

## *Lysobacter tyrosinolyticus* sp. nov. isolated from Gyeryongsan national park soil<sup>§</sup>

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A novel Gram-negative, rod-shaped (0.2–0.5  $\mu\text{m} \times 1.5\text{--}2.5 \mu\text{m}$ ), aerobic, non-motile bacterium was isolated from Gyeryongsan national park soil, Republic of Korea. The novel isolate was designated as THG-DN8.2<sup>T</sup>. The strain grows optimally at 28°C, at pH 7 and in the absence of NaCl. Phylogenetic analysis based on 16S rRNA gene sequence showed that the novel isolate shared the highest sequence similarity with *Lysobacter oryzae* KCTC 22249<sup>T</sup> followed by *Lysobacter yangpyeongensis* KACC 11407<sup>T</sup> and *Lysobacter niabensis* KACC 11587<sup>T</sup>. The DNA G+C content of strain THG-DN8.2<sup>T</sup> is 66.0 mol% and ubiquinone Q-8 is the main isoprenoid quinone. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and phosphatidyl-N-methylethanolamine. The major fatty acids of strain THG-DN8.2<sup>T</sup> were identified as iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, and C<sub>16:1</sub> $\omega$ 7c alcohol. The phylogenetic distinctiveness and phenotypic characteristics differentiated strain THG-DN8.2<sup>T</sup> from closely related *Lysobacter* species. The results of polyphasic taxonomic analysis suggest that strain THG-DN8.2<sup>T</sup> represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter tyrosinolyticus* sp. nov. is proposed. The type strain is THG-DN8.2<sup>T</sup> (=KCTC 42235<sup>T</sup> =JCM 30320<sup>T</sup>).

**Keywords:** *Lysobacter tyrosinolyticus*, Gram-negative, Ubiquinone Q-8, 16S rRNA

### Introduction

The genus *Lysobacter* was first proposed by Christensen and Cook (1978), with *Lysobacter enzymogenes* as the type species. The genus *Lysobacter* belongs to the family *Xanthomonadaceae*

within the phylum *Proteobacteria*. At the time of writing, the genus *Lysobacter* contains 26 validly published names (<http://www.bacterio.net/lysobacter.html>). The species of the genus *Lysobacter* are closely related to members of the genera *Xanthomonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Thermomonas*, *Vulcaniibacterium*, and *Xylella*, containing ubiquinone Q-8 as the major respiratory quinone (Wang *et al.*, 2009). Members of the genus have high G+C content (61.7–70.7%) and most members lack flagella (Lee *et al.*, 2006; Wei *et al.*, 2012) except *Lysobacter spongiicola* (Romanenko *et al.*, 2008), *Lysobacter arseniciresistens* (Luo *et al.*, 2012) and *Lysobacter mobilis* (Yang *et al.*, 2015). The typical characteristics of the genus *Lysobacter* were Gram-negative, rod-shaped, predominance of iso-branched fatty acids, and diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG) as the major polar lipids (Park *et al.*, 2008; Romanenko *et al.*, 2008; Wang *et al.*, 2011; Zhang *et al.*, 2011). All published species of *Lysobacter* showed negative results for urease activity and indole production (Ten *et al.*, 2009; Zhang *et al.*, 2011). *Lysobacter* species were typically found in soil and water habitats (Ten *et al.*, 2009; Srinivasan *et al.*, 2010; Liu *et al.*, 2011). Some members of the genus have the ability to be used as biocontrol agent (Ahmed *et al.*, 2003; Folman *et al.*, 2004; Kilic-Ekici and Yuen, 2004). Members of the genus are strongly proteolytic and characteristically lyse a variety of micro-organisms (both Gram-negative and Gram-positive bacteria), as well as nematodes (Yassin *et al.*, 2007), suggesting that they have a particular biological function in microbial ecosystems. Some *Lysobacter* strains could also be used to control fungal pathogens through various mechanisms, such as production of chitinases and  $\beta$ -1,3-glucanases (Zhang and Yuen, 2000b; Zhang *et al.*, 2001; Palumbo *et al.*, 2003), antibiotics (Zhang and Yuen, 2000a; Islam *et al.*, 2005) or induction of systemic resistance (Kilic-Ekici and Yuen, 2004). The aim of the present study was to determine the exact taxonomic position of strains THG-DN8.2<sup>T</sup> by means of polyphasic approaches. On the basis of chemotaxonomic and physiological data we described the new isolate THG-DN8.2<sup>T</sup> belonging to the genus *Lysobacter*. The phenotypic and genotypic characterization of the novel strain is described in this report.

