Hymenobacter deserti sp. nov., isolated from the desert of Xinjiang, China

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A Gram-negative, rod-shaped, non-motile and pink-pigmented bacterium, designated strain ZLB-3^T, was isolated from a desert soil sample collected from Xinjiang Province, China, and characterized by using a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate belongs to the phylum *Bacteroidetes* and is related to the genus *Hymenobacter*. 16S rRNA gene sequence similarities between strain ZLB-3^T and the type strains of *Hymenobacter* species with validly published names ranged from 90.0 to 94.6 %. No other taxa in the phylum *Bacteroidetes* showed more than 90 % sequence similarity to the isolate. The strain contained MK-7 as the predominant menaquinone. The major fatty acids were iso-C_{15:0} (27.8 %), anteiso-C_{17:1} B/iso-C_{17:1} I (25.2 %), iso-C_{17:0} 3-OH (9.6 %) and C_{16:1}ω7c/iso-C_{15:0} 2-OH (8.5 %). Phosphatidylethanolamine, two unknown aminophospholipids, an unknown aminolipid, an unknown glycolipid and six unknown polar lipids were detected in the polar lipid profile. The DNA G + C content was 58.6 mol%. These chemotaxonomic data supported the affiliation of strain ZLB-3^T to the genus *Hymenobacter*. However, the results of physiological and biochemical tests allowed phenotypic differentiation of the isolate from recognized *Hymenobacter* species. On the basis of the evidence presented, it is proposed that strain ZLB-3^T represents a novel species, *Hymenobacter deserti* sp. nov. The type strain is ZLB-3^T (=CCTCC AB 207171^T =NRRL B-51267^T).

The genus *Hymenobacter* was first described by Hirsch *et al.* (1998) and its description was subsequently emended by Buczolits *et al.* (2006). At the time of writing, it comprises 11 recognized species, including the recently described species *Hymenobacter soli* (Kim *et al.*, 2008) and *Hymenobacter psychrotolerans* (Zhang *et al.*, 2008). In this study, we report a pink-pigmented bacterial strain, ZLB-3^T, isolated from a desert area in Xinjiang Province, PR China, in the course of environmental investigation. On the basis of phenotypic characteristics, chemotaxonomic data and phylogenetic analysis of the 16S rRNA gene sequence, the isolate represents a novel species of *Hymenobacter*.

Strain ZLB-3^T was isolated from a desert soil sample. The soil sample was thoroughly suspended in sterilized water and the suspension was spread on tenfold-diluted tryptic soy broth (0.1 × TSB) agar plates [3.0 g TSB (Difco) l⁻¹ solidified with 15.0 g agar l⁻¹, pH 7.0] after serial dilution with sterilized water. Isolation was achieved after incubation at 28 °C for 1 week. Isolate ZLB-3^T was routinely

cultivated on the same medium at 30 °C and stored as a glycerol suspension (20 %, v/v) at –80 °C.

Chromosomal DNA was isolated and purified by using a Bacteria Genomic DNA isolation kit (ChaoShi-Bio). Primers 27f (5'-GAGTTTGATCCTGGCTCAG-3') and 1527r (5'-AGAAAGGAGGTGATCCAGCC-3') were used for amplification of the 16S rRNA gene (Lane, 1991). PCR and 16S rRNA gene sequencing were carried out as described by Lin *et al.* (2004). An almost-complete 16S rRNA gene sequence was obtained (1427 bp) and compared with sequences deposited in the GenBank database. The sequences were aligned using CLUSTAL_X software (Thompson *et al.*, 1997) and distances were calculated according to Kimura's two-parameter method (Kimura, 1980). Phylogenetic trees were inferred using the neighbour-joining method (Saitou & Nei, 1987) and bootstrap analysis was performed, based on 1000 resamplings (Felsenstein, 1985). The MEGA package version 3.1 (Kumar *et al.*, 2004) was used for all analyses.

