Pontibacter korlensis sp. nov., isolated from the desert of Xinjiang, China

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Two Gram-negative, rod-shaped, gliding and pink-pigmented bacterial strains, X14-1^T and X19-1, were isolated from a mixture of sand samples collected from the desert of Xinjiang, China, and characterized by using a polyphasic taxonomic approach. Strains X14-1^T and X19-1 contained MK-7 as the predominant menaquinone. The major cellular fatty acids included iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 and summed feature 4. The DNA G + C contents of strains X14-1^T and X19-1 were 48.2 and 48.9 mol%, respectively. 16S rRNA gene sequence analysis showed that the isolates were highly related to each other (99.2 %) and confirmed their placement in the genus *Pontibacter*. Strains X14-1^T and X19-1 exhibited 16S rRNA gene similarity levels of 95.0–97.2 % to the type strains of the two *Pontibacter* species with validly published names. DNA–DNA hybridization experiments revealed a high level of relatedness between the two new isolates (82 %), but low levels of relatedness between strain X14-1^T and the phylogenetically most closely related species *Pontibacter actiniarum* KMM 6156^T (51 %). On the basis of genotypic and phenotypic evidence, strains X14-1^T and X19-1 are considered to represent a novel species of the genus *Pontibacter*, for which the name *Pontibacter korlensis* sp. nov. is proposed. The type strain is X14-1^T (=CCTCC AB 206081^T=NRRL B-51097^T).

The genus *Pontibacter* belongs to the phylum *Bacteroidetes* and currently comprises two species: *Pontibacter actiniarum* and *Pontibacter akesuensis* (Nedashkovskaya *et al.*, 2005; Zhou *et al.*, 2007). The type strains of the recognized species of the genus are Gram-negative, aerobic, heterotrophic and pink-pigmented bacteria. These *Pontibacter* species were isolated from marine actinians and desert soil, respectively. In this study, we report on the taxonomic characterization of two pink-pigmented bacterial strains, X14-1^T and X19-1, which were isolated from a desert soil sample from Xinjiang Province, China.

Strains X14-1^T and X19-1 were isolated by using the standard dilution plating technique at 30 °C on 0.1 × tryptic soy broth (TSB) agar plates (Difco). However, the isolates grew better on marine agar 2216 (Difco). The results of a polyphasic taxonomic study of the isolates indicated that they represent a novel species of the genus *Pontibacter*.

Cell morphology was examined by phase-contrast microscopy (Olympus) and by transmission electron microscopy. 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 a 1 % solution of tetramethyl-*p*-phenylenediamine (Kovács, 1956). Catalase activity was determined by assessing the production of bubbles after the addition of a drop of 3 % H₂O₂. Growth at different temperatures (4, 7, 28, 37, 42 and 45 °C), pH (4–11) and NaCl concentration (0–10 %, w/v) was investigated on 0.1 × TSB agar for up to 1 week. Some conventional biochemical tests were performed as described by Smibert & Krieg (1994), including tests for Tween 40, Tween 80, starch and casein hydrolysis. Physiological and biochemical features of strains X14-1^T and X19-1 were also determined using the API 20E, API 20NE, API ZYM (bioMérieux) and Biolog GN2 Microplate system (Biolog) according to the manufacturers' instructions. Antimicrobial susceptibility testing was performed by using the agar-diffusion method with antibiotic-impregnated discs, as described by Buczolits *et al.* (2002).

For 16S rRNA gene sequencing and phylogenetic analysis, DNA was extracted by using a Bacteria Genomic DNA Isolation kit (ChaoShi-Bio; China). PCR and 16S rRNA gene sequencing were carried out as described by Lin *et al.*

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains X14-1^T and X19-1 are DQ888330 and DQ888331, respectively.

A transmission electron micrograph of cells of strain X14-1^T is available as a supplementary figure with the online version of this paper.

(2004). Phylogenetic analysis was performed by using MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data via CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic distances were calculated using the Kimura two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis based on 1000 replications was undertaken to test the robustness of the phylogenetic tree (Felsenstein, 1985).

