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Lysobacter pedocola sp. nov., a novel species isolated from Korean soil[§]

Jun Hyeong Jang[1,2], Dongwook Lee[1], and Taegun Seo[1]*

[1]*Department of Life Science, Dongguk University-Seoul, Goyang 10326, Republic of Korea*
[2]*Water Supply and Sewerage Research Division, National Institute of Environmental Research, Incheon 22689, Republic of Korea*

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A Gram-negative, yellow-pigmented bacterial strain, designated IPC6^T, was isolated from soil in an arid region of Goyang-si (Gyeonggi-do, South Korea). Cells were strictly aerobic, non-spore-forming, rod-shaped. The strain grew within a temperature range of 10–42°C (optimum, 30°C) and pH of 5.0–11.0 (optimum, pH 8.0) in the presence of 0–2% (w/v) NaCl. Phylogenetically, the novel strain was closely related to members of the *Lysobacter* genus based on 16S rRNA sequence similarity, and showed the highest sequence similarity to *Lysobacter niastensis* KACC 11588^T (98.5%). The predominant fatty acids were iso-$C_{15:0}$, iso-$C_{16:0}$, and summed feature 9 (iso-$C_{17:1}$ ω9*c*), with Q-8 identified as the major ubiquinone. The polar lipid content included diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, an unknown aminophospholipid, and an unidentified phospholipid. DNA-DNA hybridization results indicated that the strain IPC6^T was distinct from *Lysobacter niastensis* KACC 11588^T (37.9 ± 0.14%), *Lysobacter panacisoli* KACC 17502^T (56.4 ± 0.13%), *Lysobacter soli* KCTC 22011^T (8.1 ± 0.04%), *Lysobacter gummosus* KCTC 12132^T (9.6 ± 0.03%), and *Lysobacter cavernae* KCTC 42875^T (37.5 ± 0.14%), respectively. The DNA G + C content of the novel strain was 71.1 mol%. Based on the collective phenotypic, genotypic and chemotaxonomic data, the IPC6^T strain is considered to represent a novel species in the genus *Lysobacter*, for which the name *Lysobacter pedocola* sp. nov. (= KCTC 42811^T = JCM 31020^T) is proposed.

Keywords: DNA-DNA relatedness, *Lysobacter*, taxonomy

Introduction

The *Lysobacter* genus, a member of the family *Xanthomonadaceae*, was initially classified by Christensen and Cook (1978). At present, the *Lysobacter* genus comprises more than 40 species (www.bacterio.net/lysobacter.html) with several common characteristics, including Gram-negative staining, rod shape, and yellow pigmentation. Members of this genus contain iso-$C_{15:0}$, iso-$C_{16:0}$, and iso-$C_{17:1}$ ω9*c* as the major cellular fatty acids, ubiquinone Q-8 as the major respiratory quinone (Srinivasan *et al*., 2010; Oh *et al*., 2011; Singh *et al*., 2015; Siddiqi and Im, 2016; Weon *et al*., 2007), and the DNA G + C content of 64.0–72.0 mol%. Strain IPC6^T was isolated from an arid area in Goyang-si, Gyeonggi-do, South Korea (approximate coordinates: N′ 37.678347, E′ 126.806217), and the physiological, biochemical, and chemotaxonomic characterization and phylogenetic analysis (based on 16S rRNA gene sequences) indicated that the new isolate represents a novel species of the genus *Lysobacter*.

Materials and Methods

Isolation of culture conditions and bacteria strain

Strain IPC6^T was isolated from a soil sample collected in Goyang, Gyeonggi Province, South Korea (GPS; N′ 37.678347, E′ 126.806217; Sampling time: 31-AUG-2015). Soil samples (5 g) were mixed with 10 ml NaCl (0.85%, w/v), vortexed and serially diluted. A 0.1 ml sample of each diluted mixture was spread onto R2A agar plates (Difco Laboratories) and incubated at 30°C for 2 days. After isolation and purification, a yellow-pigmented bacterial strain, IPC6^T, was cultivated at 30°C on R2A agar. IPC6^T was deposited in the Korean Collection for Type Cultures (KCTC) of South Korea and Japan Collection of Microorganisms (JCM). The reference type strains *Lysobacter niastensis* KACC 11588^T, *Lysobacter panacisoli* KACC 17502^T, *Lysobacter soli* KCTC 22011^T, *Lysobacter gummosus* KCTC 12132^T, and *Lysobacter cavernae* KCTC 42875^T were maintained and cultivated on R2A agar at 30°C.

