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Lysobacter lycopersici sp. nov., isolated from tomato plant *Solanum lycopersicum*

Shih-Yao Lin · Asif Hameed · Cheng-Zhe Wen ·
You-Cheng Liu · Yi-Han Hsu · Wei-An Lai ·
Chiu-Chung Young

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Abstract A Gram-stain negative, aerobic, rod-shaped and non-spore-forming bacterium, CC-Bw-6^T was isolated from chopped tomato stems. Analysis of the 16S rRNA gene sequence of the strain CC-Bw-6^T showed its affiliation with the genus *Lysobacter* within the class *Gammaproteobacteria*. Strain CC-Bw-6^T was found to be most closely related to *Lysobacter panaciterrae* KCTC 12601^T (97.0 %) and *Lysobacter daecheongensis* KCTC 12600^T (96.8 %), and showed lower similarity (<96.5 %) to other *Lysobacter* species.

GenBank accession number for the 16S rRNA gene sequence of strain CC-Bw-6^T is KC820654.

Electronic supplementary material The online version of this article (doi:10.1007/s10482-015-0419-1) contains supplementary material, which is available to authorized users.

S.-Y. Lin · A. Hameed · Y.-C. Liu · Y.-H. Hsu ·
W.-A. Lai · C.-C. Young (✉)
Department of Soil and Environmental Sciences, College
of Agriculture and Natural Resources, National Chung
Hsing University, 250, Kuo Kuang Rd., Taichung 402,
Taiwan
e-mail: ccyoung@mail.nchu.edu.tw

C.-Z. Wen
Bachelor Program of Bio-Industry Management, College
of Agriculture and Natural Resources, National Chung
Hsing University, Taichung, Taiwan

W.-A. Lai · C.-C. Young
Agricultural Biotechnology Center, National Chung Hsing
University, Taichung, Taiwan

DNA–DNA relatedness between strain CC-Bw-6^T and *L. panaciterrae* KCTC 12601^T was 10.8 %, the G+C content of the genomic DNA is 69.9 mol%. Strain CC-Bw-6^T was determined to possess C_{11:0} iso, C_{11:0} iso 3OH, C_{14:0} iso, C_{15:0} anteiso, C_{15:1} ω5c, C_{16:1} ω5c, C_{16:0}, C_{15:0} iso, C_{16:0} iso, C_{17:0} iso and C_{16:0} 10-methyl/ C_{17:1} iso ω9c as predominant fatty acids. The major polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and unidentified phospholipids. The predominant polyamine is spermidine, and ubiquinone-8 is the predominant respiratory quinone. Analysis of phenotypic, geno- and phylogenetic characteristics revealed a distinct taxonomic position of strain CC-Bw-6^T with respect to other *Lysobacter* species. Based on the phylogenetic, chemotaxonomic and phenotypic data presented here, we propose a novel species with the name *Lysobacter lycopersici* sp. nov. The type strain is CC-Bw-6^T (=BCRC 80612^T = JCM 19164^T).

Keywords Polyphasic taxonomy ·
Gammaproteobacteria · *Lysobacter lycopersici*

Introduction

The genus *Lysobacter* and the family *Lysobacteraceae* was proposed in 1978 (Christensen and Cook 1978). Further taxonomic studies have shown that the genus *Lysobacter* belongs to the family *Xanthomonadaceae* (Saddler and Bradbury 2005) and the description of

the genus has been emended by Park et al. (2008). There are more than thirty validly named species of the genus *Lysobacter* including four *Lysobacter* species described in 2014, *Lysobacter panacisoli* (Choi et al. 2014), *Lysobacter terrae* (Ngo et al. 2014), *Lysobacter mobilis* (Yang et al. 2014) and *Lysobacter caeni* (Ye et al. 2014). Most of *Lysobacter* species were isolated from soil samples, except for *Lysobacter spongiicola* (Romanenko et al. 2008), *Lysobacter concretionis* (Bae et al. 2005) and *L. mobilis* (Yang et al. 2014) which were isolated from deep sea sponge, sludge and abandoned lead–zinc ore, respectively.

Some bacteria of this genus exhibit robust proteolytic activity and are able to lyse certain microorganisms such as Gram-stain positive bacteria (including actinomycetes), filamentous fungi, yeasts, green algae, and some invertebrates such as nematodes (Ahmed et al. 2003; Ryazanova et al. 2005). For example, some strains of *Lysobacter enzymogenes* were shown to be used as a potential biocontrol agent for plant fungal pathogens (Folman et al. 2004; Kilic-Ekici and Yuen 2003). Typical chemotaxonomic features of the bacteria of the genus *Lysobacter* comprise Q-8 as the major respiratory quinone, iso-branched fatty acids and DPE, PE and PG as the major polar lipids.

In this study, we aimed to clarify the taxonomic status of a novel bacterium, designated CC-Bw-6^T, which was isolated from the tomato stems. In order to confirm the taxonomic position of the novel strain, comparative physiological, biochemical, chemotaxonomic, genomic and phylogenomic analyses have been performed.