For DNA–DNA hybridization experiments and determination of the DNA G+C content, genomic DNA of the bacterial strains was prepared according to a modification of the procedure of Wilson (1987). The DNA G+C content was determined by HPLC according to the method of Mesbah *et al.* (1989). DNA–DNA hybridizations were performed using the microplate method, as described previously by Ezaki *et al.* (1989) and Willems *et al.* (2001). The respiratory quinone system was extracted and determined by HPLC as described by Xie & Yokota (2003). For the determination of the whole-cell fatty acid profiles, strains X14-1^T and X19-1 and *P. actiniarum* KMM 6156^T and *P. akesuensis* AKS 1^T were grown at 30 °C for 48 h on marine agar. Analysis of the fatty acid methyl esters was carried out according to the standard protocol of the Sherlock Microbial Identification System (MIDI).

Cells of strains X14-1^T and X19-1 were collected for morphological observations after being cultured on marine agar for 24 h. The cells of the two strains were Gram-negative, rod-shaped and motile by gliding. Cells were prepared for transmission electron microscopy as described by Golyshina *et al.* (2000). Cells of strains X14-1^T and X19-1 ranged from 0.6 to 0.7 µm in width and from 1.3 to 3.5 µm in length (see Supplementary Fig. S1, available in IJSEM Online).

The 16S rRNA genes of strains X14-1^T and X19-1 showed a high level of similarity (99.2 %). Phylogenetic analysis based on 16S rRNA gene sequences showed that the new isolates were phylogenetically most related to the genera

Pontibacter and *Effluviibacter* (Fig. 1). *P. actiniarum* KMM 6156^T was the closest relative of strains X14-1^T and X19-1. Similarity values between the 16S rRNA gene sequences of the two isolates and *P. actiniarum* KMM 6156^T were 97.2 and 96.8 %, respectively. No other recognized bacterial species showed more than 96 % 16S rRNA gene sequence similarity to the new isolates. Therefore, the two isolates appeared to belong to the genus *Pontibacter*.

The chemotaxonomic properties, i.e. fatty acid profiles and DNA G+C content, supported the result of the monothetic phylogenetic classification; namely, that the two isolates are members of the genus *Pontibacter*, and not the genus *Effluviibacter* (Nedashkovskaya *et al.*, 2005; Suresh *et al.*, 2006; Zhou *et al.*, 2007). The two strains contained large amounts of branched-chain saturated and unsaturated fatty acids; the predominant fatty acids were iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and summed feature 4 (consisting of anteiso-C_{17:1} B and/or iso-C_{17:1} I) (Table 1). These fatty acid profiles were similar to those of recognized *Pontibacter* species. The DNA G+C contents of strains X14-1^T and X19-1 were 48.2 and 48.9 mol%, respectively.

Levels of DNA–DNA relatedness were determined between strain X14-1^T and the type strain of the phylogenetically most closely related species (*P. actiniarum* KMM 6156^T) and between strains X14-1^T and X19-1. Strains X14-1^T and X19-1 showed a level of DNA–DNA relatedness of 82 %, above the level of 70 % that is accepted as the limit for species delineation (Wayne *et al.*, 1987). Strain X14-1^T clearly showed <70 % DNA–DNA reassociation (51 %) with the closest neighbour, *P. actiniarum* KMM 6156^T. The results of the DNA–DNA hybridization experiments indicate that strains X14-1^T and X19-1 represent a single novel species of the genus *Pontibacter*.

The main physiological and biochemical characteristics of strains X14-1^T and X19-1 are given in Table 2 and the species description. The features that differentiate strains X14-1^T and X19-1 from related members of the genera

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strains X14-1^T and X19-1 and some other related taxa. Percentage bootstrap values (1000 replications) greater than 50 % are shown at nodes. *Reichenbachella agariperforans* KMM 3525^T was used as the outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Cellular fatty acid compositions (%) of strains X14-1^T and X19-1 (*Pontibacter korlensis* sp. nov.) and closely related strains

Strains: 1, X14-1^T; 2, X19-1; 3, *P. actiniarum* KMM 6156^T; 4, *P. akesuensis* AKS 1^T; 5, *E. roseus* SRC-1^T. All data for *Pontibacter* species were taken from this study. Data for *E. roseus* SRC-1^T were taken from Suresh *et al.* (2006). Only fatty acids that account for more than 1 % for one of the strains are indicated. ND, Not detected/not reported.