16S rRNA sequencing and phylogenetic analysis

The gene encoding 16S rRNA of IPC6^T was amplified using the 27F, 1492R, 518F and 805R universal bacterial primer set for the colony PCR protocol described by Weisburg *et al*. (1991). The 16S rRNA gene sequence of IPC6^T was assembled using SeqMan software (DNASTAR Inc.) and compared with those of other relatively close taxa using the EzTaxon database server (Kim *et al*., 2012). Multiple alignments were performed using the Clustal X program (Thompson *et al*., 1997). The phylogenetic tree was constructed using MEGA 5 Program (Tamura *et al*., 2011) and reconstructed according to the neighbor-joining (NJ) method with the Kimura two-parameter model (Kimura, 1980; Saitou and Nei, 1987). ootstrap analysis was conducted with 1,000 replicates (Felsen-

*For correspondence. E-mail: tseo@dongguk.edu; Tel.: +82-31-961-5135; Fax: +82-31-961-5108
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The GenBank accession number for the 16S rRNA gene sequence of strain IPC6^T (= KCTC 42811^T = JCM 31020^T) is KT630892.

stein, 1985). The max-min heuristic search algorithm was applied in maximum-parsimony (MP) and maximum-likelihood (ML) analyses for comparison with the neighbor-joining phylogenetic tree (NJ) (Fitch, 1971).

Phenotypic and biochemical characteristics

Bacterial growth at 4, 10, 15, 20, 25, 30, 37, 40, 42, and 50°C was assessed after 5 days of incubation in R2A broth. Tests for hydrolysis of casein, starch, chitin and DNA were performed using the methods of Smibert and Krieg (1994). The pH range suitable for growth was determined after 5 days of incubation at 30°C in R2A broth. To examine the effects of pH variations on growth, media were adjusted to pH 4.0–11.0 with acetic acid/sodium acetate (pH 4.0), 10 mM MES (pH 5.0–6.0), 10 mM Tris (pH 7.0–9.0) or sodium carbonate/sodium bicarbonate (pH 10.0–11.0). Salt tolerance (0–10.0% at intervals of 1.0%, w/v) of the strain was tested on R2A agar plates after 3 days of incubation. Growth of strain IPC6^T was examined on the following agar media: R2A (MB cell), Luria-Bertani (LB, Difco), MacConkey (MACA, Difco), marine (MA, Difco), nutrient (NA, Difco), yeast starch and tryptic soy (TSA, Difco) at 30°C for 5 days. The Gram reaction of IPC6^T was determined using the non-staining KOH lysis method (3% KOH) (Buck, 1982) and cell morphology examined under a transmission electron microscope (LIBRA 120; Carl Zeiss) (Supplementary data Fig. S1). Cell motility was analyzed using the 0.4% agar technique as described by Bowman (2000), with growth for 3 days at 30°C on R2A agar. Other biochemical and physiological characteristics were examined with the API 20NE, API ZYM, and API 32GN systems (bioMérieux) according to the manufacturer's instructions. IPC6^T cells grown on R2A agar plates at 30°C for 3 days were employed for API tests.

Chemotaxonomic and genomic analyses

Total polar lipids were extracted from IPC6^T and analyzed as described previously by Minnikin *et al.* (1984) and *Lysobacter niastensis* KACC 11588^T grown on R2A agar plates at 30°C. Total lipids were separated using two-dimensional thin layer chromatography (TLC) with chloroform/methanol/water (65:25:4) and chloroform/methanol/acetic acid/