### Materials and Methods

#### Isolation and culture condition

Soil sample was collected from Gyeryongsan national park, South Korea. Cells were isolated by serial dilution using Reasoner's 2A agar (R2A; Difco). One gram soil sample was

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suspended in 10 ml of 0.85% (w/v) saline solution, vortexed, serially diluted, and spread on R2A agar plates. The plates were incubated at 28°C for one week. Single colonies were purified by subculturing R2A agar plates incubating under the same condition. One isolate, designated THG-DN8.2<sup>T</sup> was selected for further study. Firstly, the isolate was routinely cultured on R2A agar at 28°C and stored as glycerol suspension 25% (v/v) at -80°C. Strain THG-DN8.2<sup>T</sup> has been deposited in Korean Collection for Type Cultures (KCTC 42229<sup>T</sup>) and Japan Collection of Microorganisms (JCM 30320<sup>T</sup>). Reference strains *Lysobacter oryzae* KCTC 22249<sup>T</sup>, *Lysobacter yangpyeongensis* KACC 11407<sup>T</sup>, and *Lysobacter niabensis* KACC 11587<sup>T</sup> were obtained from Korean Collection for Type Cultures (KCTC) and Korean Agricultural Culture Collection (KACC). These reference strains were cultured as same as optimum conditions of strain THG-DN8.2<sup>T</sup>.

### 16S rRNA and phylogenetic construction

Genomic DNA 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 (5'-TACCAGGGTATCTAATCC-3') and 1492R (5'-G GTTACCTTGTACGACTT-3') (Weisburg *et al.*, 1991) and the purified PCR products were sequenced by Solgent Co. Ltd. The 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 nearly complete (1,447 bp) 16S rRNA sequence of strain THG-DN8.2<sup>T</sup> was compiled using Seq-Man software version 4.1 (DNASTAR, Inc.). The 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's two-parameter model (Kimura, 1983). The phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood methods (Felsenstein, 1981) in the MEGA 6 program package (Tamura *et al.*, 2013). The tree topology was estimated by bootstrap analysis with 1,000 resampling of datasets (Felsenstein, 1985).

### Morphological and physiological characterization

The morphological, physiological and biochemical characteristics of strain THG-DN8.2<sup>T</sup> were investigated after 3 days on R2A agar at 28°C. Gram-staining was determined using a bioMérieux (France) Gram stain Kit according to the manufacturer's instruction. Cell morphologies of novel strain were examined using transmission electron microscope. Bacterial strain was cultured in R2A broth and cells suspension were placed on carbon- and formvar-coated nickel grids for 30 sec and grids were floated on one drop of 0.1% (w/v) aqueous uranyl acetate, blotted dry and then viewed with a transmission electron microscope (Model JEM1010; JEOL) at 11,000 × magnification under standard operating conditions. 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 test was performed on R2A agar, tryptone soya agar (TSA; Oxoid), Nutrient agar (NA; Oxoid), Luria Bertani agar (LA; Oxoid), Marine agar (MB; Difco) and

