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

### Juan Du<sup>1†</sup>, Hina Singh<sup>1†</sup>, Hien T.T. Ngo<sup>1</sup>, KyungHwa Won<sup>1</sup>, Ki-Young Kim<sup>2</sup>, and Tae-Hoo Yi<sup>1\*</sup>

<sup>1</sup>Department of Oriental Medicine Biotechnology, College of Life Science, Kyung Hee University Global Campus, Gyeonggi-do 446-701, Republic of Korea

<sup>2</sup>Department of Genetic Engineering, College of Life Science, Kyung Hee University Global Campus, Gyeonggi-do 446-701, Republic of Korea

(Received Dec 23, 2014 / Revised Mar 30, 2015 / Accepted Apr 13, 2015)

A novel Gram-negative, rod-shaped (0.2–0.5  $\mu$ m × 1.5–2.5 um), 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- $C_{15:0}$ , iso- $C_{16:0}$ , and  $C_{16:1}\omega7c$  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>1</sup> represents a novel species of the genus Lysobacter, for which the name Lysobacter tyrosinelyticus sp. nov. is proposed. The type strain is THG-DN8.2<sup>1</sup> (=KCTC  $42235^{T} = JCM \ 30320^{T}$ ).

*Keywords: Lysobacter tyrosinelyticus*, 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 *Xanthomona*-

daceae 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 Gramnegative, 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 controls 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

<sup>&</sup>lt;sup>†</sup>These authors equally contributed to this work.

<sup>\*</sup>For correspondence. E-mail: drhoo@khu.ac.kr; Tel.: +82-31-201-2609; Fax: +82-31-206-2537

<sup>&</sup>lt;sup>§</sup>Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

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>1</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>1</sup> has been deposited in Korean Collection for Type Cultures (KCTC 42229<sup>1</sup>) and Japan Collection of Microorganisms (JCM 30320<sup>1</sup>). Reference strains Lysobacter oryzae KCTC 22249<sup>1</sup>, Lysobacter yangpyeongensis KACC 11407<sup>1</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 GTTACCTTGTTACGACTT-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>1</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 SunFireTM C18 ( $4.6 \times 250 \text{ mm} \times 5 \mu \text{m}$ ), 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 (Hewlet 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  $22249^{T}$  were analyzed as described by Minnikin *et al.* (1984). Two-dimensional thin layer chromatography (2<sub>D</sub>-TLC) performed using TLC Kiesel gel 60 F254 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%  $\alpha$ -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

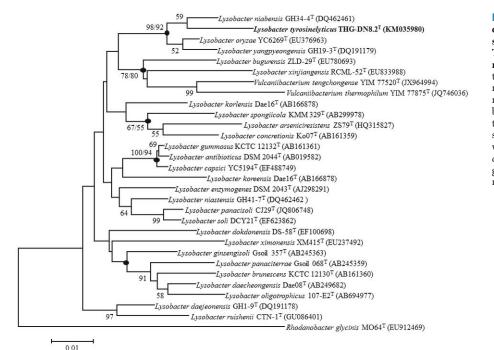


Fig. 1. The neighbor-joining tree based on 16S rRNA gene sequence analysis, showing relationships between strain THG-DN8.2<sup>T</sup> and members of the genus Lysobacter. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. 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, value arylamidase, acid phosphatase and Naphtol-*AS-BI*-phosphohydrolase but negative for  $\beta$ -glucuronidase and *a*-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.* (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	+	+	+
a-Chymotrypsin	-	+	+	-
α-Galactosidase	-	-	-	+
$\beta$ -Galactosidase	-	-	-	+
α-Glucosidase	-	+	+	+
$\beta$ -Glucosidase	-	-	-	+
N-acetyl- $\beta$ -glucosaminidase	+	-	+	W
α-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  $\mu$ m × 1.5–2.5  $\mu$ 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- $C_{17:1}$  w9c and/or  $C_{16:0}$  10-methyl. Fatty acids amounting to less than 1.0% in all strains were not listed. ND, not detected; Tr, trace amount (<0.5%).

(<0.570).				
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>15:1ω</sub> 9c	2.9	2.3	3.2	ND
C <sub>16:1</sub> w7calcohol	11.8	3.5	7.9	Tr
anteiso-C <sub>17:1</sub> ω9c	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  $C_{16:1\omega}7c$  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 isobranched chain fatty acids as major fatty acids. However, strain THG-DN8.2<sup>T</sup> also contains unsaturated  $C_{16:1}\omega7c$  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  $42235^{T}$  =JCM  $30320^{T}$ ) is considered to represents a novel species of the genus *Lysobacter*, for which name *Lysobacter tyrosinelyticus* sp. nov. is proposed.

