA–DNA relatedness values were significantly lower than the threshold value of 70 % recommended for recognition of separate species (Wayne *et al.*, 1987). On the basis of DNA–DNA relatedness results, it is evident that the novel strain should belong to the genus *Lysobacter*.

### Description of Lysobacter *novalis* sp. nov.

Lysobacter *novalis* (no.va'lis. L. gen. n. *novalis* of fallow land)

Cells are Gram-stain-negative, aerobic, gliding and rod-shaped. Cell size is approximately 1.0–1.5 μm long × 0.5–0.8 μm wide. Colonies on R2A agar are yellow, round, smooth, convex and have a diameter of 1–2 mm. Cells grow at 18–37 °C (optimum 25–35 °C), at pH 6.0–7.5 (optimum pH 7.0) and at 0–1.0 % (w/v) NaCl (optimum 0 % NaCl). Catalase-positive and oxidase-negative. Flexirubin-type pigments are present. Growth occurs on R2A, TSA and NA, but not on LB, MA and MacConkey agar. Positive for the hydrolysis of Tween 20, Tween 80, L-tyrosine, casein, and CMC, but negative for the hydrolysis of starch, chitin, DNA, urea, gelatin and aesculin. Tests for nitrate reduction and indole production are negative. The following compounds are utilized as the sole carbon source: D-glucose, D-mannose, *N*-acetylglucosamine and maltose; but not L-arabinose, D-mannitol, gluconate, caprate, adipate, malate, citrate and phenyl-acetate. Negative for glucose acidification and fermentation of arginine hydrolysis. Positive for the following enzyme activities: alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and *N*-acetyl-β-glucosaminidase. Negative for the following enzyme activities: α-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. The major isoprenoid quinone is ubiquinone-8. The predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unidentified aminophospolipid. The major cellular fatty acids (>10 %) are iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and iso-$C_{17:1}\omega 9c$.

The type strain, THG-PC7$^T$ (=KACC 18276$^T$=CCTCC AB 2014319$^T$), was isolated from fallow farmland soil in Yongin, South Korea. The DNA G+C content of the type strain is 62.5 mol%.

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