MacConkey Agar (Oxoid) and after incubation at 28°C for 7 days. The temperature range of strain THG-DN8.2<sup>T</sup> was determined on R2A agar and cultures were incubated at different temperatures 4, 10, 15, 18, 25, 28, 30, 35, 37, and 42°C. The pH range was determined using different pH conditions (pH 4.0–10.0, at intervals of 0.5 pH units) in R2A broth for 4 days at 28°C. The following pH buffers were used to set pH values: acetic acid was used for pH 4.0–4.5, acetate buffer was used for pH 5.0–6.5 and phosphate buffer was used for pH 7.0–10.0. pH of R2A broth was confirmed after autoclaving. The salt tolerance was tested with 0 to 5% (w/v) NaCl (at 0.5% intervals) in R2A broth after 4 days at 28°C. Growth was estimated by monitoring the optical density at 600 nm. Anaerobic growth was tested in serum bottles containing R2A broth supplemented with thioglycolate (0.1%) and in which the air was substituted with nitrogen gas. Production of flexirubin-type pigments was determined by the reversible color shift to red, purple or brown when yellow or orange colonies are covered with aqueous 20% KOH solution (Fautz and Reichenbach, 1980). Methyl red and Voges-Proskauer reaction were tested in clark-Lubs' medium (Scharlau). Catalase activity was determined by the production of bubble from 3% (v/v) H<sub>2</sub>O<sub>2</sub> solution mixed with freshly grown cells and oxidase activity was determined by using of 1% (w/v) *N,N,N,N*-tetramethyl-*p*-phenylenediamine reagent (Sigma) according to the manufacturer's instructions. Hydrolysis of following substrate were performed on R2A agar containing: casein (2% skim milk, Oxoid), starch (1%, Difco), esculin (Bile Esculin agar, Difco), Tween 80 (0.01% CaCl<sub>2</sub>·2H<sub>2</sub>O and 1% Tween 80, Sigma), Tween 20 (0.01% CaCl<sub>2</sub>·2H<sub>2</sub>O and 1% Tween 20, Sigma), chitin (1%, Sigma), L-tyrosine (0.5%, Sigma), carboxymethyl-cellulose (CMC) (0.1%, Sigma) and DNA (DNase agar, Scharlau, DNase activity revealed by flooding the plates with 1 N HCl) were evaluated after 4 days of incubation at 28°C. Nitrate reduction was tested in nitrate broth containing 0.2 % KNO<sub>3</sub> (Skerman, 1967). Indole production was analyzed using Kovács's reagent in 1% tryptone broth (Skerman, 1967). Urease activity was evaluated in Christensen's medium (Christensen, 1946). The substrate utilization profile and enzyme activity for novel isolate and all reference strains were determined using API 20NE and API ZYM strips, according to the manufacturer's instructions (bioMérieux). API 20NE were recorded after incubation for 48 h, under the optimal conditions for each strain while API ZYM was recorded after incubation for 10 h.

### DNA G+C mol% content

For determination of the DNA G+C mol%, genomic DNA of strain THG-DN8.2<sup>T</sup> was extracted and purified by the method described by Moore and Dowhan (1995). Ten microliters volume of solution containing ten microgram DNA was heated in a boiling water bath for 5 min and then cooled in ice-water bath. The denatured DNA solution was mixed with Ten microliters of nuclease P1 solution (100 U/ml, Sigma), and incubated at 37°C for 1 h. Ten microliters of alkaline phosphatase (1,000 U/ml, Sigma) were added to the sample to remove the phosphate group from purified sample, and the mixture was incubated for 3 h at 37°C. The obtained nucleosides were analyzed using a reverse-phase HPLC sys-

tem (Alliance 2690 system, Waters) as described previously (Mesbah *et al.*, 1989) with reversed-phase column SunFire™ C18 (4.6 × 250 mm × 5 µm), flow rate of 1.0 ml/min, solvent mixture of 200 mM (NH<sub>4</sub>)H<sub>2</sub>PO<sub>4</sub>/acetonitrile (97 : 3, v/v) as mobile phase, and wavelength at 270 nm. The genomic DNA of *Escherichia coli* strain B (Sigma-Aldrich D4889) was used as a standard.