Fatty acid	1	2	3	4	5
iso-C _{15:1} F	0.5	0.6	ND	1.2	ND
iso-C _{15:0}	15.0	15.8	22.8	22.8	15.8
anteiso-C _{15:0}	0.3	0.4	0.2	0.7	3.5
C _{15:1} ω6c	1.1	0.9	0.4	3.1	ND
iso-C _{16:1} H	1.3	1.0	0.3	2.8	4.4
iso-C _{16:0}	1.1	0.9	0.3	2.0	5.9
C _{16:1} ω5c	0.9	1.0	0.7	1.2	2.1
C _{16:0}	0.4	0.4	0.3	1.3	1.7
iso-C _{15:0} 3-OH	2.6	2.7	2.6	2.7	2.4
iso-C _{17:1} ω9c	0.3	0.3	0.2	1.1	1.0
iso-C _{17:0}	1.9	2.0	1.3	1.2	1.3
anteiso-C _{17:0}	0.1	0.2	ND	ND	1.3
C _{17:1} ω6c	6.2	6.0	1.6	2.9	5.6
iso-C _{16:0} 3-OH	0.4	0.4	ND	0.5	1.2
iso-C _{18:1} H	0.3	0.3	ND	0.2	3.1
C _{16:0} 3-OH	ND	ND	ND	0.1	1.3
C _{18:1} ω9c	1.8	1.3	2.0	1.3	ND
C _{18:1} ω7c	1.2	0.8	1.3	0.9	ND
C _{18:0}	1.2	0.9	1.4	2.3	ND
iso-C _{17:0} 3-OH	9.6	10.1	6.2	6.9	10.3
Summed features*					
2	1.7	1.9	2.5	6.0	ND
3	10.3	11.3	16.2	4.2	ND
4	33.4	33.4	31.4	26.7	36.7
5	1.6	1.6	2.2	1.7	ND

Summed features represent groups of two or three fatty acids that could not be separated using gas-liquid chromatography 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; summed feature 5 contains anteiso-C_{18:0} and/or C_{18:2}ω6,9c.

Pontibacter and *Effluviibacter* are shown in Table 2. The results of the phenotypic examination showed that the strains studied have many traits in common with *P. actiniarum* KMM 6156^T. However, the habitats occupied by these two taxa are different: strains X14-1^T and X19-1 were isolated from desert soil samples, whereas *P. actiniarum* KMM 6156^T was isolated from a marine habitat. In contrast to *P. actiniarum* KMM 6156^T, strains X14-1^T and X19-1 could hydrolyse starch, and utilize carbohydrates such as D-fructose, D-galactose and D-mannose as the sole carbon source. Strains X14-1^T and X19-1 produced enzymes such as α-galactosidase and β-glucosidase, whereas *P. actiniarum* KMM 6156^T produced trypsin, but not α-galactosidase or β-glucosidase. Differences were also observed in the cellular fatty acid compositions of the novel strains and the two recognized *Pontibacter* species (Table 1).

Based on the phenotypic and genotypic evidence presented, strains X14-1^T and X19-1 should be assigned to the genus *Pontibacter* as representing a single novel species, for which the name *Pontibacter korlensis* sp. nov. is proposed.

Description of *Pontibacter korlensis* sp. nov.

Pontibacter korlensis (ko.r.len'sis. N.L. masc. adj. *korlensis* pertaining to Korla, a city of Xinjiang Province in the north-west of China from where the type strain was isolated).

