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MINI-REVIEW

Lysobacter gilvus sp. nov., isolated from activated sludge

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Abstract

Strain HX-5-24^T was isolated from the sludge collected from the outlet of the biochemical treatment facility of an agricultural chemical plant in Maanshan city, Anhui province, PR China (118° 28′ N, 31° 47′ E). Cells were observed to be Gram-reaction-negative, rod-shaped, non-motile and aerobic. Strain HX-5-24^T shared 99.1% 16S rRNA gene sequence similarity with *Lysobacter dokdonensis* DS-58^T and less than 97% similarities with other type strains. The phylogenetic analysis based on 16S rRNA indicated that strain HX-5-24^T belonged to the genus *Lysobacter* and formed a subclade with *L. dokdonensis* DS-58^T. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between strain HX-5-24^T and *L. dokdonensis* DS-58^T were 87.5% and 35.3%, respectively. The genomic DNA G + C content of the strain was 66.4%. The major fatty acids (> 5%) were iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, $C_{16:0}$ and summed feature 9 (iso-$C_{17:1}$ ω9c and/or $C_{16:0}$ 10-methyl). The predominant quinone was ubiquinone Q-8. The polar lipid profile consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phospholipids (PL). On the basis of phenotypic and phylogenetic evidences, strain HX-5-24^T is considered as a novel species in the genus *Lysobacter*, for which the name *Lysobacter gilvus* sp. nov. is proposed. The type strain is HX-5-24^T (= KCTC 72470^T = CCTCC AB 2019228^T).

Keywords *Lysobacter gilvus* sp. nov. · Sludge · Polyphasic taxonomy · 16S rRNA gene sequence

Introduction

The genus *Lysobacter*, belongs to the family of *Xanthomonadaceae* in the class *Gammaproteobacteria*, was first proposed by Christensen and Cook (1978) and emended by Park et al. (2008). At the time of writing, the genus embraces 48 strains with validly published names in the genus (https://www.bacterio.net/lysobacter.html). In this study, we investigated the taxonomic position of a *Lysobacter*-like strain, which was designed HX-5-24 ^{T}, using a polyphasic taxonomic approach.

Material and methods

Isolation and culture conditions

Strain HX-5-24^T was isolated from the sludge collected from the outlet of the biochemical treatment facility of an agricultural chemical factory in Maanshan city, Anhui province, PR China (118° 28′ N, 31° 47′ E), the outlet was located outside the factory and connected to a river. Isolation was carried out using the standard dilution plating method (Reasoner and

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences and the whole genome of strains HX-5-24^T are MN786796 and WOXT00000000, respectively.

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Geldreich 1985) on R2A (BD Difco). After incubation at 30 °C for 5 days, individual colonies were selected, purified and maintained on R2A at 30 °C, and preserved as glycerol suspensions (30%, w/v) at − 80 °C. A bacterial strain that formed pale yellow colonies, designated as HX-5-24^T, was selected for further study. The reference strain, *L. dokdonensis* KCTC 12822^T, was obtained from Korean Collection for Type Cultures (KCTC).

Phenotypic characteristics

Cell morphology and flagella were observed by transmission electron microscopy (H-7650; Hitachi) after 3 days of incubation on R2A at 30 °C. Motility was determined by hanging drop method (Bernadet et al. 2002). Gram staining was performed according to the standard Gram reaction (Powers 1995). The temperature range was tested at 4, 10, 15, 25, 30, 35, 37, 40 and 50 °C. Salt tolerance was measured in R2A supplemented with various concentrations of NaCl (0, 0.2, 0.4, 0.5, and 1.0–5.0% at intervals of 0.5%, w/v). The pH range was measured in R2A from pH 3.0 to 10.0 with an interval of 1.0 unit using the buffer system described by Xu et al. (2005). Growth on different medium was observed on R2A agar, nutrient agar (NA, BD Difco), tryptic soy agar (TSA, BD Difco), MacConkey agar (BD Difco) and lysogeny broth (LB, BD Difco). Anaerobic growth was assessed in serum bottles containing R2A broth supplemented with potassium nitrate (0.1%, w/v), the broth was prepared anaerobically under a nitrogen atmosphere (Zhang et al. 2012). Sensitivity to various antibiotics was tested on R2A using different antibiotic discs (μg per disc): amikacin (30), spectinomycin (100), neomycin (30), amoxicillin (10), rifampin (5), streptomycin (10), benzylpenicillin (10), ampicillin (25), tetracycline (30), erythromycin (15), vancomycin (30), gentamicin (10), chloramphenicol (30), norfloxacin (10), lincomycin (2), and kanamycin (30). Oxidase activity was determined using the oxidase reagent (bioMérieux). Catalase activity was tested by observing bubble production in 3% (v/v) H_2O_2 solution (Hiroyuki and Tsutomu 1983). Biochemical properties of HX-5-24^T were analyzed using API ZYM, API 20E (bioMérieux) and Biolog GEN III (Biolog) kits respectively, according to the manufacturer's instructions.