#### Description of Lysobacter tyrosinelyticus sp. nov

Lysobacter tyrosinelyticus (ty.ro.sine.ly'tic.us. M. L. n. tyrosine; M. L. adj. lyticus, dissolving; M. L. adj. tyrosinelyticus, 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 Mac-Conkey 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  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase  $\beta$ -glucuronidase,  $\alpha$ -mannosidase, and  $\alpha$ -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- $C_{15:0}$ , iso- $C_{16:0}$ , and  $C_{16:1}\omega7c$  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  $42235^{T}$  =JCM  $30320^{T}$ ), which was isolated from Gyeryongsan national park soil, South Korea.

#### References

- Ahmed, K., Chohnan, S., Ohashi, H., Hirata, T., Masaki, T., and Sakiyama, F. 2003. Purification, bacteriolytic activity, and specificity of b-lytic protease from *Lysobacter* sp. IB-9374. *J. Biosci. Bioeng.* 95, 27–34.
- Aslam, Ž., Yasir, M., Jeon, C.O., and Chung, Y.R. 2009. Lysobacter oryzae sp. nov., isolated from the rhizosphere of rice (Oryza sativa L.). Int. J. Syst. Evol. Microbiol. 59, 675–680.
- **Christensen, W.B.** 1946. Urea decomposition as a means of differentiating proteus and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J. Bacteriol.* **52**, 461–466.
- Christensen, P. and Cook, F.D. 1978. Lysobacter, a new genus of nonfruiting, gliding bacteria with a high base ratio. Int. J. Syst. Bacteriol. 28, 367–393.
- Collins, M.D. and Jones, D. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol. Rev.* 45, 316–354.
- Fautz, E. and Reichenbach, H. 1980. A simple test for flexirubintype pigments. *FEMS Microbiol. Ecol.* 8, 87–91.
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. 20, 406–416.
- Folman, L.B., De Klein, M.J.E.M., Postma, J., and Van Veen, J.A. 2004. Production of antifungal compounds by *Lysobacter enzy-mogenes* isolate 3.1 T8 under different conditions in relation to its efficacy as a biocontrol agent of *Pythium aphanidermatum* in cucumber. *Biol. Control* **31**, 145–154.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
- Hiraishi, A., Ueda, Y., Ishihara, J., and Mori, T. 1996. Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J. Gen. Appl. Microbiol.* **42**, 457–469.
- Islam, M.T., Hashidoko, Y., Deora, A., Ito, T., and Tahara, S. 2005. Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soil borne peronosporomycetes. *Appl. Environ. Microbiol.* 71, 3786–3796.
- Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., *et al.* 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* 62, 716–721.
- Kilic-Ekici, O. and Yuen, G.Y. 2004. Comparison of strains of Lysobacter enzymogenes and PGPR for induction of resistance against Bipolaris sorokiniana in tall fescue. Biol. Control 30, 446–455.
- Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge.
- Lee, J.W., Im, W.T., Kim, M.K., and Yang, D.C. 2006. Lysobacter koreensis sp. nov., isolated from a ginseng field. Int. J. Syst. Evol. Microbiol. 56, 231–235.

- Liu, M., Liu, Y., Wang, Y., Luo, X., Dai, J., and Fang, C. 2011. *Lysobacter xinjiangensis* sp. nov., a moderately thermotolerant and alkalitolerant bacterium isolated from a gamma-irradiated sand soil sample. *Int. J. Syst. Evol. Microbiol.* **61**, 433–437.
- Luo, G., Shi, Z., and Wang, G. 2012. *Lysobacter arseniciresistens* sp. nov., an arsenite-resistant bacterium isolated from iron-mined soil. *Int. J. Syst. Evol. Microbiol.* **62**, 1659–1665.
- Mesbah, M., Premachandran, U., and Whitman, W.B. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int. J. Syst. Bacteriol.* 39, 159–167.
- Minnikin, D.E., ODonnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J. Microbiol. Methods 2, 233–241.
- Moore, D.D. and Dowhan, D. 1995. Preparation and analysis of DNA. *In* Ausubel F.W., Brent R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (eds.). Current protocols in molecular biology, pp 2–11. Wiley, New York, USA.
- Park, J.H., Kim, R., Aslam, Z., Jeon, C.O., and Chung, Y.R. 2008. Lysobacter capsici sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus Lysobacter. Int. J. Syst. Evol. Microbiol. 58, 387–392.
- **Palumbo, J.D., Sullivan, R.F., and Kobayashi, D.Y.** 2003. Molecular characterization and expression in *Escherichia coli* of three  $\beta$ -1,3-glucanase genes for *Lysobacter enzymogenes* strain N4-7. *J. Bacteriol.* **185**, 4362–4370.
- Romanenko, L.A., Uchino, M., Tanaka, N., Frolova, G.M., and Mikhailov, V.V. 2008. Lysobacter spongiicola sp. nov., isolated from a deep-sea sponge. Int. J. Syst. Evol. Microbiol. 58, 370– 374.
- Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425.
- Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. DE: MIDI Inc, Newark, USA.
- Skerman, V.B.D. 1967. A guide to the identification of the genera of bacteria, 2nd edition, Williams and Wilkins, Baltimore.
- Srinivasan, S., Kim, M.K., Sathiyaraj, G., Kim, H.B., Kim, Y.J., and Yang, D.C. 2010. Lysobacter soli sp. nov., isolated from soil of a ginseng field. Int. J. Syst. Evol. Microbiol. 60, 1543–1547.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729.
- Tamaoka, J., Katayama-Fujiruma, A., and Kuraishi, H. 1983. Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J. Appl. Bacieriol.* **54**, 31–36.
- Ten, L.N., Jung, H., Im, W.T., Yoo, S.A., Oh, H.M., and Lee, S.T. 2009. Lysobacter panaciterrae sp. nov., isolated from soil of ginseng field. Int. J. Syst. Evol. Microbiol. 59, 958–963.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL\_X windows interface: flexi-