Comparative 16S rRNA gene sequence analysis showed that strain ZLB-3^T was most closely related to members of the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ZLB-3^T is EU325941.

genus *Hymenobacter*. In a phylogenetic tree based on the neighbour-joining algorithm, strain ZLB-3^T joined the clade comprising *Hymenobacter* species at a bootstrap confidence value of 100 % (Fig. 1). Strain ZLB-3^T showed the highest 16S rRNA gene sequence similarity to *Hymenobacter ocellatus* Myx 2105^T (94.6 %); this value is well below the threshold for demarcating bacterial species (Stackebrandt & Goebel, 1994). 16S rRNA gene sequence similarities between strain ZLB-3^T and the type strains of other *Hymenobacter* species ranged from 90.0 to 92.3 %. No other recognized bacterial species showed more than 90 % 16S rRNA gene sequence similarity to the new isolate. These results suggest that strain ZLB-3^T represents a novel species within the genus *Hymenobacter*.

Cell morphology was examined by using phase-contrast microscopy (BX51 microscope; Olympus). The Gram reaction was carried out according to the classical Gram procedure described by Doetsch (1981). Gliding motility was examined as described by Bowman (2000). Growth was assessed at 4, 10, 25, 30, 37, 40 and 42 °C, at pH 4–11 (increments of 1 pH unit) and at 0, 1, 2, 3, 5 and 10 % NaCl. Oxidase activity was tested using a 1 % solution of tetramethyl-*p*-phenylenediamine (Kovács, 1956). Catalase activity was detected by assessing the production of bubbles after the addition of a drop of 3 % H₂O₂. Hydrolysis of starch and casein was tested as described by Smibert & Krieg (1994). Other physiological properties and enzyme activities were tested using the API 20E, API 20NE and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Antibiotic-sensitivity tests were performed by spreading bacterial suspensions on culture plates and applying discs impregnated with different antibiotics (Buczolits *et al.*, 2002).

The ability of strain ZLB-3^T to utilize 95 different compounds was tested by using the Biolog GN2 automatic identification system. In order to compare its ability to utilize carbon sources with other species of the genus *Hymenobacter*, the type strains of five *Hymenobacter* species were used as reference strains: *H. ocellatus* DSM 11117^T and

Hymenobacter chitinivorans DSM 11115^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), *Hymenobacter rigui* KCTC 12533^T was obtained from the Korean Collection for Type Cultures (Taejeon, Korea), *Hymenobacter norwichensis* NS/50^T was obtained from Dr Hans-Jürgen Busse (Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität Wien, Austria) and *Hymenobacter xinjiangensis* CCTCC AB 206080^T was obtained from the China Center for Type Culture Collection (Wuhan, PR China). All strains were grown on R2A agar at 30 °C for 48 h and suspended in pre-warmed sterile GN/GP-IF medium [0.40 % NaCl, 0.03 % Pluronic F-68 (Sigma), 0.02 % gellan gum (Sigma)] within the density range specified by the manufacturer (determined with a Biolog model 21907 photometer). Biolog GN2 MicroPlates were inoculated with 150 µl cell suspension per well. Inoculated Biolog GN2 plates were incubated at 30 °C for 24 h and the results were read with a MicroPlate reader, using MicroLog 3 computer software to perform automated reading and identification.

Strain ZLB-3^T formed visible colonies on 0.1 × TSB agar after incubation at 30 °C for 48 h. The strain grew well on nutrient-reduced media such as 0.1 × TSB agar, R2A and PYES agar, but did not grow on TSA or marine agar 2216 (Difco). Colonies were pink, convex and circular. Cells were Gram-negative, strictly aerobic, non-motile, 1.1–2.5 µm long and 0.6–0.8 µm wide. The physiological and biochemical characteristics of strain ZLB-3^T are summarized in the species description and a comparison of selective characteristics with related type strains is given in Table 1.