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA of strain HX-5-24^T was extracted using the method described by Marmu (1963). The 16S rRNA was amplified by PCR using a pair of universal primers, 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′) (Frank et al. 2008). The amplicon was cloned into pMD19-T (TaKaRa Biotechnology) and then sequenced using an automated sequencer (3730; Applied Biosystems). Comparison of 16S rRNA with related strains was conducted by EzTaxon server (https://www.ezbiocloud.net/identify) (Kim et al. 2012). Multiple alignments with corresponding sequences of the related strains were aligned using CLUSTAL X 1.83 (Thompson et al. 1994). Phylogenetic analyses were performed by MEGA software (version 7.0.26) (Kumar et al. 2016) using neighbor-joining (NJ) (Saitou and Nei 1987), minimum-evolution (ME) (Rzhetsky and Nei 1992), and maximum-likelihood (ML) (Felsenstein 1981) methods, with bootstrap values based on 1000 replications (Felsensten 1985). Evolutionary distance matrices were calculated using Kimura's two-parameter model (Kimura 1980).

The genome sequence of HX-5-24^T was determined using the sequencing platform (Illumina PE150) by Beijing Novogene Bioinformatics Technology Co., Ltd. The digital DNA–DNA hybridization (dDDH) between HX-5-24^T and *L. dokdonensis* DS-58^T was determined by genome-to-genome distance calculator (https://ggdc.dsmz.de/ggdc.php/) (Meier-Kolthoff et al. 2013). The average nucleotide identity (ANI) was calculated using the OrthoANIu algorithm (https://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017).

Chemotaxonomic characterization

Strain HX-5-24^T and the reference strain *L. dokdonensis* KCTC 12822^T were cultured in R2A broth at 30 °C. Cells were harvested in the exponential growth phases (OD_{600}, 0.7–0.8) and then lyophilized immediately for chemotaxonomic analyses. Respiratory quinones were extracted and purified as described by Collins et al. (1977) and analyzed by HPLC (Agilent 1260) as described before (Groth et al. 1996). The cellular fatty acids were extracted and analyzed according to the manufacturer's instructions of the Sherlock Microbial Identification System (MIS; MIDI) (Sasser 2006). Polar lipids analysis was carried out using two-dimensional TLC and HPLC (Minnikin et al. 1984).

Results and discussion

Phenotypic characteristics

Cell of strain HX-5-24^T was short rod, polar flagellum and spore were not observed, (Fig. S1), Gram stain reaction was negative. The strain was resistant to erythromycin and lincomycin, and sensitive to amikacin, spectinomycin, neomycin, amoxicillin, rifampin, streptomycin, benzylpenicillin, ampicillin, tetracycline, vancomycin, gentamicin, chloramphenicol, norfloxacin and kanamycin. A phenotypic and biochemical characteristics comparison between HX-5-24^T and *L. dokdonensis* KCTC 12822^T are summarized in Table 1.

Table 1 Comparative characteristics of strain HX-5-24^T and *L. dokdonensis* KCTC 12822^T

Characteristic	1	2
Colony color	Pale yellow	Light yellow
Growth temperature (°C)	10–35	4–38
Assimilation of		
Tween 40	−	+
N-Acetyl-D-galactosamine	+	−
N-Acetyl-D-glucosamine	+	−
D-Fructose	+	−
Gentiobiose	+	−
Turanose	+	−
Acetic acid	+	−
Propionic acid	+	−
Quinic acid	+	−
L-Aspartic acid	+	−
L-Histidine	+	−
D-Glucose 6-phosphate	+	−
Trypsin	+	−
DNA G+C content mol%	66.4	68.1

All data were obtained in this study except for assimilation of carbon sources and DNA G+C content of *L. dokdonensis* KCTC 12822^T from Oh et al. (2011). Both strains were positive for oxidase, L-alanine, L-glutamic acid, gelatin, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and acid phosphatase; both strains were negative for *β*-galactosidase, cellobiose, D-galactose, D-glucose, lactose, D-mannose, melibiose, D-sorbitol, trehalose, succinic acid monomethyl ester, citric acid, formic acid, D-galacturonic acid, DL-lactic acid, L-ornithine, L-serine, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-mannosidase and *α*-fucosidase. Strains: 1, HX-5-24^T; 2, *L. dokdonensis* KCTC 12822^T. +, Positive; −, negative

The other characteristics of HX-5-24 were shown in the species description. All negative reactions of Biolog GEN III, API 20E and API ZYM are listed in Table S1.