### Chemotaxonomic characterization

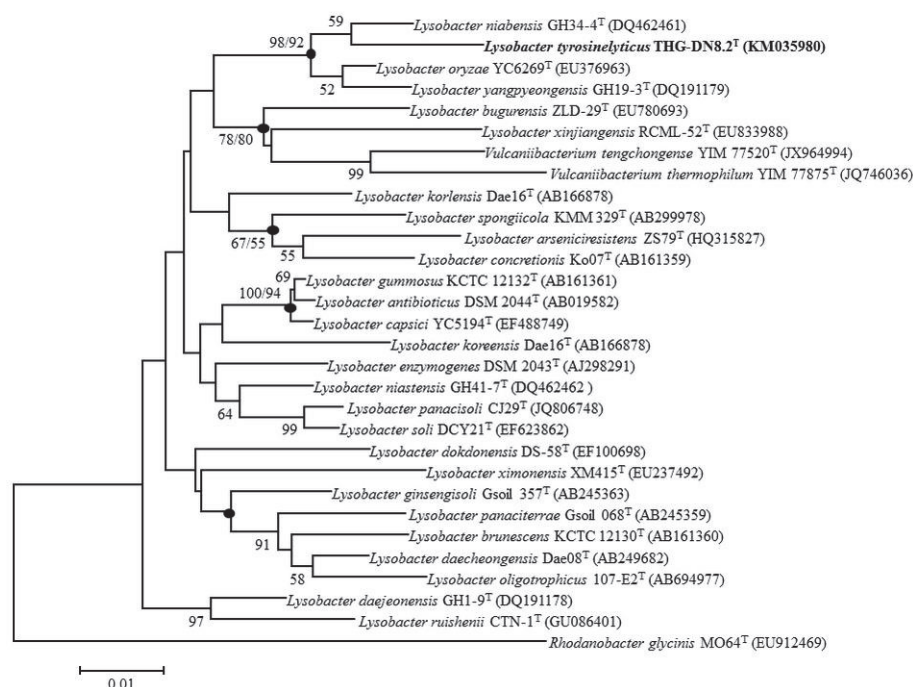
For fatty acid analysis, strain THG-DN8.2<sup>T</sup> and all three reference strains were cultured on R2A agar at 28°C for 48 h. Biomass from third quadrant of each plate were collected. Fatty acids were extracted, methylated and saponified by method described by Sasser (1990) and analyzed by the Sherlock Microbial Identification system (MIDI) with GC (Hewlett Packard 6890).

For quinone and polar lipids analyses, strain THG-DN8.2<sup>T</sup> and *L. oryzae* KCTC 22249<sup>T</sup> were grown in R2A broth at 28°C, shaken at 180 rpm for two days, centrifuged and freeze dried. Isoprenoid quinones were extracted from 300 mg freeze-dried cells with chloroform: methanol (2:1, v/v), separated by using hexane and eluted with hexane: diethyl ether (90:10, v/v), then eluent was evaporated by rotatory evaporator dissolved in acetone. Ubiquinone purification was subsequently analyzed by a RP-HPLC system (Alliance 2690 system, Waters) [solvent; methanol: 2-propanol (7:5, v/v), flow rate; 1.0 ml/min] as previously described (Collins and Jones, 1981; Tamaoka *et al.*, 1983; Hiraishi *et al.*, 1996). Polar lipids of strain THG-DN8.2<sup>T</sup> and *L. oryzae* KCTC 22249<sup>T</sup> were analyzed as described by Minnikin *et al.* (1984). Two-dimensional thin layer chromatography (2D-TLC) performed using TLC Kiesel gel 60 F<sub>254</sub> plates (10 × 10 cm, Merck), plates were developed in the first direction using chloroform: methanol: water (65:25:4, v/v/v) followed by

the second development of chloroform: methanol: acetic acid: water (80:12:15:4, v/v/v/v). Separately, each sample was spotted on the corner of the plates. For the presence of total and specific lipids, the plates developed in the solvent system were done using a spray of 5% molybdatophosphoric acid (total lipids, Sigma), 0.2% ninhydrin (aminolipids, Sigma), and 2.5 % α-naphthol-sulfuric acid (glycolipids, Sigma) followed by drying at 120°C for 5–10 min. TLC plates also sprayed with molybdenum blue reagent (Sigma) for detecting phospholipids. No heating step needed for this reagent.