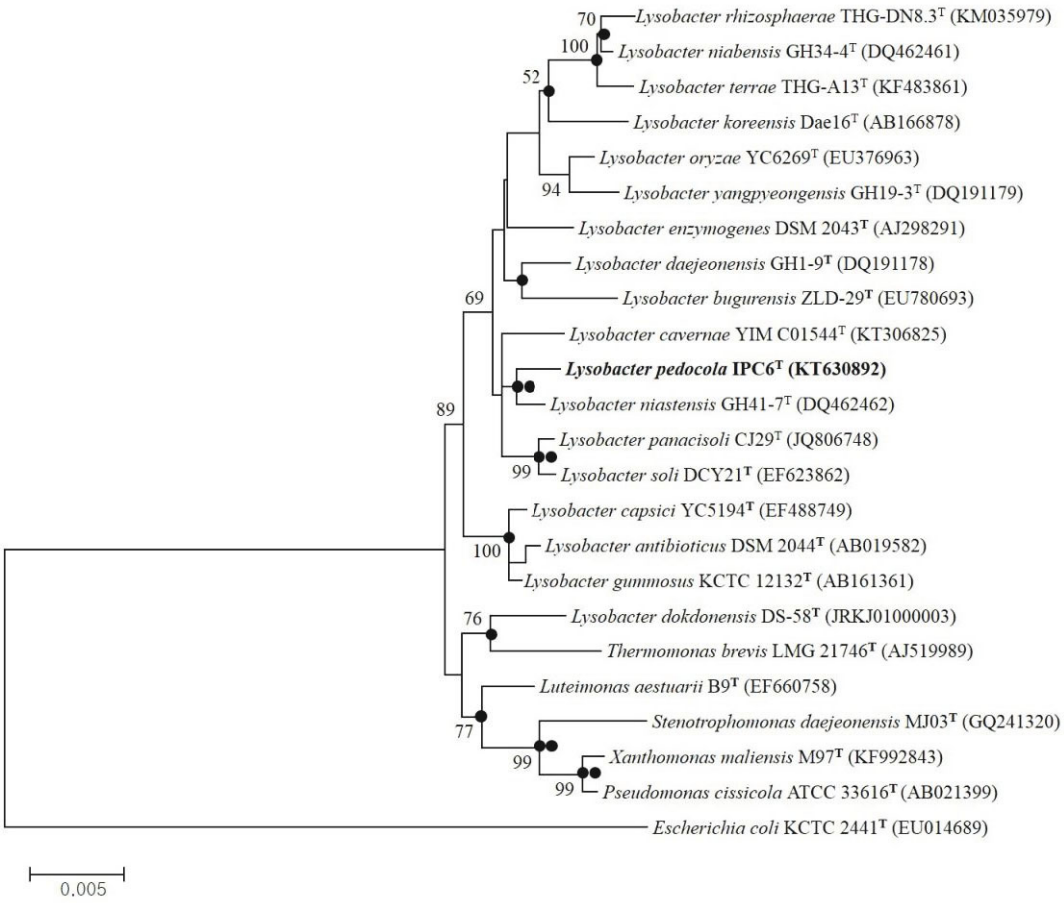

Fig. 1. Neighbor-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships between IPC6^T and members of *Lysobacter* genus. Evolutionary distances were computed using the Kimura 2-parameter method. Bootstrap values base on 1,000 replicates are expressed as percentages; greater than 50% are shown at branch points. The dots indicate that the corresponding branches were also recovered in both the maximum parsimony (MP) and maximum-likelihood (ML) trees. *Escherichia coli* KCTC 2441^T (EU014689) was used as the out-group. Numbers near the nodes indicate the bootstrap values (> 50%) calculated on the basis of NJ/MP/ML tree algorithms and expressed as a percentage of 1,000 replicates. Bar, 0.005 nucleotide substitutions per position.

water (80:12:15:4) as the first and second developing solvents, respectively. After total lipid separation, various lipids were observed by spraying with the appropriate detection reagents (Komagata and Suzuki, 1987). Total lipids were detected after spraying with molybdophosphoric acid solution followed by heating at 150°C for 10 min (Sigma-Aldrich). For fatty acid methyl ester analysis, cells were allowed to grow on R2A agar at 30°C for 3 days, after which two loops of well-grown cells were harvested. Fatty acids were identified following the protocol of the Sherlock Microbial Identification System V6.01 (MIS, database SBA6, MIDI Inc.). Extraction and analysis procedures were performed as described previously by Kuykendall *et al*. (1988). Polar lipids and isoprenoid quinones were identified according to previously reported methods (Collins and Jones, 1981; Hiraishi *et al*., 1996). Total genomic DNA was isolated following the method of CTAB/NaCl-standard protocol. The DNA GC content (mol%) was determined with a simple and rapid fluorimetric protocol (Gonzalez and Saiz-Jimenez, 2002) using a real-time PCR thermocycler (Rotor-Gene Q; Qiagen) and SYBR Green I (Life Technologies). Genomic DNA of the following species was used as calibration references: *Bacillus*

Table 1. Differential phenotypic characteristics of strain IPC6^T and its related species in the *Lysobacter* genus

Strains: 1, IPC6^T (data from this study); 2, *L. niastensis* KACC 11588^T (data from this study); 3, *L. panacisoli* KACC 17502^T (data from this study); 4, *L. soli* KCTC 22011^T (data from this study); 5, *L. gummosus* KCTC 12132^T (data from this study); 6, *Lysobacter cavernae* KCTC 42875^T (data from this study). All strains were Gram-negative and oxidase- and catalase-positive. +, positive; -, negative.