The DNA G + C content of strain ZLB-3^T was determined by HPLC (UltiMate 3000; Dionex) according to the method of Mesbah *et al.* (1989). Respiratory quinones were extracted and identified by HPLC as described by Xie & Yokota (2003). For fatty acid methyl ester analysis, strain ZLB-3^T and the five reference strains were grown on R2A agar at 30 °C for 48 h. Fatty acids were extracted, methylated and analysed according to the standard

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain ZLB-3^T and related taxa. Bootstrap values (1000 replications) are shown as percentages at each node only if they are 50 % or greater. The sequence of *Flavobacterium aquatile* ATCC 11947^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Phenotypic characteristics that differentiate strain ZLB-3^T from related members of the genus *Hymenobacter*

Strains: 1, strain ZLB-3^T; 2, *H. ocellatus* Myx 2105^T; 3, *H. norwichensis* NS/50^T; 4, *H. chitinivorans* Txc1^T; 5, *H. xinjiangensis* X2-1g^T; 6, *H. rigui* WPCB131^T. Data were taken from Buczolits *et al.* (2006), Baik *et al.* (2006), Zhang *et al.* (2007) and this study. All data for single carbon-source utilization on Biolog GN2 plates were taken from this study using the strains indicated in the text. +, Positive; −, negative; (+), weakly positive; NR, not reported. All strains were negative for α-galactosidase and β-galactosidase.

Characteristic	1	2	3	4	5	6
Growth at/in:						
4 °C	(+)	−	(+)	+	+	+
37 °C	+	+	−	−	(+)	+
1 % NaCl	(+)	+	−	(+)	−	+
3 % NaCl	−	+	−	(+)	−	−
Carbon-source utilization on Biolog GN2						
N-Acetyl-D-glucosamine	(+)	−	−	−	(+)	−
Cellobiose	(+)	−	(+)	−	+	+
i-Erythritol	+	−	−	−	+	−
Gentiobiose	−	(+)	−	(+)	−	+
Lactulose	(+)	−	+	−	+	−
D-Mannose	+	(+)	+	−	(+)	−
Trehalose	+	−	(+)	(+)	+	+
Acetic acid	+	−	−	−	+	−
Formic acid	−	−	−	(+)	−	+
D-Glucosaminic acid	−	−	−	(+)	+	(+)
α-Ketobutyric acid	+	−	−	−	−	−
L-Serine	+	−	−	−	+	−
Glucose 6-phosphate	(+)	−	−	(+)	−	(+)
Enzyme activity (API ZYM)						
Esterase (C4)	−	−	(+)	(+)	+	+
Valine arylamidase	+	−	(+)	−	+	+
Cystine arylamidase	+	−	−	−	+	(+)
Acid phosphatase	+	−	(+)	−	+	+
α-Glucosidase	−	−	−	−	−	+
N-Acetyl-β-glucosaminidase	+	−	−	−	+	+
α-Mannosidase	−	−	−	−	+	−
DNA G + C content (mol%)	58.6	65	NR	61	54	65

protocol of the Sherlock Microbial Identification System (MIDI). Polar lipids were extracted and analysed as described by Tindall (1990) and Ventosa *et al.* (1993).

The DNA G + C content of strain ZLB-3^T was 58.6 mol%, which is consistent with the range reported for the genus *Hymenobacter* (55–65 mol%; Buczolits *et al.*, 2006). The predominant isoprenoid quinone was MK-7. The major fatty acids were iso-C_{15:0} (27.8 %), summed feature 4 (anteiso-C_{17:1} B/iso-C_{17:1} I) (25.2 %), iso-C_{17:0} 3-OH (9.6 %) and summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH) (8.5 %). The fatty acid profile of the new isolate was similar to those of *Hymenobacter* species. However, some obvious quantitative differences were observed. The novel strain differed from the other *Hymenobacter* species especially in the relative amounts of iso-C_{17:0} 3-OH, C_{16:1}ω5c, anteiso-C_{15:0} and summed feature 3. Detailed fatty acid profiles are shown in Table 2. The polar lipid profile (Fig. 2) consisted mainly of phosphatidylethanolamine, two unknown aminophospholipids (APL1, APL2) and an unknown polar lipid (L3). An unknown aminolipid (AL1), an unknown glycolipid (GL1) and three unknown polar lipids (L2, 5, 6) occurred at

moderate levels. Trace amounts of two further unknown polar lipids (L1, 4) were also detected. The polar lipid profile may be useful for confirming strain ZLB-3^T as a representative of a novel species within the genus *Hymenobacter*.