### Results and Discussion

A phylogenetic analysis based on 16S rRNA sequence revealed that strain THG-DN8.2<sup>T</sup> fell within the genus *Lysobacter* and family *Xanthomonadaceae*. The highest sequence similarity was *L. oryzae* KCTC 22249<sup>T</sup> (96.7%) followed by *L. yangpyeongensis* KACC 11407<sup>T</sup> (96.5%), *L. niabensis* KACC 11587<sup>T</sup> (96.5%). Strain THG-DN8.2<sup>T</sup> also shows less than 96% similarity with other members of the family *Xanthomonadaceae*. Neighbor-joining phylogenetic tree shows the position of strain THG-DN8.2<sup>T</sup> is clustered within the genus *Lysobacter* (Fig. 1). The maximum-parsimony tree also supported the clustering of strain THG-DN8.2<sup>T</sup> shown in neighbor-joining tree. Other phylogenetic tree built using maximum-likelihood method also available on Supplementary data Fig. S1. Strain THG-DN8.2<sup>T</sup> formed the biphyletic cluster with the genus *Vulcaniibacterium*. The genus *Vulcaniibacterium* has been recently described by (Yu *et al.*, 2013) following the reclassification of *Lysobacter thermophilus* as *Vulcaniibacterium thermophilum*. Both genus *Lysobacter* and *Vulcaniibacterium* are very closely related to each other and which is also evident from the tree. As both species of the genus *Vulcaniibacterium* formed a monophyletic cluster with



**Fig. 1.** The neighbor-joining tree based on 16S rRNA gene sequence analysis, showing relationships between strain THG-DN8.2<sup>T</sup> and members of the genus *Lysobacter*. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Numbers at nodes indicate bootstrap percentages (based on 1,000 resampled datasets). Bootstrap values less than 50% were not indicated. *Rhodanobacter glycinis* NBRC 105007<sup>T</sup> was used as an out group. Scale bar, 0.01 substitutions per nucleotide position.



**Table 1. Differential characteristics of strain THG-DN8.2<sup>T</sup> and related type strains**

Strains: 1, THG-DN8.2<sup>T</sup>; 2, *Lysobacter oryzae* KCTC 22249<sup>T</sup>; 3, *Lysobacter yangpyeongensis* KACC 11407<sup>T</sup>; 4, *Lysobacter niabensis* KACC 11587<sup>T</sup>. All strains are positive for following characteristics: hydrolysis of Tween 80, L-Tyrosine, Casein and gelatin. All strains are negative for following characteristics: nitrate reduction, indole production, glucose acidification and arginine dihydrolase; hydrolysis of chitin, urea and esculin. In API 20 NE strips, all strains were negative for the assimilation of all the substrates. In API ZYM strips, all strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and Naphtol-AS-BI-phosphohydrolase but negative for  $\beta$ -glucuronidase and  $\alpha$ -fucosidase. All data were obtained from this study except the DNA G+C content of the reference strains: <sup>a</sup> Aslam *et al.* (2009), <sup>b</sup> data from Weon *et al.* (2006) and <sup>c</sup> data from Weon *et al.* (2007).

Characteristics	1	2	3	4
Color	pale-yellow	yellow	yellow	yellow
Aerobic/Facultative anaerobic	aerobic	facultative anaerobic	aerobic	aerobic
Motility (gliding)	-	+	+	-
Hydrolysis of:				
Tween 20	+	-	-	+
Starch	+	-	+	+
CMC	-	+	-	-
DNA	+	-	+	-
Enzyme activity:				
Lipase (C14)	+	-	-	+
Cystine arylamidase	+	-	+	+
Trypsin	w	+	+	+
$\alpha$ -Chymotrypsin	-	+	+	-
$\alpha$ -Galactosidase	-	-	-	+
$\beta$ -Galactosidase	-	-	-	+
$\alpha$ -Glucosidase	-	+	+	+
$\beta$ -Glucosidase	-	-	-	+
N-acetyl- $\beta$ -glucosaminidase	+	-	+	w
$\alpha$ -Mannosidase	-	-	-	+
DNA G+C content (mol%)	66.0	67.4 <sup>a</sup>	67.3 <sup>b</sup>	62.5 <sup>c</sup>

+, Positive; w, weakly positive; -, negative.

each other. The phylogenetic data of novel isolate indicated that strain THG-DN8.2<sup>T</sup> represents a novel species of the genus *Lysobacter*.