Characteristics	1	2	3	4	5	6
Motility	-	+	+	+	+	-
Colony	Yellow	Light beige	Bright yellow	Yellow	Pale yellow	Yellow
Temperature (°C) range for growth	10–42	10–37	10–30	10–37	10–40	10–37
pH range for growth	5.0–11.0	7.0–9.0	7.0–9.0	7.0–9.0	4.0–9.0	6.0–10.0
NaCl (%, w/v) range for growth	0–2.0	0–4.0	0–1.0	0–2.0	0–1.0	0–2.0
Nitrate reduction to N_2	-	-	+	+	-	-
Enzyme activity						
N–Acetyl-β-glucosaminidase	-	-	-	+	+	+
Arginine dihydrolase	-	-	+	-	-	-
α-Chymotrypsin	-	+	+	+	-	+
Cystine arylamidase	-	-	+	+	-	+
α-Galactosidase	-	-	-	-	+	-
β-Galactosidase (ONPG)	+	+	+	-	-	-
β-Galactosidase (PNPG)	+	+	+	-	+	+
α-Glucosidase	+	+	+	+	-	-
β-Glucosidase	+	+	+	+	+	-
Naphtol-AS-BI-phosphohydrolase	+	+	+	+	+	-
Assimilation of:						
Urease	-	+	+	-	-	-
Valine arylamidase	-	+	+	+	-	+
D-Glucose	+	-	-	+	+	+
Glycogen	+	-	-	-	+	+
D-Mannose	+	-	+	+	+	+
D-Melibiose	+	-	-	-	+	+
D-Sucrose	+	-	-	-	+	+
Acetate	+	+	+	+	-	+
Gluconate	+	-	-	+	+	+
D,L-3-Hydroxybutyrate	-	-	+	+	+	-
L-Malate	+	+	-	-	+	+
Phenyl acetate	-	-	-	-	-	+
Propionate	+	-	+	+	-	+
n-Valerate	+	-	+	+	+	+
L-Alanine	+	-	-	+	-	+
L-Histidine	+	-	-	-	-	-
L-Proline	-	+	+	+	+	+
L-Serine	+	+	-	+	+	+
myo-Inositol	+	-	-	-	-	-
D-Mannitol	+	-	-	-	-	-
D-Sorbitol	+	-	-	-	-	-
Salicin	-	-	-	-	+	+
DNA G + C content (mol%)	71.1	66.2	64.9	65.4	65.8	65.1

licheniformis KACC 10476^T, *Lactococcus lactis* KACC 13877^T, *Corynebacterium glutamicum* KACC 20786^T, *Bacillus subtilis* KACC 17796^T, *Pseudomonas aeruginosa* ATCC 15442^T, *Micrococcus luteus* KACC 13377^T, and *Escherichia coli* KACC 14818^T. The taxonomic relationships between IPC6^T and strains *Lysobacter niastensis* KACC 11588^T, *Lysobacter panacisoli* KACC 17502^T, *Lysobacter soli* KCTC 22011^T, *Lysobacter gummosus* KCTC 12132^T, and *Lysobacter cavernae* KCTC 42875^T were further examined using DNA-DNA hybridization based on the spectroscopic method by Loveland-Curtze *et al.* (2011). Experiments were performed in triplicate, and hybridization values expressed as Means ± SD of three independent measurements.

Results and Discussion

Phylogenetic analysis

Sequence analysis of the 16S rRNA gene revealed 96.2–98.5% sequence similarities of IPC6^T (1,444 bp) with members of the genus *Lysobacter*. Specifically, the novel strain showed > 97% sequence similarity with *Lysobacter niastensis* KACC 11588^T (98.5%), *Lysobacter panacisoli* KACC 17502^T (98.0%), *Lysobacter soli* KCTC 22011^T (96.9%), *Lysobacter gummosus* KCTC 12132^T (96.2%), and *Lysobacter cavernae* KCTC 42875^T (97.3%) similarity with other members of the genus *Lysobacter*. Accordingly, other strains of *Lysobacter* were used as references in this study. The neighbor-joining tree showed grouping of IPC6^T with members of *Lysobacter* (Fig. 1).