Materials and methods

Bacterial strains and growth conditions

Tomato plant (*Solanum lycopersicum* L.) was collected from a greenhouse belonging Taichung senior high school of National Chung Hsing University (24°12'N, 120°68'E), Taichung, Taiwan. The chopped tomato stems were suspended in sterile water and shaken at 30 °C for 30 min. Subsequently, standard serial dilution was carried out and spread on R2A agar plates (BD, U.S.A.). Several bacterial strains were isolated, identified and preserved as glycerol suspension (30 %, v/v) at –80 °C for further characterization.

For comparative purposes, the most closely related type strains *Lysobacter panaciterrae* KCTC 12601^T (Ten et al. 2009), *Lysobacter daecheongensis* KCTC 12600^T (Ten et al. 2008), *Lysobacter ginsengisoli* KCTC 12602^T (Jung et al. 2008) and *L. enzymogenes* BCRC 11654^T (Kawamura et al. 2009) were obtained from Korean Collection for Type Cultures (KCTC, Korean) and Bioresource Collection and Research Center (BCRC, Taiwan), and used as reference strains. Due to reported phylogenetic heterogeneity of the genus *Lysobacter* (Romanenko et al. 2013), e.g., *L. panaciterrae* KCTC 12601^T was clustered with members of the genus *Luteimonas* as a separate line sharing a high 16S rRNA gene sequence similarity to *Luteimonas aquatica* LMG 24212^T (98.8 %), *L. aquatica* BCRC 17731^T (Chou et al. 2008), *Luteimonas composti* BCRC 17598^T (Young et al. 2007), *Luteimonas mephitis* BCRC 17539^T (Finkmann et al. 2000) and *Thermomonas brevis* BCRC 17538^T (Mergaert et al. 2003) were also obtained from BCRC. All reference strains were grown on R2A at 30 °C for 2 days, unless described otherwise.

Morphological tests and biochemical characterization

Colony morphology, flagellation, and morphology of the cells of strain CC-Bw-6^T were investigated using the colonies/cells grown on R2A agar for 3 days. Growth of the strain CC-Bw-6^T was also tested on nutrient agar (NA, Hi-Media) and tryptic soy broth (TSB, BD, U.S.A.) agar. Cell morphology was observed by using light microscopy (model A3000, Zeiss) and transmission electron microscopy (JEOL JEM-1400), and cells were stained with 0.2 % uranyl acetate. Gram staining was carried out using a Gram staining kit as described by Murray et al. (1994). Growth was tested using R2A broth at temperature 4, 10, 15, 20, 25, 30, 37, 40, 42, 45 and 50 °C, and pH 5–10 (1 unit increment). Salt tolerance was determined by cultivating the organism in R2A broth supplemented with NaCl at final concentrations of 0–5 % (1 % increment). The presence of flexirubin-type pigments was investigated as described by Bernardet et al. (2002). Catalase activity was determined by assessing bubble production by cells in 3 % (v/v) H₂O₂ and oxidase activity was determined by using 1 % (w/v) *N,N,N,N*-tetramethyl-1,4-phenylenediamine reagent (bioMérieux). Carbon sources utilization pattern was

determined by using GN2 microplate system (Bio-Log). Nitrate reduction, indole production, activities of β -galactosidase, urease, hydrolysis of esculin and gelatin and assimilation of 12 substrates were tested with API 20 NE strips (bioMérieux). The activities of various enzymes were determined by using API ZYM system (bioMérieux).

Sequencing and analysis of the 16S rRNA gene

A commercial DNA extraction kit UltraClean™ (MO BIO, USA) was used to extract the genomic DNA of CC-Bw-6^T for 16S rRNA gene amplification. The 16S rRNA gene of strain CC-Bw-6^T was amplified using the universal bacterial primers 1F and 9R as described by Edwards et al. (1989). Primers 3F, 6F and 4R were used for sequencing reaction. The amplified gene fragments were screened by 2.0 % (w/v) agarose gel electrophoresis. Gene sequencing was performed by using the Bigdye terminator kit (Heiner et al. 1998), and nucleotide sequence of PCR product was genotyped by direct sequencing in an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The DNA sequences were then assembled using the Vector NTI 9.0 software (IBI, USA) and uploaded to EzBioCloud server (EzTaxon-e Database, Kim et al. 2012) and NCBI for identification.

For the phylogenetic analysis, the almost-complete 16S rRNA gene sequence (1512 bp) of strain CC-Bw-6^T was uploaded to EzBioCloud (EzTaxon-e Database, Kim et al. 2012) and NCBI servers for BLAST search. To ascertain the phylogenetic position of the novel isolate, the 16S rRNA gene sequence of strain CC-Bw-6^T was compared with sequences obtained from GenBank. Multiple alignments of the sequences were performed using CLUSTAL_X (version 1.83) (Thompson et al. 1997). The phylogenetic analysis was performed with MEGA 6 software (Molecular Evolutionary Genetics Analysis, version 6.0; Tamura et al. 2013) and the topology of the resultant neighbor-joining (Saitou and Nei 1987), maximum likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) trees were evaluated by bootstrap analyses (Felsenstein 1985) after 1000 replications.