Cells are Gram-negative, rod-shaped, motile by gliding, 1.3–3.5 µm long and 0.6–0.7 µm wide. Colonies are circular, 2–3 mm in diameter, convex, shiny, pink and smooth on marine agar. Does not require Na⁺ or seawater for growth. Growth occurs at 7–45 °C; optimal temperature for growth is 30–37 °C. Growth occurs in 0–8 %

Table 2. Phenotypic characteristics that differentiate strains X14-1^T and X19-1 (*Pontibacter korlensis* sp. nov.) from related members of the genera *Pontibacter* and *Effluviibacter*

Strains: 1, X14-1^T and X19-1; 2, *P. actiniarum* KMM 6156^T; 3, *P. akesuensis* AKS 1^T; 4, *E. roseus* SRC-1^T. Data were taken from Nedashkovskaya *et al.* (2005), Suresh *et al.* (2006), Zhou *et al.* (2007) and this study. +, Positive; −, negative; NR, not reported.

Characteristic	1	2	3	4
Habitat	Soil	Marine	Soil	Muddy water
Cell shape	Rod	Rod	Rod	Irregular rod
Cell size (µm)	0.6–0.7 × 1.3–3.5	0.3–0.4 × 1.2–1.9	0.7–0.75 × 1.5–1.6	0.3–0.5 × 1.0–3.0
Gliding motility	+	+	−	−
Growth at:				
4 °C	−	−	+	+
42 °C	+	+	−	−
Salinity range (%)	0–8	0–10	0–4	0–8
Hydrolysis of:				
Starch	+	−	+	+
Casein	−	−	−	+
Carbon-source utilization				
D-Cellobiose	+	−	+	NR
D-Fructose	+	−	+	+
L-Fucose	−	−	+	NR
Maltose	+	−	+	NR
α-D-Glucose	+	−	+	+
D-Mannitol	−	−	+	NR
D-Galactose	+	−	+	+
α-D-Lactose	+	−	−	+
D-Mannose	+	−	−	NR
Sucrose	+	−	+	+
α-Ketobutyric acid	−	+	+	NR
α-D-Glucose 1-phosphate	+	−	−	NR
D-Glucose 6-phosphate	+	−	+	NR
Enzyme activity (API ZYM)				
α-Galactosidase	+	−	+	NR
β-Glucosidase	+	−	NR	NR
Trypsin	−	+	+	NR
DNA G + C content (mol%)	48.2–48.9	48.7	51.4	59.5

NaCl and at pH 5.5–11.0, with optimal growth at pH 7.0–8.0. Pigment can be extracted with organic solvents. Oxidase- and catalase-positive. Gelatin, aesculin and starch are decomposed, but casein and Tweens 40 and 80 are not hydrolysed. Nitrate reduction, H₂S production, citrate utilization, indole production and urease are negative. In Biolog GN2 tests, dextrin, glycogen, *N*-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, sucrose, trehalose, turanose, succinic acid monomethyl ester, acetic acid, α-ketovaleric acid, DL-lactic acid, succinic acid, succinamic acid, L-alaninamide, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, urocanic acid, α-D-glucose 1-phosphate and D-glucose 6-phosphate are utilized. Does not utilize α-cyclodextrin, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, adonitol, D-arabitol, i-erythritol, L-fucose, *myo*-inositol, D-mannitol, L-rhamnose, xylitol, pyruvic acid methyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone,

D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-, β- and γ-hydroxybutyric acids, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, glucuronamide, D-alanine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-proline, L-pyroglyutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol or DL-α-glycerol phosphate. According to the API ZYM gallery, produces alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase, but not trypsin, α-chymotrypsin, β-glucuronidase, α-mannosidase or α-fucosidase. Susceptible to chloramphenicol, colistin sulfate, erythromycin, gentamicin, penicillin G, polymyxin

B sulfate, tetracycline and vancomycin. Resistant to kanamycin and streptomycin. The predominant menaquinone is MK-7. Major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and summed feature 4 (consisting of anteiso-C_{17:1} B and/or iso-C_{17:1} I). The DNA G + C content of the type strain is 48.2 mol%.

The type strain, X14-1^T (=CCTCC AB 206081^T=NRRL B-51097^T), was isolated from the surface layer of a desert soil from Korla, Xinjiang Province, China. Strain X19-1 is a reference strain.

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