ble strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

- Wang, Y., Dai, J., Zhang, L., Luo, X., Li, Y., Chen, G., Tang, Y., Meng, Y., and Fang, C. 2009. Lysobacter ximonensis sp. nov., isolated from soil. Int. J. Syst. Evol. Microbiol. 59, 786–789.
- Wang, G.L., Wang, L., Chen, H.H., Shen, B., Li, S.P., and Jiang, J.D. 2011. Lysobacter ruishenii sp. nov., a chlorothalonil degrading bacterium isolated from a long-term chlorothalonil-contaminated soil. Int. J. Syst. Evol. Microbiol. 61, 674–679.
- Wei, D.Q., Yu, T.T., Yao, J.C., Zhou, E.M., and Song, Z.Q. 2012. Lysobacter thermophilus sp. nov., isolated from a geothermal soil sample in Tengchong, south-west China. Antonie van Leeuwenhoek 102, 643–651.
- Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**, 697–703.
- Weon, H.Y., Kim, B.Y., Baek, Y.K., Yoo, S.H., Kwon, S.W., Stackebrandt, E., and Go, S.J. 2006. Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter* yangpyeongensis sp. nov., isolated from Korean greenhouse soils. *Int. J. Syst. Evol. Microbiol.* 56, 947–951.
- Weon, H.Y., Kim, B.Y., Kim, M.K., Yoo, S.H., Kwon, S.W., Go, S.J., and Stackebrandt, E. 2007. Lysobacter niabensis sp. nov. and Lysobacter niastensis sp. nov., isolated from greenhouse soils in Korea. Int. J. Syst. Evol. Microbiol. 57, 548–551.
- Yang, S.Z., Feng, G.D., Zhu, H.H., and Wang, Y.H. 2015. Lysobacter mobilis sp. nov., isolated from abandoned lead-zinc ore. Int. J. Syst. Evol. Microbiol. doi: 10.1099/ijs.0.000026.
- Yassin, A.F., Chen, W.M., Hupfer, H., Siering, C., Kroppenstedt, R.M., Arun, A.B., Lai, W.A., Shen, F.T., Rekha, P.D., and Young, C.C. 2007. Lysobacter defluvii sp. nov., isolated from municipal solid waste. Int. J. Syst. Evol. Microbiol. 57, 1131– 1136.
- Yu, T.T., Zhou, E.M., Yin, Y.R., Yao, J.C., Ming, H., Dong, L., Li, S., Nie, G.X., and Li, W.J. 2013. Vulcaniibacterium tengchongense gen. nov., sp. nov. isolated from a geothermally heated soil sample, and reclassification of Lysobacter thermophiles Wei et al. 2012 as Vulcaniibacterium thermophilum comb. nov. Antonie van Leeuwenhoek 104, 369–376.
- Zhang, L., Bai, J., Wang, Y., Wu, J.L., Dai, J., and Fang, C.X. 2011. Lysobacter korlensis sp. nov. and Lysobacter bugurensis sp. nov., isolated from soil. Int. J. Syst. Evol. Microbiol. 61, 2259–2265.
- Zhang, Z. and Yuen, G.Y. 2000a. Effects of culture fluids and preinduction of chitinase production on biocontrol of Bipolaris leaf spot by *Stenotrophomonas maltophilia* C3. *Biol. Control* 18, 277–286.
- Zhang, Z. and Yuen, G.Y. 2000b. The role of chitinase production by *Stenotrophomonas maltophilia* strain C3 in biological control of *Bipolaris sorokiniana*. *Phytopathology* **90**, 384–389.
- Zhang, Z., Yuen, G.Y., Sarath, G., and Penheiter, A. 2001. Chitinases from the plant disease biocontrol agent, *Stenotrophomonas maltophilia* C3. *Phytopathology* **91**, 204–211.