Pontibacter populi sp. nov., isolated from the soil of a Euphrates poplar (*Populus euphratica*) forest

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A Gram-negative-staining, rod-shaped, non-motile and pink bacterial strain was isolated from the soil of a *Populus euphratica* forest located in Xinjiang, China. The strain, designated strain HYL7-15^T, was subjected to a taxonomic analysis using a polyphasic approach. 16S rRNA gene sequence analyses indicated that the isolate belonged to the phylum *Bacteroidetes* and was related to the genus *Pontibacter*, with sequence similarities ranging from 93.1 to 95.0% with other species of the genus *Pontibacter*. Strain HYL7-15^T contained MK-7 as the predominant menaquinone and its DNA G+C content was 44.9 mol%. The major cellular fatty acids of the novel strain were iso-C_{15:0} (16.49%), iso-C_{17:0} 3-OH (10.96%) and summed feature 4 (comprising anteiso-C_{17:1} B and/or iso-C_{17:1} I, 18.46%). The major polar lipids were phosphatidylethanolamine (PE) and phosphatidylglycerol (PG); diphosphatidylglycerol (DPG), three unknown aminophospholipids (APLs) and two unknown phospholipids (PLs) were also detected. On the basis of the evidence presented, it is concluded that strain HYL7-15^T represents a novel species of the genus *Pontibacter*, for which the name *Pontibacter populi* sp. nov. is proposed. The type strain is HYL7-15^T (=CCTCC AB 206239^T=NRRL B-59488^T).

The genus *Pontibacter*, which belongs to the phylum *Bacteroidetes*, was first described by Nedashkovskaya *et al.* (2005). At the time of writing, the genus *Pontibacter* comprised five species with validly published names, including *Pontibacter actiniarum* (Nedashkovskaya *et al.*, 2005), *P. akesuensis* (Zhou *et al.*, 2007), *P. korlensis* (Zhang *et al.*, 2008), *P. xinjiangensis* (Wang *et al.*, 2010) and *P. roseus* (Suresh *et al.*, 2006; Wang *et al.*, 2010). An additional species, *Pontibacter niistensis*, had been proposed in a paper in press in *IJSEM* and the name has subsequently been validly published (Dastager *et al.*, 2010). The type strains of the recognized species of the genus are Gram-negative, rod-shaped, aerobic, heterotrophic and pink-coloured bacteria. Species of the genus *Pontibacter* have been isolated from marine actinians, desert soil, soil, muddy water and forest soil. In this study, the taxonomic characterization of a Gram-negative-staining strain, HYL7-15^T, is described.

Strain HYL7-15^T was isolated from a soil sample from a *Populus euphratica* forest in Xinjiang, China. The soil sample was diluted with sterile water and the dilutions

were plated onto 0.3 × marine broth 2216 (MB, Difco) agar plates. The strain was isolated after incubation for two days at 30 °C.

Cell morphology was examined by phase-contrast microscopy (BX51; Olympus). Gram staining of cells was carried out according to the classical Gram procedure described by Doetsch (1981). Gliding motility was determined as described by Bowman (2000). Oxidase activity was determined using 1% solution of tetramethyl-*p*-phenylenediamine (Kovács, 1956). Catalase activity was determined by measurements of bubble production after the application of 3% (v/v) hydrogen peroxide solution. Tests were also made for hydrolysis of starch (1%, w/v) and casein (5%, w/v) as described by Smibert & Krieg (1994). Tolerance of NaCl concentrations (0–6%, w/v) was determined using increments of 1% NaCl. Growth was examined at different temperatures (4, 10, 20, 28, 30, 37 and 42 °C) and pH values (5.0–11.0 with 1.0 pH unit increments). Carbon-source oxidation was investigated by using the Biolog GN2 Microplate system as recommended by the manufacturer. Additional enzyme activities and biochemical features were determined by API kits (API 20NE and API ZYM) according to the manufacturer's instructions (bioMérieux). The phenotypic features of strain HYL7-15^T are given in the species description and in Table 1.

Abbreviations: APL, aminophospholipids; DPG, diphosphatidylglycerol; FAME, fatty acid methyl esters; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the strain HYL7-15^T is HQ223078.

Table 1. Phenotypic comparison between strain HYL7-15^T and other species of the genus *Pontibacter*

Taxa: 1, HYL7-15^T; 2, *P. xinjiangensis* NRRL B-51335^T; 3, *P. actiniarum* KMM 6156^T; 4, *P. akesuensis* AKS 1^T; 5, *P. korlensis* X14-1^T; 6, *P. roseus* SRC-1^T. Data for reference strains were taken from Wang *et al.* (2010), Nedashkovskaya *et al.* (2005), Zhou *et al.* (2007), Zhang *et al.* (2008) and Suresh *et al.* (2006). +, Positive; –, negative; ND, not reported.