Phenotypic analysis showed that strain THG-DN8.2<sup>T</sup> cells are Gram-stain-negative, non-motile (does not show gliding motility either), aerobic and rod shaped with size range approximately 0.2–0.5  $\mu\text{m}$   $\times$  1.5–2.5  $\mu\text{m}$  (Supplementary data Fig. S2). Colonies on R2A agar were pale-yellow, circular, convex with entire margin and with a diameter of 2–3 mm. Novel isolate grows well on R2A and NA but not on TSA, MA, LA, and MacConkey agar. Growth occurred at temperature 18–28°C, at pH 6.0–8.0 and in the presence of 0–2.0% (w/v) NaCl. Optimum growth occurs at 28°C, at pH 7.0 and in absence of NaCl. The novel isolate was able to hydrolyze Tween 80, Tween 20, L-tyrosine, starch, casein, gelatin, and DNA but not CMC, esculin, urea, and chitin. Positive for oxidase and catalase activity but negative for nitrate reduction and indole production. The results of the phenotypical and biochemical properties also suggested that the novel isolate represents a novel species belonging to the genus *Lysobacter*. The results of biochemical and physiological tests of novel isolate and closely related type strains were shown on Table 1.

The DNA G+C content of strain THG-DN8.2<sup>T</sup> was 66.0 mol%, which is consistent with the members of the genus *Lysobacter* known with high G+C content range 61.7–70.7 mol% (Lee *et al.*, 2006; Wei *et al.*, 2012). Novel isolate contains ubiquinone Q-8 as the predominant isoprenoid quinone which is in line with all other recognized members of

**Table 2. Cellular fatty acid profiles of strain THG-DN8.2<sup>T</sup> and reference strains of the genus *Lysobacter***

Strains: 1, THG-DN8.2<sup>T</sup>; 2, *Lysobacter oryzae* KCTC 22249<sup>T</sup>; 3, *Lysobacter yangpyeongensis* KACC 11407<sup>T</sup>; 4, *Lysobacter niabensis* KACC 11587<sup>T</sup>. For fatty acid analysis, novel isolate and all reference strains were cultured on R2A at 28°C for 48 h and then cells were harvested used for analysis. All data were obtained from this study. Summed feature 9\* could not be separated by the Microbial Identification System (MIDI) and consisted of iso-C<sub>17:1</sub> $\omega$ 9c and/or C<sub>16:0</sub> 10-methyl. Fatty acids amounting to less than 1.0% in all strains were not listed. ND, not detected; Tr, trace amount (<0.5%).

Fatty acid	1	2	3	4
Straight				
C <sub>16:0</sub>	5.5	6.9	4.1	11.8
C <sub>18:00</sub>	2.7	2.0	3.9	3.8
Branched				
iso-C <sub>10:0</sub>	1.5	Tr	Tr	ND
iso-C <sub>11:0</sub>	3.9	5.2	3.2	1.3
iso-C <sub>12:0</sub>	1.9	ND	1	ND
iso-C <sub>14:0</sub>	2.9	1.7	1.5	1.4
iso-C <sub>15:0</sub>	10.0	14.7	12.9	13.5
anteiso-C <sub>15:0</sub>	2.8	2.9	4.2	33.9
iso-C <sub>16:0</sub>	29.4	17.3	23.5	22.9
iso-C <sub>18:0</sub>	1.3	Tr	Tr	Tr
Hydroxy				
iso-C <sub>11:0</sub> 3OH	5.9	6.6	5.2	1.8
Unsaturated				
iso-C <sub>15:1</sub> $\omega$ 9c	2.9	2.3	3.2	ND
C <sub>16:1</sub> $\omega$ 7alcohol	11.8	3.5	7.9	Tr
anteiso-C <sub>17:1</sub> $\omega$ 9c	1.4	Tr	Tr	Tr
Summed Feature 9*	6.0	16.5	5.7	5.1