DNA–DNA hybridization

DNA–DNA hybridization (Graham et al. 1991; Wayne et al. 1987) is considered to be a standard

method for species definition. DNA–DNA hybridization was performed between strain CC-Bw-6^T and *L. panaciterrae* KCTC 12601^T (97.0 % 16S rRNA gene sequences similarity) using the DIG-High Prime DNA Labelling and Detection Starter kit II according to the manufacturer's instructions (Roche). DNA samples were loaded on to positively charged membranes as described by Seldin and Dubnau (1985). Chromosomal DNA of strain CC-Bw-6^T and *L. panaciterrae* KCTC 12601^T were used to construct hybridization probes by labelling with digoxigenin–11-dUTP (DIG). Hybridization was conducted in triplicate with reciprocal probes.

DNA base composition

For the analysis of DNA G+C content, DNA samples were prepared as described by Mesbah et al. (1989). The nucleoside mixtures obtained were then separated and analyzed via HPLC [Hitachi L-2130 chromatograph equipped with a Hitachi L-2200 autosampler, Hitachi L-2455 Diode array detector, and a reverse-phase C18 column (Phenomenex® Synergi 4 μ Fusion-RP80 250 \times 4.60 mm)].

Determination of the predominant quinones and polar lipids

For the investigation of chemotaxonomic characteristics, strain CC-Bw-6^T and the reference strains were grown on R2A agar plates for 2 days at 30 °C and cells of these strains were harvested at a similar physiological age given that all strains showed similar growth kinetics. Isoprenoid quinones were purified according to the methods of Minnikin et al. (1984) and analysed by HPLC as described by Collins (1985). Biomass subjected to polar lipid analysis was grown on R2A broth and harvested at the stationary growth phase. Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and analyzed by two-dimensional TLC followed by spraying with appropriate detection reagents. Phospholipids were detected with the Zinzadze reagent of Dittmer and Lester (1964). Whole lipid profiles were detected by spraying with molybdophosphoric acid followed by heating at 150 °C (Worliczek et al. 2007). Determination of the respiratory quinone system was carried out as described by Collins (1985).