Phenotypic characteristics and morphological

Cells of IPC6^T were Gram-negative, aerobic and rod-shaped with sizes of 0.6–0.7 × 0.5–1.0 μm (Supplementary data Fig. S1). Colonies grew in a circular, convex and smooth manner with detectable yellow pigmentation on R2A agar plates. Strains grew on various media, including R2A agar (MB cell), trypticase soy agar (Difco), Luria-Bertani agar (Difco) and nutrient agar (Difco). The range of temperatures suitable for growth was 10–42°C with optimum growth at 30°C. The pH range for growth was 5.0–11.0 with optimum growth at pH 8.0, while the NaCl tolerance range was 0–2% (w/v). The strain was positive for the presence of oxidase and catalase. The biochemical, physiological and morphological characteristics that differentiate IPC6^T from closely related species in the *Lysobacter* genus are listed in Table 1.

Chemotaxonomic and genomic analyses

Cellular fatty acids of IPC6^T and other strains within *Lysobacter*, including *L. niastensis* KACC 11588^T, *L. panacisoli* KACC 17502^T, *L. soli* KCTC 22011^T, and *L. gummosus* KCTC 12132^T, are depicted in Table 2. All species contained iso-$C_{15:0}$, iso-$C_{16:0}$, and summed feature 9 (iso-$C_{17:1}$ *ω9c*), with $C_{16:1}$ *ω5c*, and iso-$C_{15:0}$ as the major cellular fatty acids. The predominant fatty acids of IPC6^T were similar to those of

Table 2. Cellular fatty acid profiles of IPC6^T and related species in *Lysobacter* genus

Strains: 1, IPC6^T (data from this study); 2, *L. niastensis* KACC 11588^T (data from this study); 3, *L. panacisoli* KACC 17502^T (data from this study); 4, *L. soli* KCTC 22011^T (data from this study); 5, *L. gummosus* KCTC 12132^T (data from this study); 6, *Lysobacter cavernae* KCTC 42875^T. Summed feature 1 consisted of $C_{13:0}$ 3OH and/or $C_{15:1}$ H; Summed feature 3 consisted of $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*; Summed feature 9 consisted of iso-$C_{17:1}$ *ω9c*. Values signify percentages of total fatty acids, and only fatty acids representing > 1% for at least one of the strains are shown. -, not detected or no data; ND, not determined; tr, trace amounts (< 1%).

Fatty acids	1	2	3	4	5	6*
Saturated						
Iso-$C_{11:0}$	5.9	4.9	4.3	3.4	4.1	*4.3
Iso-$C_{11:0}$ 3-OH	8.1	8.5	7.6	5.7	10.4	*7.0
$C_{14:0}$	tr	tr	1.0	tr	1.64	*-
Iso-$C_{14:0}$	2.2	1.9	2.0	2.5	-	*tr
Iso-$C_{15:0}$	24.3	34.4	29.5	22.0	28.5	*47.8
Anteiso-$C_{15:0}$	2.3	2.0	1.3	2.9	4.5	*3.5
$C_{16:0}$	2.6	1.2	3.6	tr	3.0	*1.9
Iso-$C_{16:0}$	18.9	12.9	13.9	17.6	4.7	*2.6
$C_{16:0}$ N alcohol	-	tr	1.3	tr	4.1	*-
Iso-$C_{17:0}$	5.0	4.6	7.5	3.5	6.3	*3.9
Iso-$C_{17:0}$ 3-OH	2.1	1.6	1.1	1.3	11.4	*-
Unsaturated						*
$C_{14:1}$ *ω5c*	2.5	1.9	1.6	1.8	14.4	*-
Iso-$C_{15:1}$	-	-	29.5	-	-	*-
Iso-$C_{15:1}$ F	1.3	-	2.2	tr	-	*-
Iso-$C_{15:1}$ *ω9c*	-	1.1	-	-	-	*tr
Iso-$C_{16:1}$ H	-	tr	tr	1.1	-	*-
Anteiso-$C_{17:1}$ *ω9c*	-	-	-	7.2	-	*-
Summed Feature 1	-	tr	-	1.14	-	*-
Summed Feature 3	3.0	4.5	2.7	4.0	2.6	*4.3
Summed Feature 9	19.4	15.6	18.3	15.5	8.6	*10.3