the genus *Lysobacter* (Wang *et al.*, 2009). The major polar lipids detected were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidyl-*N*-methylethanolamine (PME). The polar lipid profile of strain THG-DN8.2<sup>T</sup> and closest reference strain *L. oryzae* KCTC 22249<sup>T</sup> were shown in Supplementary data Fig. S3. In addition, some unidentified polar lipids (PL1-2) were also detected. Thus, the polar lipid results of novel isolate shows predominance of similar polar lipids with the genus *Lysobacter* (Park *et al.*, 2008; Romanenko *et al.*, 2008; Wang *et al.*, 2011; Zhang *et al.*, 2011). The main fatty acids of strain THG-DN8.2<sup>T</sup> were iso-C<sub>15:0</sub> (10.0%), iso-C<sub>16:0</sub> (29.4%), and C<sub>16:1</sub>ω7c alcohol (11.8%). Our results are similar to the previously described cellular fatty acids profile of other members of *Lysobacter* genus which is known to contain iso-branched chain fatty acids as major fatty acids. However, strain THG-DN8.2<sup>T</sup> also contains unsaturated C<sub>16:1</sub>ω7c alcohol (11.8%) as one of the major fatty acids which were not observed in the reference species. The cellular fatty acid profile of strain THG-DN8.2<sup>T</sup> and the nearest reference strains were shown on Table 2.

On the basis of the polyphasic taxonomy data obtained in this study including phylogenetic, phenotypic, biochemical and chemotaxonomic properties, strains THG-DN8.2<sup>T</sup> (=KCTC 42235<sup>T</sup> =JCM 30320<sup>T</sup>) is considered to represent a novel species of the genus *Lysobacter*, for which name *Lysobacter tyrosinolyticus* sp. nov. is proposed.

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

*Lysobacter tyrosinolyticus* (ty.ro.sine.ly'tic.us. M. L. n. tyrosine; M. L. adj. lyticus, dissolving; M. L. adj. tyrosinolyticus, decomposing tyrosine) is Gram-negative, rod-shaped, aerobic and non-motile bacterium. Cells size is approximately 0.2–0.5 μm in width and 1.5–2.5 μm in length. On R2A agar, colonies are pale-yellow, circular, convex with entire margin and with a diameter of 2–3 mm. Positive for oxidase and catalase test but negative for nitrate reduction and indole production. Anaerobic growth does not occur. Strain THG-DN8.2<sup>T</sup> grows on R2A agar and NA but not on TSA, LA, MA, and MacConkey agar. Growth occurs at 18–28°C and optimum is 28°C. Growth occurs at pH 6.0–8.0 and optimum is pH 7.0. Optimal growth occurs in absence of NaCl, but could tolerate up to 2.0% NaCl (w/v). Tween 80, Tween 20, L-tyrosine, starch, casein, gelatin and DNA are hydrolyzed but CMC, esculin, urea and chitin are not. Flexirubin-type pigments are absent. Negative for MR-VP test and assimilation of the following substrates in API 20 NE tests: D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Positive for following enzyme activities alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, leucine arylamidase, acid phosphatase, Naphtol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase and weakly positive for trypsin but negative for α-chymotrypsin, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase β-glucuronidase, α-mannosidase, and α-fucosidase. The predominant isoprenoid quinone is ubiquinone Q-8. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine

and phosphatidyl-*N*-methylethanolamine. The main cellular fatty acids contents are iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, and C<sub>16:1</sub>ω7c alcohol. The DNA G+C content of the type strain, THG-DN8.2<sup>T</sup> is 66.0 mol%.

The type strain is THG-DN8.2<sup>T</sup> (=KCTC 42235<sup>T</sup> =JCM 30320<sup>T</sup>), which was isolated from Gyeryongsan national park soil, South Korea.

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