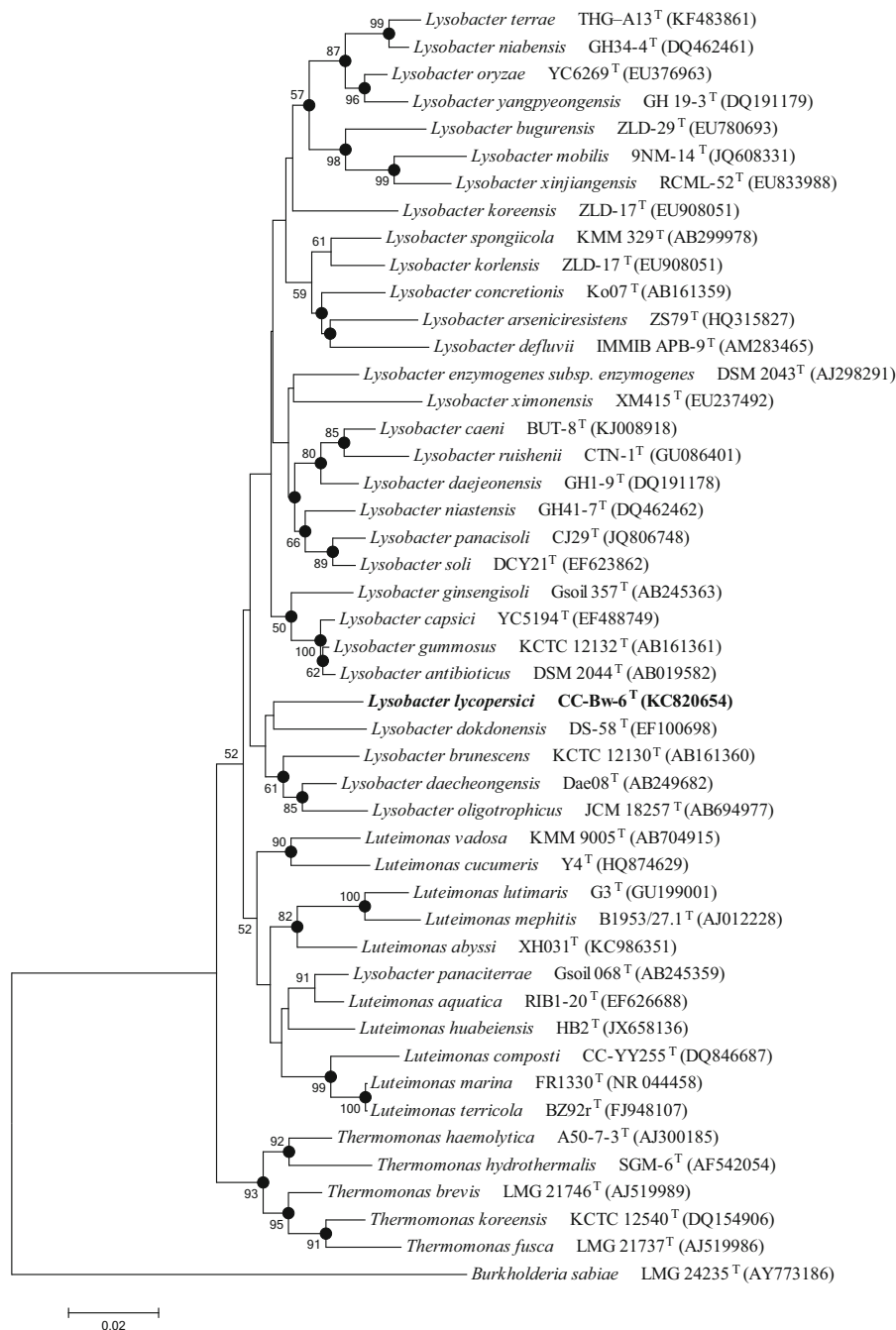


Fig. 1 Neighbor-joining phylogenetic tree showing taxonomic position of strain CC-Bw-6^T and other closely related species of the genus *Lysobacter* based on 16S rRNA gene sequences. Filled circles indicate that the corresponding nodes were also

recovered in the tree constructed based on maximum parsimony and maximum likelihood algorithm. Bootstrap values (>50 %) based on 1000 replications are listed as percentages at the branching points

Cellular polyamines and fatty acid methyl ester (FAME) analyses

Biomass subjected to polyamine analysis was grown on R2A and harvested at the late exponential growth phase. Polyamines were extracted as described by Scherer and Kneifel (1983), analyzed by using HPLC. The dansyl derivatives were separated by using a Hitachi L-2130 equipped with a Hitachi L-2200 autosampler, Hitachi L-2485 fluorescence detector (excitation at 360 nm and emission at 520 nm), and a reverse-phase C18 column (Phenomenex® Synergi Fusion-RP80, 250 × 4.60 mm, 4 µm particle size). For the extraction of fatty acid methyl esters (FAMES), strain CC-Bw-6^T and reference type strains were cultured simultaneously on R2A agar for 48 h at 30 °C (the type strains exhibited similar growth rates). Samples were prepared, separated and identified according to the standard protocol (Paisley 1996) of the Microbial Identification System (MIDI) (Sasser 1990) by gas chromatograph (Agilent 7890A) fitted to a flame ionization detector. Harvested biomasses were subjected to saponification, methylation and extraction (Miller 1982). Identification and comparison were made by using the Aerobe (RTSBA6) database of the MIDI System (Sherlock version 6.0).

Results and discussion

Analysis of the 16S rRNA gene sequences indicated that strain CC-Bw-6^T shows the highest similarity to *L. panaciterrae* KCTC 12601^T (97.0 %) followed by *L. daecheongensis* KCTC 12600^T (96.8 %) and other recognized species of the genus *Lysobacter* (<96.8 %). The similarity values of the 16S rRNA gene suggested that strain CC-Bw-6^T could be considered as a novel species since sequence divergence values of ≥3 % are recommended for the species delineation (Stackebrandt and Goebel 1994). DNA–DNA hybridization value between strain CC-Bw-6^T and *L. panaciterrae* KCTC 12601^T was found to be 10.8 ± 1.5 % (reciprocal value was 5.1 ± 0.9 %). This value is below the 70 % cut-off recommended for genomic species discrimination (Wayne et al. 1987), clearly suggesting that strain CC-Bw-6^T represents a novel species of the genus *Lysobacter*.

The 16S rRNA gene sequence similarities between strain CC-Bw-6^T and other related species of the

genera *Lysobacter* (25 species), *Luteimonas* (10 species) and *Thermomonas* (5 species) were found to be 93–97, 95–97 and 93–96 %, respectively, which is at the interspecies level. The phylogenetic position of strain CC-Bw-6^T is shown in Fig. 1. Similar topology of the phylogenetic trees was obtained using neighbor-joining, maximum likelihood and maximum-parsimony algorithms.

Colonies of strain CC-Bw-6^T were observed to be circular, smooth and yellow-colored after three-days of incubation on R2A agar. Cell morphology of strain CC-Bw-6^T is presented in Fig. 2. Strain CC-Bw-6^T was found to produce flexirubin-type pigments. In the biochemical tests, strains CC-Bw-6^T, *L. enzymogenes* subsp. *enzymogenes* BCRC 11654^T and *L. panaciterrae* KCTC 12601^T utilize glycogen, Tween 40, pyruvic acid methyl ester, acetic acid, formic acid, β-hydroxybutyric acid, α-keto butyric acid, α-keto glutaric acid, α-keto valeric acid, propionic acid, L-glutamic acid, glycyl-L-aspartic acid, L-serine, L-threonine and urocanic acid as carbon sources; positive for β-glucosidase and protease (hydrolysis of gelatin); activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, α-chymotrypsin, acid phosphatase and Naphthol-AS-BI-phosphohydrolase were detected; but negative for α-galactosidase, β-galactosidase and β-glucuronidase.