Characteristic	1	2	3	4	5	6
Habitat	Soil	Soil	Marine actinians	Soil	Soil	Muddy water
Cell size (µm)	0.4–0.6 × 1.2–2.2	0.4–0.6 × 1.2–2.0	0.3–0.4 × 1.2–1.9	0.7–0.75 × 1.5–1.6	0.6–0.7 × 1.3–3.5	0.3–0.5 × 1.0–3.0
Gliding Motility	–	–	+	–	+	–
Colony colour	Pink	Pink	Pink	Pink	Pink	Dark pink
Oxidase	+	–	+	+	+	+
Growth on:						
4 °C	+	–	+	–	+	–
42 °C	–	+	–	+	–	+
Salinity range (%)	0–4	0–10	0–5	0–10	0–4	0–8
Hydrolysis of:						
Aesculin	+	+	+	+	+	–
Gelatin	+	+	+	+	+	+
Starch	+	+	–	+	+	+
Casein	+	+	–	–	–	+
Carbon-source oxidation (Biolog GN2):						
Cellobiose	–	+	–	+	+	+
D-Fructose	–	+	–	–	+	+
L-Fucose	–	–	–	+	–	ND
D-Galactose	–	+	–	–	+	+
α-D-Glucose	–	+	–	–	+	+
α-Lactose	–	+	–	–	+	+
Sucrose	+	+	–	+	+	+
Maltose	–	+	–	+	+	+
D-Mannitol	–	+	–	+	–	–
D-Mannose	+	+	–	–	+	ND
α-Ketobutyric acid	+	–	+	–	–	–
L-Histidine	–	–	–	+	–	–
L-Pyroglutamic acid	–	–	–	+	–	–
α-D-Glucose	–	+	–	–	+	ND
1-Phosphate						
D-Glucose	+	+	–	+	+	+
6-phosphate						
Enzyme activity (API ZYM):						
Lipase (C14)	–	–	–	–	–	+
Trypsin	+	+	+	+	+	–
α-Chymotrypsin	+	+	+	–	+	–
α-Galactosidase	–	–	+	–	–	+
β-Galactosidase	–	–	+	–	–	+
α-Glucosidase	–	–	–	+	+	+
β-Glucosidase	–	–	–	–	–	+
N-Acetyl-β-glucosaminidase	–	–	+	+	+	+
DNA G + C content (mol%)	44.9	47.8	48.7	51.4	48.2–48.9	52.0–52.3

Genomic DNA was isolated from freshly grown culture following the methods of Sambrook *et al.* (1989). PCR amplification of the 16S rRNA gene from strains HYL7-15^T was performed using an established method (Lane, 1991). The PCR product was sequenced by Invitrogen Biotechnology Co. Ltd. Preliminary phylogenetic analysis

was performed with the EzTaxon database (Chun *et al.*, 2007). Selected sequences were loaded into the MEGA version 4.0 software package (Tamura *et al.*, 2007), and phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Evolutionary distances for the neighbour-joining algorithm

were calculated with the Kimura two-parameter model (Kimura, 1980) and close-neighbour-interchange (search level=2, random additions=100) was applied in the maximum-parsimony analysis. The topology of the neighbour-joining tree was evaluated by bootstrap analysis on the basis of 1000 replications (Felsenstein, 1985).

The DNA G + C content of the novel strain was determined by HPLC according to the method of Mesbah *et al.* (1989). The respiratory quinone system was extracted and determined by HPLC (UltiMate 3000; Dionex) as described by Xie & Yokota (2003). For analysis of cellular fatty acids, strain HYL7-15^T and other related type strains were grown on 0.3 × MB agar plates at 30 °C for 2 days. The harvesting of the cells and the preparation of the fatty acid methyl esters (FAME) were performed as described by Kuykendall *et al.* (1988). The separation, identification and quantification of the individual FAME was made according to the standard protocol of the Sherlock Microbial Identification System (MIDI) and analysed by GC (6890N; Agilent) using the MIDI Sherlock system and the TSBA6 version of the database (Sasser, 1990). Polar lipids were extracted and analysed as described by Tindall (1990) and Ventosa *et al.* (1993) by using two-dimensional TLC (silica gel 60 F₂₅₄ plates, layer thickness 0.2 mm, Merck).