In summary, the characteristics of strain ZLB-3^T are consistent with the description of the genus *Hymenobacter* with regard to morphological, biochemical and chemotaxonomic properties. However, on the basis of phylogenetic distance from known *Hymenobacter* species indicated by 16S rRNA gene sequence similarities and the combination of unique phenotypic characteristics (Table 1), strain ZLB-3^T should be placed in the genus *Hymenobacter* as a representative of a novel species, for which the name *Hymenobacter deserti* sp. nov. is proposed.

Description of *Hymenobacter deserti* sp. nov.

Hymenobacter deserti (de.ser'ti. L. gen. n. *deserti* of a desert).

Cells are Gram-negative, rod-shaped, non-spore-forming, 1.1–2.5 µm long and 0.6–0.8 µm wide. Motility is not

Table 2. Cellular fatty acid compositions of the type strains of *Hymenobacter* species

Strains: 1, strain ZLB-3^T (data from this study); 2, *H. ocellatus* DSM 11117^T (this study); 3, *H. soli* PB17^T (Kim *et al.*, 2008); 4, *H. roseosalivarius* AA-718^T (Buczolits *et al.*, 2002); 5, *H. norwichensis* NS/50^T (this study); 6, *H. chitinivorans* DSM 11115^T (this study); 7, *H. actinosclerus* CCUG 39621^T (Buczolits *et al.*, 2002); 8, *H. aerophilus* I/26-Cor1^T (Buczolits *et al.*, 2002); 9, *H. psychrotolerans* Tibet-IIU11^T (Zhang *et al.*, 2008); 10, *H. gelipurpurascens* Txg1^T (Buczolits *et al.*, 2006); 11, *H. xinjiangensis* CCTCC AB 206080^T (this study); 12, *H. rigui* KCTC 12533^T (this study). Values are percentages of total fatty acids; only fatty acids accounting for more than 0.5 % in at least one of the strains are indicated. ND, Not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
C _{14:0}	ND	ND	1.2	ND	1.6	1.1	ND	ND	0.8	ND	0.9	1.0
iso-C _{14:0}	0.1	0.5	ND	ND	0.3	0.4	ND	ND	ND	ND	0.7	0.3
iso-C _{15:1} G	0.3	0.7	ND	ND	0.9	0.7	ND	ND	1.8	0.8	ND	ND
anteiso-C _{15:1} A	0.1	0.5	ND	ND	0.4	0.3	ND	ND	0.6	1.1	0.3	0.2
iso-C _{15:0}	27.8	27.5	51.8	8.3	18.9	22.3	22.3	10.8	24.5	17.3	22.5	25.1
anteiso-C _{15:0}	1.6	6.8	6.2	ND	6.8	6.4	25.8	22.3	8.4	23.1	4.6	5.2
iso-C _{16:1} H	1.1	0.8	1.0	2.7	1.6	2.3	1.5	1.5	1.2	3.7	2.4	1.7
iso-C _{16:0}	0.8	0.9	ND	2.1	0.8	1.5	ND	0.8	2.1	2.7	1.8	0.7
C _{16:1} ω5c	4.9	7.5	11.0	23.3	12.5	15.2	3.7	7.9	12.7	11.3	20.5	14.2
C _{16:0}	2.0	0.6	4.2	1.1	2.6	1.4	ND	1.7	2.3	ND	1.3	2.1
iso-C _{15:0} 3-OH	5.1	3.5	2.1	2.7	3.1	2.9	1.6	1.6	2.7	2.3	3.2	4.1
iso-C _{17:1} ω9c	0.7	1.1	ND	ND	0.2	0.7	ND	ND	ND	ND	0.2	0.2
iso-C _{17:0}	2.1	3.1	1.1	1.7	2.3	1.8	1.8	4.5	0.4	1.7	1.3	1.9
anteiso-C _{17:0}	0.4	0.9	ND	ND	0.5	0.4	0.7	2.3	ND	2.0	0.3	0.3
C _{17:1} ω6c	0.8	1.2	ND	1.1	0.6	0.8	0.7	ND	ND	ND	0.6	0.4
iso-C _{16:0} 3-OH	0.6	0.3	ND	1.0	0.3	0.3	ND	ND	ND	ND	0.4	0.2
C _{16:0} 3-OH	0.6	0.4	1.0	1.2	0.8	0.9	ND	ND	1.4	ND	1.3	1.0
iso-C _{17:0} 3-OH	9.6	7.0	1.2	5.8	4.3	4.1	3.1	3.5	3.5	3.7	4.5	4.5
C _{17:0} 2-OH	0.3	0.2	ND	ND	0.4	0.3	2.0	1.3	ND	1.7	0.3	0.2
Summed feature 2*	1.8	2.6	0.9	ND	1.4	1.5	2.3	0.8	1.5	ND	1.0	2.5
Summed feature 3*	8.5	9.6	14.4	29.8	24.1	15.0	13.1	21.4	20.0	17.6	19.9	15.8
Summed feature 4*	25.2	14.1	3.5	18.5	7.3	13.5	19.9	17.7	14.0	9.4	5.7	9.7