Strain CC-Bw-6^T can be distinguished from other phylogenetically related species by its ability to utilize of α-keto valeric acid, L-alanine, L-phenylalanine, L-histidine, L-leucine, L-ornithine and L-proline; negative for lipase, cystine arylamidase, trypsin and *N*-

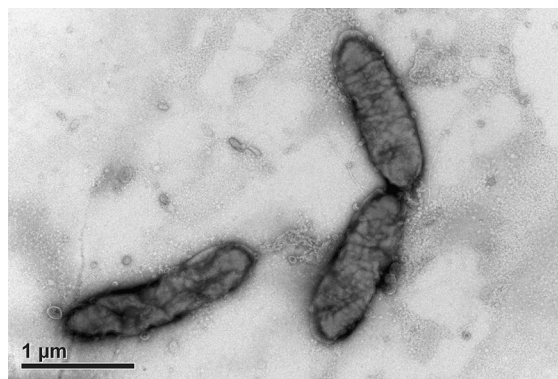


Fig. 2 Transmission electron micrograph of a negatively stained cell of strain CC-Bw-6^T. The cells appeared as short rods (0.3–0.4 × 1.5–1.7 µm) with a single polar flagellum. Bar 1.0 µm

Table 1 Phenotypic characteristics of strain CC-Bw-6^T and closely related species

Characteristic	1	2	3	4	5	6	7
Ranges for growth							
pH	5.0–8.0	6.0–8.0	5.0–9.0	5.0–9.0	6.0–8.0	5.0–9.0	5.0–9.0
NaCl (% w/w)	<1.0	<1.0	<1.0	<1.0	0–0.5	0–2.0	0–1.0
Temperature (°C)	20–37	20–37	20–37	20–30	4–38	2–40	20–30
Reduction							
NO ³⁻ to NO ²⁻	+	–	–	–	–	+	–
NO ³⁻ to N ₂	–	+	–	–	ND	ND	+
Carbon source utilization							
Dextrin	–	+	–	+	+	–	+
D-Cellobiose	–	+	+	+	–	+	+
D-Fructose	–	+	–	+	–	+	+
α-D-Glucose	–	+	–	+	–	+	+
Maltose	–	+	+	+	w	+	+
α-Keto valeric acid	+	+	–	–	–	+	+
L-Alanine	+	–	–	–	+	+	+
L-Aspartic acid	–	+	–	–	–	+	+
L-Phenylalanine	+	–	–	–	–	ND	–
L-Histidine	+	–	–	–	–	+	+
L-Leucine	+	–	–	+	+	ND	+
L-Ornithine	+	–	–	–	–	+	+
L-Proline	+	–	–	+	–	+	+
Glycerol	–	+	+	–	ND	ND	+
Enzymatic activities							
Lipase (C14)	–	–	–	–	–	ND	+
Cystine arylamidase	–	+	+	–	–	ND	+
Trypsin	–	+	–	–	–	ND	+
N-acetyl-β-glucosaminidase	–	+	–	–	ND	ND	+
Assimilation							
D-Glucose	–	+	–	–	–	+	+
D-Mannose	–	–	–	–	–	+	+
N-acetyl glucosamine	–	+	–	–	–	+	+
D-Maltose	–	–	–	–	w	+	+
Malic acid	–	w	–	–	ND	+	+
DNA G+C content	69.9	67.0 ^a	69.3 ^a	69.3 ^a	68.1 ^a	69.2 ^a	69.0 ^a

Data were obtained in this study unless indicated otherwise

1 strain CC-Bw-6^T; 2 *Lysobacter panaciterrae* KCTC 12601^T; 3 *Lysobacter daecheongensis* KCTC 12600^T; 4 *Lysobacter ginsengisoli* KCTC 12602^T; 5 *Lysobacter dokdonensis* DS-58^T (Oh et al. 2011); 6 *Lysobacter antibioticus* BCRC 11653^T (Weon et al. 2007); 7 *Lysobacter enzymogenes* subsp. *enzymogenes* BCRC 11654^T (type species)

+ positive; – negative; w weak reaction, ND no data available

^a Data obtained from Mergaert et al. (2003); Weon et al. (2007); Young et al. (2007); Chou et al. (2008); Park et al. (2008); Ten et al. (2009); Zhang et al. (2011); Oh et al. (2011)

acetyl-β-glucosaminidase in enzymatic activities; reduction of nitrate (NO³⁻) to nitrite (NO²⁻). The detail phenotypic characteristics of strain CC-Bw-6^T are

given in the species description. Additionally, strain CC-Bw-6^T showed several distinct physiological and biochemical characteristics, a comparison of the