Analysis of the 16S rRNA gene sequence of strain HYL7-15^T resulted in a sequence of 1439 bp. The sequence similarity values of the sequence of the novel strain with those of recognized members of the genus *Pontibacter* were below the threshold recommended for the demarcation of bacterial species (Stackebrandt & Goebel, 1994), as follows: *P. korlensis* NRRL B-51097^T (95.0 %), *P. actiniarum* KMM 6156^T (93.7 %), *P. roseus* SRC-1^T (93.8 %), *P. xinjiangensis* NRRL B-51335^T (93.6 %), *P. akesuensis* AKS 1^T (93.1 %) and

P. niistensis NCIM 5339^T (94.0 %). No other recognized bacterial species showed more than 91 % 16S rRNA gene sequence similarity with the new isolate. To elucidate the phylogenetic relationship between the novel isolate and other species of the genus *Pontibacter*, a neighbour-joining phylogenetic tree (Fig. 1) was constructed. The same relationship was also evident in the 16S rRNA gene sequence dendrogram generated with the maximum-parsimony algorithm (data not shown). Strain HYL7-15^T formed a distinct branch with the clade comprising *P. korlensis* X14-1^T and *P. niistensis* NII-0905^T in the phylogenetic tree, in which all species of the genus *Pontibacter* reported to date were included. This branching pattern demonstrated that strain HYL7-15^T represented a novel species within the genus *Pontibacter*.

The DNA G + C content of strain HYL7-15^T was 44.9 mol%, a value slightly lower than those reported for the other species of the genus *Pontibacter* (Table 1). The predominant isoprenoid quinone was MK-7 in line with all other members of the genus *Pontibacter*. Strain HYL7-15^T had a fatty acid profile similar to those of other species of the genus *Pontibacter*, having a predominance of branched-chain saturated and unsaturated fatty acids, with iso-C_{15:0} (16.49 %), iso-C_{17:0} 3-OH (10.96 %) and summed feature 4 (comprising anteiso-C_{17:1} B and/or iso-C_{17:1} I, 18.46 %) as the major constituents. In addition, iso-C_{16:0} (4.25 %), C_{16:0} (9.11 %) and C_{18:0} (7.87 %) were produced in moderate amounts. A comparison of fatty acid profiles among related species is shown in Table 2. The presence of C_{16:0} (9.11 %) and C_{18:0} (7.78 %) distinguished strain HYL7-15^T from recognized species in phylogenetically related genera. Strain HYL7-15^T could also be differentiated from the closest type species by the absence of C_{17:0} (2.03 %)

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain HYL7-15^T and representative members of the phylum *Bacteroidetes*. Bootstrap values ≥ 70 % (1000 replications) are shown as percentages at each node. Bar, 0.02 substitutions per nucleotide position.

Table 2. Cellular fatty acid profiles (%) of strain HYL7-15^T and other members of the genus *Pontibacter*

Taxa: 1, HYL7-15^T; 2, *P. xinjiangensis* NRRL B-51335^T; 3, *P. actiniarum* KMM 6156^T; 4, *P. akesuensis* AKS 1^T; 5, *P. korlensis* X14-1^T; 6, *P. roseus* MTCC 7260^T. All data for reference strains were taken from this study. Only fatty acids that account for >1 % for one of the strains are indicated. ND, Not detected/not reported.

Fatty acid	1	2	3	4	5	6
iso-C _{13:0} 3-OH	1.00	ND	ND	ND	ND	ND
iso-C _{15:1} F	1.08	ND	ND	ND	ND	ND
iso-C _{15:0}	16.49	18.13	22.55	11.86	20.80	10.13
anteiso C _{15:0}	ND	ND	ND	ND	ND	1.96
C _{15:1} ω6c	1.38	ND	ND	ND	1.87	ND
iso-C _{16:1} H	1.74	1.83	ND	5.98	3.04	4.12
iso-C _{16:0}	4.25	1.92	ND	3.53	2.48	5.90
C _{16:1} ω5c	2.17	2.83	1.04	2.12	2.93	1.43
C _{16:0}	9.11	5.56	3.21	9.70	1.38	1.64
iso-C _{15:0} 3-OH	1.75	2.50	2.73	2.05	2.77	1.75
iso-C _{17:0}	2.56	2.20	1.89	1.17	1.09	2.39
anteiso C _{17:0}	ND	ND	ND	ND	ND	2.18
C _{17:1} ω6c	3.54	5.24	1.42	2.61	7.75	2.24
C _{17:0}	2.03	ND	ND	ND	ND	ND
iso-C _{16:0} 3-OH	1.07	ND	ND	ND	ND	ND
C _{18:1} ω9c	1.65	1.20	1.21	ND	ND	1.62
C _{18:0}	7.87	5.59	3.69	9.28	1.35	4.08
iso-C _{17:0} 3-OH	10.96	8.40	7.75	5.71	9.61	9.27
Summed features*						
1	1.47	1.21	2.86	2.99	1.99	1.06
3	2.36	8.63	15.72	3.96	2.98	ND
4	18.46	26.76	30.21	27.61	32.09	39.55
5	ND	ND	ND	ND	1.58	ND
8	3.33	1.42	ND	2.19	3.69	1.63