* Data from Chen *et al.* (2016).

other members of the *Lysobacter* genus. However, other strains of *Lysobacter* contained $C_{16:0}$ N-alcohol, which was not detected in IPC6^T. The predominant ubiquinone of IPC6^T was Q-8, typical of other strains of the *Lysobacter* genus. The polar lipids of IPC6^T included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), four an unknown aminophospholipid (APL), and an unidentified phospholipid (PL) (Supplementary data Fig. S2). DPG, PE, and PG have consistently been determined as the predominant polar lipids of other *Lysobacter* species (Park *et al*., 2008; Choi *et al*., 2014). The DNA G + C content of IPC6^T was 71.1 mol%, which lies within the range of all other species of *Lysobacter* with validly published names.

Strain IPC6^T displayed similarities to the following strains (with the degree of genetic similarity specified in parentheses): *Lysobacter niastensis* KACC 11588^T (37.9 ± 0.14%), *Lysobacter panacisoli* KACC 17502^T (56.4 ± 0.13%), *Lysobacter soli* KCTC 22011^T (8.1 ± 0.04%), *Lysobacter gummosus* KCTC 12132^T (9.6 ± 0.03%), and *Lysobacter cavernae* KCTC 42875^T (37.5 ± 0.14%). These values support the conclusion that strain IPC6^T represents a novel species distinct from closely related species of the genus *Lysobacter*. The DNA-DNA hybridization levels between IPC6^T and other strains were determined as lower than 70%, which represents the threshold for delineating a genomic species (Stackebrandt and Goebel, 1994).

Taxonomic conclusion

All characteristics determined for strain IPC6^T are in accordance with those of the genus *Lysobacter*. However, it could be differentiated from the closely related species by its phenotypic characteristics. Furthermore, the DNA-DNA relatedness values, lower than the accepted threshold, provided another means to distinguish IPC6^T and the type strains of the related *Lysobacter* species. Phylogenetic analyses based on 16S rRNA sequences, polar lipids, major fatty acid composition, predominant ubiquinone and the DNA G + C content further support the classification of IPC6^T as a member of *Lysobacter*. The strain was distinguishable from other *Lysobacter* species using a combination of biochemical and physiological features. The physiological characters that differentiating the strain IPC6^T from the other reference species are shown in Table 1. On the basis of the polyphasic data obtained, we suggest that strain IPC6^T represents a novel species in the *Lysobacter* genus, for which the name *Lysobacter pedocola* sp. nov. is proposed.

Description of *Lysobacter pedocola* sp. nov.

Lysobacter pedocola sp. nov. (*pe.do'co.la*. Gr. n. *pedon*, soil; L. suff. *-cola*, a dweller, inhabitant; N.L. n. *pedocola*, a soil dweller).

Cells of the novel bacterial strain IPC6^T are Gram-negative, aerobic, rod-shaped and 0.6–0.7 × 0.5–1.0 μm in size after cultivation at 30°C for 3 days on R2A agar plates. Colonies on R2A agar plates appear circular, convex, smooth and yellow-pigmented. Growth occurs on a wide range of agar media (R2A agar, trypticase soy, Luria-Bertani agar, yeast starch agar, and nutrient agar) at temperatures of 10–42°C (optimally at 30°C) and pH levels of 5.0–11.0 (optimally at pH 8.0) in the presence of 0–2% (w/v) NaCl. No growth is evident on MacConkey and marine agar plates. IPC6^T is oxidase- and catalase-positive, produces alkaline phosphatase, esterase (C4, weakly), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, and β-glucosidase. The strain is negative for lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-fucosidase, and α-mannosidase. According to API 20NE, cells are positive for esculin hydrolysis, gelatin hydrolysis, D-Mannose, D-Mannitol, N-acetyl-D-glucosamine, D-maltose, gluconate, adipate, L-malate, and citrate but negative for nitrate reduction, glucose fermentation, arginine dihydrolase activity, indole production, phenyl acetate and urea hydrolysis. IPC6^T and degrade casein, chitin and starch, but not DNA. IPC6^T can assimilate N-acetylglucosamine, inositol, D-sucrose, D-maltose, acetate, L-alanine, glycogen, L-serine, D-mannitol, D-glucose, D-melibiose, D-sorbitol, propionate, n-valerate and L-histidine but not L-rhamnose, D-ribose, itaconate, suberate, malonate, D,L-lactate, 5-ketogluconate, 3-hydroxybenzoate, salicin, L-fucose, L-arabinose, caprate, 2-ketogluconate, D,L-3-hydroxybutyrate, 4-hydroxybenzoate and L-proline. The major cellular fatty acid components are iso-$C_{15:0}$, iso-$C_{16:0}$, and summed feature 9 (iso-$C_{17:1}$ *ω9c*) while the major ubiquinone is Q-8. The polar lipids of IPC6^T include diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), four an unknown aminophospholipid (APL), and an unidentified phospholipid (PL). The DNA G + C content of IPC6^T is 71.1 mol%. The strain under investigation, IPC6^T, was isolated from an arid area of Goyang-si, Gyeonggi-do, South Korea (approximate coordinates: N′ 37.678347, E′ 126.806217). The NCBI GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IPC6^T (= KCTC 42811^T = JCM 31020^T) is KT630892.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