Table 2 Cellular fatty acid composition (%)

Fatty acid	1	2	3	4	5	6	7
C _{11:0} iso	5.8	12.8	6.0	3.4	5.9	3.3	9.7
C _{11:0} iso 3OH	8.0	28.3	9.7	6.6	7.8	7.3	22.6
C _{12:0} iso 3OH	tr	2.7	tr	tr	–	–	tr
C _{14:0} iso	2.3	tr	3.6	1.6	4.6	tr	tr
C _{14:0}	–	–	tr	tr	–	tr	–
C _{15:1} iso F*	tr	1.2	tr	tr	–	–	tr
C _{15:0} iso	15.7	11.7	24.4	4.8	18.7	28.3	32.3
C _{15:0} anteiso	2.9	1.9	3.0	4.6	4.9	3.6	tr
C _{15:1} ω5c	3.6	3.8	6.0	tr	–	4.3	8.9
C _{16:1} iso H	tr	1.1	tr	1.6	1.1	tr	tr
C _{16:0} iso	27.5	8.0	22.4	35.0	30.5	6.1	1.6
C _{16:1} ω5c	4.8	1.6	4.9	6.0	–	tr	tr
C _{16:0}	2.0	tr	1.4	3.5	1.9	5.8	2.0
C _{15:0} iso 3OH	tr	tr	tr	1.1	–	2.1	1.7
C _{17:0} iso	5.4	3.0	2.9	2.9	2.5	3.4	2.0
C _{17:0} anteiso	–	–	–	1.3	–	tr	–
C _{17:0} cyclo	–	1.3	–	–	–	1.4	1.5
C _{18:0} iso	–	–	tr	1.2	–	tr	–
Summed feature 1	–	–	tr	tr	–	tr	–
Summed feature 2	1.9	–	–	–	–	–	–
Summed feature 3	1.4	1.7	1.3	4.5	–	11.7	5.7
Summed feature 8	–	–	–	tr	–	1.7	–
Summed feature 9	13.0	11.8	8.5	13.7	14.0	8.6	5.1

Data were obtained in this study unless indicated otherwise

1 strain CC-Bw-6^T; 2 *Lysobacter panaciterrae* KCTC 12601^T; 3 *Lysobacter daecheongensis* KCTC 12600^T; 4 *Lysobacter ginsengisoli* KCTC 12602^T; 5 *Lysobacter dokdonensis* DS-58^T (Oh et al. 2011); 6 *Lysobacter antibioticus* BCRC 11653^T (Weon et al. 2007); 7 *Lysobacter enzymogenes* subsp. *enzymogenes* BCRC 11654^T (type species)

tr trace (<1 %), – not detected

Fatty acids found in amounts <1.0 % in all strains are not shown

C_{15:1} iso F* should correspond to either C_{15:1} iso v6c and/or C_{15:1} iso v5c. The double bond position is presumptive (Yassin et al. 2007)

Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system

Summed feature 1 C_{15:1} iso H/C_{13:0} 3-OH, Summed feature 2 C_{14:0} 3-OH/C_{16:1} iso I, Summed feature 3 C_{16:1} ω7c/C_{16:1} ω6c, Summed feature 8 C_{18:1} ω7c/C_{18:1} ω6c, Summed feature 9 C_{16:0} 10-methyl/C_{17:1} iso

phenotypic properties between strain CC-Bw-6^T and the type strains of validly named species in the genus *Lysobacter* is given in Table 1.

Strain CC-Bw-6^T was determined to possess 69.9 ± 0.4 mol% DNA, which is within the range described for the genus *Lysobacter* (61.7–70.1 mol%). Similar polyamine patterns with spermidine as the major compound, were also observed for several type strains of species of the genera *Lysobacter*, *Luteimonas* and *Thermomonas*. The major polar lipids were

identified as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), unidentified phospholipids (PL1–6), unidentified aminophospholipids (APL1–3); a moderate amount of phosphatidylmethylethanolamine (PME) was also identified (Supplementary data Fig. S1). The major fatty acids in strain CC-Bw-6^T were determined to be C_{11:0} iso, C_{11:0} iso 3OH, C_{15:0} iso, C_{16:0} iso, C_{17:0} iso and C_{16:0} 10-methyl/C_{17:1} iso ω9c, which are commonly detected in *Lysobacter* species lipids (Table 2).

Based on the results of the polyphasic taxonomic study, strain CC-Bw-6^T is proposed to represent a novel species *Lysobacter lycopersici* sp. nov. CC-Bw-6^T (=BCRC 80623^T = JCM 19164^T) within the genus *Lysobacter*.

Description of *Lysobacter lycopersici* sp. nov

Lysobacter lycopersici (ly.co.per'si.ci N.L. gen. neut. n. lycopersici of *Lycopersicon*, the systematic name of the tomato).