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 contains C_{13:0} 3-OH and/or iso-C_{15:1} I; summed feature 3 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 contains anteiso-C_{17:1} B and/or iso-C_{17:1} I.

observed. Colonies grown on 0.1 × TSB agar for 5 days are circular, 1–2 mm in diameter, convex, pink and smooth. Growth occurs at 4–40 °C (optimum 30 °C), 0–2 % (w/v) NaCl (optimum 0 %) and pH 5–11 (optimum pH 7). Oxidase- and catalase-positive. Gelatin, starch and casein are hydrolysed, but aesculin is not. Nitrate reduction is weakly positive without production of N₂. H₂S, indole and acetoin (Voges–Proskauer reaction) are not produced. Activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease are not detected. Biolog GN2 tests show that the type strain oxidizes α-cyclodextrin, dextrin, glycogen, N-acetyl-D-glucosamine, L-arabinose, cellobiose, i-erythritol, D-fructose, D-galactose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, D-sorbitol, trehalose, turanose, monomethyl succinate, acetic acid, citric acid, D-galacturonic acid, α-ketobutyric acid, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-serine, L-threonine, uridine and glucose 6-phosphate. Does not oxidize Tweens 40 or 80, N-acetyl-D-galactosamine, adonitol, D-arabitol, L-fucose, gentio-

biose, myo-inositol, D-mannitol, melibiose, methyl β-D-glucoside, D-psicose, raffinose, sucrose, cis-aconitic acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-, β- or γ-hydroxybutyric acids, p-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebamic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate or glucose 1-phosphate. According to the API ZYM gallery (bioMérieux), produces alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase, but not esterase (C4), lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase or α-fucosidase.

Fig. 2. Polar lipid profile of strain ZLB-3^T after separation in two-dimensional TLC. PE, Phosphatidylethanolamine; APL1–2, unknown aminophospholipids; AL1, unknown aminolipid; GL1, unknown glycolipid; L1–6, unknown polar lipids.

Susceptible to chloramphenicol, erythromycin, gentamicin, penicillin G, polymyxin B sulfate, tetracycline, kanamycin and vancomycin. The predominant menaquinone is MK-7. The major fatty acids are iso-C_{15:0}, summed feature 4 (anteiso-C_{17:1} B/iso-C_{17:1} I), iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1} ω7c/iso-C_{15:0} 2-OH). The polar lipid profile is composed of the major compound phosphatidylethanolamine and two unknown aminophospholipids, an aminolipid, a glycolipid and six polar lipids. The DNA G + C content of the type strain is 58.6 mol%.

The type strain, ZLB-3^T (=CCTCC AB 207171^T =NRRL B-51267^T), was isolated from the surface layer of a desert soil from Xinjiang Province, China.

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