References

Bowman, J.P. 2000. Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int. J. Syst. Evol. Microbiol.* **50**, 1861–1868.

Buck, J.D. 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* **44**, 992–993.

Chen, W., Zhao, Y.L., Cheng, J., Zhou, X.K., Salam, N., Fang, B.Z., Li, Q.Q., Hozzein, W.N., and Li, W.J. 2016. *Lysobacter cavernae* sp. nov., a novel bacterium isolated from a cave sample. *Antonie van Leeuwenhoek* **109**, 1047–1053.

Choi, J.H., Seok, J.H., Cha, J.H., and Cha, C.J. 2014. *Lysobacter panacisoli* sp. nov., isolated from ginseng soil. *Int. J. Syst. Evol. Microbiol.* **64**, 2193–2197.

Christensen, P. and Cook, F.D. 1978. *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int. J. Syst. Evol. Microbiol.* **28**, 367–393.

Collins, M.D. and Jones, D. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol. Rev.* **45**, 316–354.

Felsenstein, J. 1985. Confidence limit 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. Biol.* **20**, 406–416.

Gonzalez, J.M. and Saiz-Jimenez, C. 2002. A fluorimetric method for the estimation of G + C mol% content in microorganisms by thermal denaturation temperature. *Environ. Microbiol.* **4**, 770–773.

Hiraishi, A., Ueda, Y., Ishihara, J., and Mori, T. 1996. Comparative lipoquinone analysis of influent sewage and activated sludge by highperformance liquid chromatography and photodiode array detection. *J. Gen. Appl. Microbiol.* **42**, 457–469.

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.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120.

Komagata, K. and Suzuki, K.I. 1987. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol.* **19**, 161–207.

Kuykendall, L.D., Roy, M.A., O'Neill, J.J., and Devine, T.E. 1988. Fatty acids, antibiotic resistance and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int. J. Syst. Bacteriol.* **38**, 358–361.

Loveland-Curtze, J., Miteva, V.I., Brenchley, J.E. 2011 Evaluation of a new fluorimetric DNA-DNA hybridization method. *Can. J. Microbiol.* **57**, 250–255.

Minnikin, D.E., O'Donnell, 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.

Oh, K.H., Kang, S.J., Jung, Y.T., Oh, T.K., and Yoon, J.H. 2011. *Lysobacter dokdonensis* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.* **61**, 1089–1093.

Park, J.H., Kim, R.M., 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.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Siddiqi, M.Z. and Im, W.T. 2016. *Lysobacter hankyongensis* sp. nov., isolated from activated sludge and *Lysobacter sediminicola* sp. nov., isolated from freshwater sediment. *Int. J. Syst. Evol. Microbiol.* **66**, 212–218.

Singh, H., Won, K., Du, J., Yang, J.E., Akter, S., Kim, K.Y., and Yi, T.H. 2015. *Lysobacter agri* sp. nov., a bacterium isolated from soil. *Antonie van Leeuwenhoek* **108**, 553–561.

Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization, pp. 607–654. *In* Gerhadt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. (eds.), Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, USA.

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.

Stackebrandt, E. and Goebel, B.M. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Evol. Microbiol.* **44**, 846–849.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

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., 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.