Cells are Gram-stain negative, rod-shaped, 1.5–1.7 µm in length and 0.3–0.4 µm in diameter. Colonies are circular, smooth and yellow-colored after three-days of incubation on R2A agar. The growth temperature ranges from 20 to 37 °C, pH 5.0–8.0 but tolerates less than 1 % (w/v) NaCl concentration. Oxidase and catalase tests give a positive reaction. The flexirubin pigments are produced. Nitrates are reduced to nitrites, but nitrites are not reduced. The following carbon sources are utilized in the BioLog GN2 system: glycogen, Tween 40, pyruvic acid methyl ester, acetic acid, formic acid, β-hydroxybutyric acid, α-keto butyric acid, α-keto glutaric acid, α-keto valeric acid, propionic acid, L-alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine and urocanic acid. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase are positive in the API-ZYM system. The fatty acid profiles consists of C_{11:0} iso, C_{11:0} iso 3OH, C_{14:0} iso, C_{15:0} anteiso, C_{15:1} ω5c, C_{16:1} ω5c, C_{16:0}, C_{15:0} iso, C_{16:0} iso, C_{17:0} iso and C_{16:0} 10-methyl/C_{17:1} iso ω9c. The polar lipid profile constitutes PE, PG and DPG as major lipids. The predominant polyamine is spermidine (Spd) and the predominant ubiquinone is (Q-8). The DNA G+C content is 69.9 ± 0.4 mol%.

The type strain is CC-Bw-6^T (=BCRC 80612^T = JCM 19164^T), isolated from chopped tomato stems, in Taiwan.

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References

- Ahmed K, Chohnan S, Ohashi H, Hirata T, Masaki T, Sakiyama F (2003) Purification, bacteriolytic activity, and specificity of β-lytic protease from *Lysobacter* sp. IB-9374. *J Biosci Bioeng* 95:27–34
- Bae HS, Im WT, Lee ST (2005) *Lysobacter concretions* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Microbiol* 55:1155–1161
- Bernardet JF, Nakagawa Y, Holmes B (2002) Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 52:1049–1070
- Choi J-H, Seok J-H, Cha J-H, Cha C-J (2014) *Lysobacter panacisoli* sp. nov., isolated from ginseng soil. *Int J Syst Evol Microbiol* 64:2193–2197
- Chou JH, Cho NT, Arun AB, Young CC, Chen WM (2008) *Luteimonas aquatica* sp. nov., isolated from fresh water from Southern Taiwan. *Int J Syst Evol Microbiol* 58:2051–2055
- Christensen P, Cook FD (1978) *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* 28:367–393
- Collins MD (1985) Isoprenoid quinone analysis in classification and identification. In: Goodfellow M, Minnikin DE (eds) *Chemical methods in bacterial systematics*. Academic Press, London, pp 267–287
- Dittmer JC, Lester RL (1964) A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. *J Lipid Res* 15:126–127
- Edwards U, Rogall T, Blocker H, Emde M, Bottger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* 17:7843–7853
- 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
- Finkmann W, Altendorf K, Stackebrandt E, Lipski A (2000) Characterization of N₂O-producing *Xanthomonas*-like isolates from biofilters as *Stenotrophomonas nitritireducens* sp. nov., *Luteimonasmephitis* gen. nov., sp. nov. and *Pseudoxanthomonas broegbernensis* gen. nov., sp. nov. *Int J Syst Evol Microbiol* 50:273–282
- Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20:406–416
- Folman LB, De Klein MJEM, Postma J, van Veen JA (2004) Production of antifungal compounds by *Lysobacter enzymogenes* isolate 3.1T8 under different conditions in relation to its efficacy as a biocontrol agent of *Pythium aphanidermatum* in cucumber. *Biol Control* 31:145–154
- Graham PH, Sadowsky MJ, Keyser HH, Barnet YM, Bradley RS, Cooper JE, De Ley DJ, Jarvis BDW, Roslycky EB et al (1991) Proposed minimal standards for the description of