16S rRNA gene sequencing and phylogenetic analysis

The almost-complete 16S rRNA gene sequence of strain HX-5-24^T (1533 nts) was obtained, which was 100% identity with the sequence retrieved from the genome of strain HX-5-24^T. Strain HX-5-24^T displayed 99.1% 16S rRNA gene sequence identity with *L. dokdonensis* DS-58^T and less than 97% identities with other type strains. The phylogenetic tree based on NJ algorithm showed that strain HX-5-24^T was located within the genus *Lysobacter* and formed a distinct clade with the related type strain *L. dokdonensis* DS-58^T (Fig. 1). The overall affiliation was also supported by the ME and ML trees (Fig. S2).

The draft genome sequence of strain HX-5-24^T was acquired, the number of contigs was 8, and the N50 length was 0.78 Mb, the genome coverage was 100×. The size of the genome is 3.46 Mb, which is bigger than that of *L. dokdonensis* DS-58^T (3.27 Mb) (Oh et al. 2011). The genome contains 3280 coding sequences (CDSs), 2 complete rRNA genes, 52 tRNA genes, 2 23S rRNA and 2 identical copies of the 5S rRNA gene. The G+C content was 66.4 mol%, which was lower than that of *L. dokdonensis* DS-58^T (68.1%) (Oh et al. 2011). The ANI and dDDH value between strain HX-5-24^T and *L. dokdonensis* DS-58^T were 87.5% and 35.5%, respectively, which are clearly lower than the threshold (< 95% and 70%) generally accepted for species delineation (Goris et al. 2007; Meier-Kolthoff et al. 2013).

Chemotaxonomic characterization

The major respiratory quinones of HX-5-24^T was Q-8, which was consistent with other type strains in the genus *Lysobacter* (Weon et al. 2007; Choi et al. 2014). The predominant polar lipids included diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phospholipids (Fig. S4). As shown in Table 2, the major fatty acids (> 5%) of strain HX-5-24^T were iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, $C_{16:0}$ and iso-$C_{17:1}$ ω9c, while those of *L. dokdonensis* KCTC 12822^T were iso-$C_{11:0}$, iso-$C_{11:0}$3-OH, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:1}$ω9c. The fatty acid profile of strain HX-5-24^T were similar to that of strain *L. dokdonensis* KCTC 12822^T. However, some qualitative and quantitative differences in fatty acid concentrations could be observed between strains HX-5-24^T and *L. dokdonensis* KCTC 12822^T. Strain HX-5-24^T contains relatively higher levels of $C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:1}$ω9c, iso-$C_{17:0}$ and anteiso-$C_{15:0}$, and summed feature 3, and lower levels of iso-$C_{16:0}$, iso-$C_{11:0}$, iso-$C_{11:0}$ 3-OH, iso-$C_{14:0}$. In addition, $C_{16:0}$ alcohol N was detected in strain HX-5-24^T but absent in strain *L. dokdonensis* KCTC 12822^T.

Taxonomic conclusion

In summary, the phylogenetic analysis showed that strain HX-5-24^T belongs to the genus *Lysobacter*, and is most closely related to type stain *L. dokdonensis* DS-58^T. However, strain HX-5-24^T could be clearly distinguished from *L. dokdonensis* DS-58^T in terms of colony color, growth temperature, assimilation of carbon sources (Table 1), DNA G+C content and fatty acid profile (Table 2). In addition, the ANI and dDDH values between strain HX-5-24^T and *L. dokdonensis* KCTC 12822^T were significantly below the proposed cut-offs for a species boundary. Therefore, strain HX-5-24^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter gilvus* sp. nov. is proposed.