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 contains C_{13:0} 3-OH and/or iso-C_{15:1} H; summed feature 3 contains C_{16:1}ω6c and/or C_{16:1}ω7c; summed feature 4 contains anteiso-C_{17:1} B and/or iso-C_{17:1} I; summed feature 5 contains anteiso-C_{18:0} and/or C_{18:2}ω6, 9c; summed feature 8 contains C_{18:1}ω7c or C_{18:1}ω6c.

and iso-C_{16:0} (1.07 %) and by some quantitative differences in the fatty acid composition. The major polar lipids of strain HYL7-15^T were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG); diphosphatidylglycerol (DPG), three unknown aminophospholipids (APLs) and two unknown phospholipids (PLs) were also detected (Fig. 2). The polar lipid pattern of strain HYL7-15^T was identical to that found for the type strain of the type species of the genus *Pontibacter*, *Pontibacter actiniarum* KMM 6156^T.

Phylogenetic analyses based on 16S rRNA gene sequences and chemotaxonomic data revealed that strain HYL7-15^T could be assigned to the genus *Pontibacter* (Nedashkovskaya *et al.*, 2005) as a novel species. This conclusion was supported by a number of phenotypic differences between the isolate

Fig. 2. Polar lipid profiles of strain HYL7-15^T (a) and *P. actiniarum* KMM 6156^T (b) after separation by two-dimensional TLC. DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; APL1–3, unknown aminophospholipids; PL1–5, unknown phospholipids.

and its phylogenetic neighbours (Table 1), as well as fatty acid compositions (Table 2). On the basis of the phenotypic and genotypic evidences, strain HYL7-15^T represents a novel member of the genus *Pontibacter*, for which the name *Pontibacter populi* sp. nov. is proposed.

Description of *Pontibacter populi* sp. nov.

Pontibacter populi (po'pu.li. L. gen. n. *populi* of a poplar, pertaining to *Populus*, the Latin name for the poplars that grow in the forest from which the strain was isolated).

Cells are Gram-negative-staining, aerobic, non-motile, rod-shaped, 0.4–0.6 µm in diameter and 1.0–2.2 µm in length. Colonies on 0.3 × MB agar plates are circular, convex, shiny, pink and smooth. Growth occurs at 4–37 °C (optimum, 30 °C) and pH 5.0–10.0 (optimum, pH 7.0). NaCl concentrations for growth are 0–4 % (w/v). Catalase and oxidase activities are present. Hydrolyses gelatin, aesculin, starch and casein. Tests for nitrate reduction, indole production, glucose acidification, arginine dihydrolase and

urease give a negative result. According to API ZYM tests, the type strain is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), α -galactosidase, β -galactosidase, *N*-acetyl- β -glucosaminidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase. In the Biolog GN2 microplate system, strain HYL7-15^T gives a positive result for glycogen, D-mannose, sucrose, D-psicose, trehalose, succinic acid monomethyl ester, α -ketobutyric acid, α -ketovaleric acid, succinamic acid, L-glutamic acid, L-leucine, L-ornithine, urocanic acid and D-glucose 6-phosphate. Does not utilize α -cyclodextrin, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, D-arabinose, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, α -D-glucose, α -lactose, maltose, D-mannitol, xylitol, pyruvic acid methyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-glucuronic acid, α - or γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, malonic acid, propionic acid, quinic acid, sebacic acid, D-alanine, L-histidine, L-pyrroglutamic acid, D-serine, γ -aminobutyric acid, 2-aminoethanol or α -D-glucose 1-phosphate. The major fatty acids are iso-C_{15:0}, C_{16:0}, C_{18:0}, iso-C_{17:0} 3-OH and summed feature 4 (comprising anteiso-C_{17:1} B and/or iso-C_{17:1} I). MK-7 is the predominant menaquinone. The polar lipids are DPG, PE, PG, three unknown APLs and two unknown PLs.

The type strain, HYL7-15^T (=CCTCC AB 206239^T=NRRL B-59488^T), was isolated from a soil sample from a Euphrates poplar forest in Xinjiang, China. The G + C content of the genomic DNA of the type strain is 44.9 mol%.

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