- new genera and species of root-and stem-nodulating bacteria. *Int J Syst Bacteriol* 41:582–587
- Heiner CR, Hunkapiller LK, Chen SM, Glass JI, Chen EY (1998) Sequencing multimegabase-template DNA using BigDye terminator chemistry. *Genome Res* 8:557–561
- Jung HM, Ten LN, Im WT, Yoo SA, Lee ST (2008) *Lysobacter ginsengisoli* sp. nov., a novel species isolated from soil in Pocheon Province, South Korea. *J Microbiol Biotechnol* 18:1496–1499
- Kawamura Y, Tomida J, Morita Y, Naka T, Mizuno S, Fujiwara N (2009) '*Lysobacter enzymogenes* ssp. cookii' Christensen 1978 should be recognized as an independent species, *Lysobacter cookii* sp. nov. *FEMS Microbiol Lett* 298:118–123
- Kilic-Ekici O, Yuen GY (2003) Induced resistance as a mechanism of biological control by *Lysobacter enzymogenes* strain C3. *Biol Control* 93:1103–1110
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (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
- Mergaert J, Cnockaert MC, Swings J (2003) *Thermomonas fusca* sp. nov. and *Thermomonas brevis* sp. nov., two mesophilic species isolated from a denitrification reactor with poly(ϵ -caprolactone) plastic granules as fixed bed, and emended description of the genus *Thermomonas*. *Int J Syst Evol Microbiol* 53:1961–1966
- Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* 39:159–167
- Miller LT (1982) Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxyl acids. *J Clin Microbiol* 16:584–586
- Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaak K, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 2:233–241
- Murray RGE, Doetsch RN, Robinow CF (1994) Determination and cytological light microscopy. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) *Methods for general and molecular bacteriology*. American Society for Microbiology, Washington DC, pp 31–32
- Ngo HTT, Won K, Du J, Son H-M, Park Y, MooChang K, Kim K-Y, Jin F-X, Yi T-H (2014) *Lysobacter terrae* sp. nov. isolated from *Aglaia odorata* rhizosphere soil. *Int J Syst Evol Microbiol*. doi:10.1099/ij.s.0.067397-0
- Oh KH, Kang SJ, Jung YT, Oh TK, Yoon JH (2011) *Lysobacter dokdonensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 61:1089–1093
- Paisley R (1996) MIS whole cell fatty acid analysis by gas chromatography training manual. MIDI, Newark
- Park JH, Kim R, Aslam Z, Jeon CO, Chung YR (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
- Romanenko LA, Uchino M, Tanaka N, Frolova GM, Mikhailov VV (2008) *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int J Syst Evol Microbiol* 58:370–374
- Romanenko LA, Tanaka N, Svetashev VI, Kurilenko VV, Mikhailov VV (2013) *Luteimonas vadosa* sp. nov., isolated from seashore sediment. *Int J Syst Evol Microbiol* 63:1261–1266
- Ryazanova LP, Stepnaya OA, Suzina NE, Kulaev IS (2005) Antifungal action of the lytic enzyme complex from *Lysobacter* sp. XL1. *Process Biochem* 40:557–564
- Saddler GS, Bradbury JF (2005) Family I. *Xanthomonadaceae* fam. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) *Bergey's manual of systematic bacteriology (The Proteobacteria)*, part B (The *Gammaproteobacteria*). Springer, New York, p 63
- Saitou N, 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. MIDI Inc, Newark, DE
- Scherer P, Kneifel H (1983) Distribution of polyamines in methanogenic bacteria. *J Bacteriol* 154:1315–1322
- Seldin L, Dubnau D (1985) Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int J Syst Bacteriol* 35:151–154
- Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* 44:846–849
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- Ten LN, Jung H-M, Im W-T, Yoo S-A, Lee S-T (2008) *Lysobacter daecheongensis* sp. nov., isolated from sediment of stream near the Daechung Dam in south Korea. *J Microbiol* 46:519–524
- Ten LN, Jung H-M, Im W-T, Yoo S-A, Oh HM, Lee S-T (2009) *Lysobacter panaciterrae* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* 59:958–963
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882
- Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE (1987) International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37:463–464
- Weon HY, Kim BY, Kim MK, Yoo SH, Kwon SW, Go SJ, 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
- Worliczek HL, Kämpfer P, Rosengarten R, Tindall BJ, Busse H-J (2007) Polar lipid and fatty acid profiles-revitalizing old approaches as a modern tool for the classification of mycoplasmas? *Syst Appl Microbiol* 30:355–370
- Yang S-Z, Feng G-D, Zhu H-H, Wang Y-H (2014) *Lysobacter mobilis* sp. nov., isolated from abandoned lead-zinc ore. *Int J Syst Evol Microbiol*. doi:10.1099/ij.s.0.000026
- Yassin AF, Chen W-M, Hupfer H, Siering C, Kroppenstedt RM, Arun AB, Lai W-A, Shen F-T, Rekha PD, Young C-C

- (2007) *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. *Int J Syst Evol Microbiol* 57:1131–1136
- Ye X-M, Chu C-W, Shi C, Zhu J-C, He Q, He J (2014) *Lysobacter caeni* sp. nov., isolated from the sludge of pesticide manufacturing factory. *Int J Syst Evol Microbiol*. doi:[10.1099/ijse.0.000026](https://doi.org/10.1099/ijse.0.000026)
- Young C-C, Kämpfer P, Chen W-M, Yen W-S, Arun AB, Lai W-A, Shen F-T, Rekha PD, Lin K-Y, Chou J-H (2007) *Luteimonas composti* sp. nov., a moderately thermophilic bacterium isolated from food waste. *Int J Syst Evol Microbiol* 57:741–744
- Zhang L, Bai J, Wang Y, Wu GL, Dai J, Fang C (2011) *Lysobacter korlensis* sp. nov. and *Lysobacter bugurensis* sp. nov., isolated from soil in north-west China. *Int J Syst Evol Microbiol* 61:2259–2265