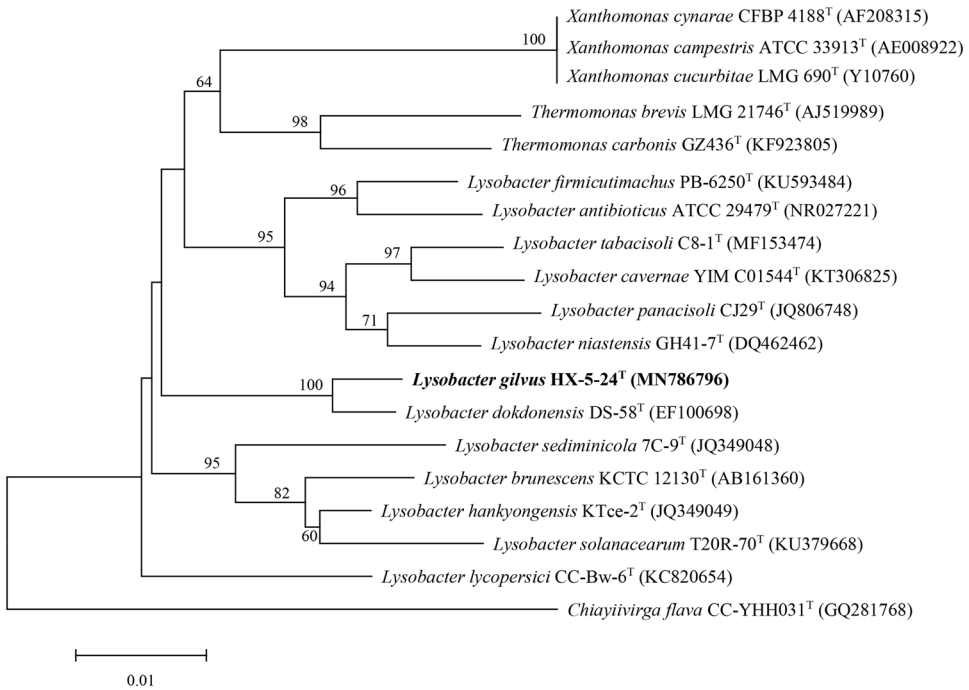

Fig. 1 Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain HX-5-24^T and closely related species. Numbers at nodes represent percentages of bootstrap support based on a neighbor-joining analysis of 1000 resampled datasets. Values below 50% are not indicated at branch points. Bar, 0.01 substitutions per nucleotide position

Table 2 Cellular fatty acids profiles (%) of the strains HX-5-24^T (strain 1) and *L. dokdonensis* KCTC 12822^T (strain 2)

Fatty acid	1	2
$C_{14:0}$	1.1	tr
$C_{15:0}$	–	tr
$C_{16:0}$	**6.3**	1.9
iso-$C_{11:0}$	4.2	**5.9**
iso-$C_{11:0}$ 3-OH	4.3	**7.8**
iso-$C_{14:0}$	2.8	4.6
iso-$C_{15:0}$	**20.7**	**18.7**
anteiso-$C_{15:0}$	**6.8**	4.9
iso-$C_{16:1}$ H*	tr	1.1
iso-$C_{16:0}$	**15.9**	**30.5**
$C_{16:0}$ alcohol N	4.5	–
iso-$C_{17:0}$	3.5	2.5
$C_{16:1}\omega7c$ alcohol	tr	–
iso-$C_{17:1}\omega9c$	**19.6**	**14.0**
$C_{17:0}$	tr	–
Summed feature 3[a]	4.1	2.8

All data were obtained from this study. Only fatty acids that account for > 1% are listed. Values > 5% are given in bold

tr trace (< 1%), – not detected

[a]Summed feature 3 contains C16:1 ω7c and/or C16:1 ω6c

Description of *Lysobacter gilvus* sp. nov.

Lysobacter gilvus (gil′vus. L. masc. adj. *gilvus* pale yellow, referring to the pale yellow pigmentation of the type strain).

Cells are Gram-negative, non-spore-forming, aerobic, rod-shaped (0.4–0.5 × 1.0–1.1 μm) and non-flagellated. Growth occurs on R2A, NA and LB. Colonies are circular, convex, slimy and pale yellow on R2A. Growth is observed at 10–35 °C (optimum 30 °C), at pH 6.0–8.0 (optimum 7.0) and with 0–0.5% (w/v) NaCl (optimum at 0.2%). Catalase and oxidase tests are positive. In API 20E tests, positive for hydrolysis of gelatin, and negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylases, citrate utilization, H_2S production, urease, tryptophan deaminase, indole production, Voges–Proskauer, D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin and L-arabinose. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase, cystine arylamidase and trypsin, and negative for lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}$ ω9c, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and $C_{16:0}$. The respiratory quinone is Q-8. The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phospholipids. The DNA G + C content is 66.4%.

The type strain, HX-5-24^T (= KCTC 72470^T = CCTCC AB 2019228^T) was isolated from activated sludge of agricultural chemical plant in Nanjing, Jiangsu province, PR China.

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