Pontibacter xinjiangensis sp. nov., in the phylum 'Bacteroidetes', and reclassification of [*Effluviibacter*] *roseus* as *Pontibacter roseus* comb. nov.

Yang Wang, Kundi Zhang, Feng Cai, Lei Zhang, Yali Tang, Jun Dai and Chengxiang Fang

Correspondence
Chengxiang Fang
cxfang@whu.edu.cn

China Center for Type Culture Collection (CCTCC), College of Life Sciences, Wuhan University, Wuhan 430072, PR China

A novel strain, designated 311-10^T, isolated from soil of Xinjiang, China, was characterized by using a polyphasic taxonomic approach. The isolate was Gram-negative, aerobic, rod-shaped, non-motile, oxidase-negative and catalase-positive. The predominant menaquinone of strain 311-10^T was MK-7 and the genomic DNA G+C content was 47.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate formed a cluster with the genera *Pontibacter* and [*Effluviibacter*] in the phylum 'Bacteroidetes', with sequence similarities of 93.9–95.6%. Phylogenetic evidence and the results of phenotypic, genotypic and chemotaxonomic analyses support the reclassification of [*Effluviibacter*] *roseus* as *Pontibacter roseus* comb. nov. (type strain, SRC-1^T=MTCC 7260^T=DSM 17521^T) and the establishment of a novel species, *Pontibacter xinjiangensis* sp. nov., with strain 311-10^T (=CCTCC AB 207200^T=NRRL B-51335^T) as the type strain.

The genus *Pontibacter*, a member of the phylum 'Bacteroidetes' with menaquinone 7 (MK-7) as the main respiratory quinone, accommodates heterotrophic, aerobic, rod-shaped, Gram-negative and pink-pigmented bacteria. At the time of writing, *Pontibacter* species with validly published names comprise *Pontibacter actiniarum*, isolated from marine actinians (Nedashkovskaya *et al.*, 2005), and *Pontibacter akesuensis* (Zhou *et al.*, 2007) and *Pontibacter korlensis* (Zhang *et al.*, 2008) from desert soil. Members of the genus *Pontibacter* form a phylogenetic cluster with the genus [*Effluviibacter*], first proposed for irregular rod-shaped and non-motile bacteria by Suresh *et al.* (2006). Up to now, [*Effluviibacter*] *roseus*, isolated from muddy water, is the sole species of the genus [*Effluviibacter*].

During the course of studying the bacterial communities associated with soil in Xinjiang, China, bacterial strain 311-10^T was isolated. The strain appeared as pink-pigmented, circular, convex, shiny colonies with a smooth surface after 48 h cultivation at 30 °C on 0.3 × marine agar 2216 (MA; Difco). After primary isolation and purification, the novel strain was cultivated at 30 °C for 2 days on

0.3 × MA and cellular morphology was observed by phase-contrast microscopy (Olympus). Except where indicated, the isolate was routinely grown aerobically on 0.3 × MA for 2 days at 30 °C.

The gliding-motility test was performed as described by Bowman (2000). Oxidase activity was evaluated via the oxidation of 1 % *p*-aminodimethylaniline oxalate. 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), cellulose (0.1 %, w/v), chitin from crab shells (1 %, w/v), casein (5 %, w/v) and tyrosine (0.5 %, w/v) as described by Smibert & Krieg (1994). Tolerance of NaCl concentrations (0–6 %, w/v) was determined at 1 % NaCl increments. Growth was examined at different temperatures (4, 10, 20, 28, 30, 37 and 42 °C) and pH values (5.0–11.0 at 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, API 20E and API ZYM) according to the manufacturer's instructions (bioMérieux).

Isoprenoid quinones were extracted from lyophilized cells and analysed by HPLC (UltiMate 3000; Dionex) as described by Xie & Yokota (2003). Whole-cell fatty acids

Abbreviations: MA, marine agar 2216; MK, menaquinone.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 311-10^T is FJ004994.

A supplementary figure showing a maximum-parsimony phylogenetic tree is available with the online version of this paper.

of all strains were analysed according to the instructions of the Microbial Identification System (MIDI) after cultivation on $0.3 \times \text{MA}$ for 2 days at 30°C .

Whole genomic DNA was prepared according to the method of Wilson (1987). The DNA G+C content was determined by HPLC (Mesbah *et al.*, 1989). PCR amplification of the 16S rRNA gene from strain 311-10^T was performed by using an established method (Lane, 1991). The PCR product was sequenced by Invitrogen. Preliminary phylogenetic analysis was done in the EzTaxon database (Chun *et al.*, 2007). Selected sequences were loaded into the software package MEGA version 4.0 (Tamura *et al.*, 2007) and phylogenetic trees were constructed by 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).

Strain 311-10^T was oxidase-negative and catalase-positive. Cells of the isolate were $0.4\text{--}0.6 \times 1.2\text{--}2.0\ \mu\text{m}$. Growth of the strain occurred at $4\text{--}37^\circ\text{C}$. NaCl concentrations and pH range for growth of the isolate were 0–5 % (w/v) and pH 6.0–10.0, respectively. Strain 311-10^T hydrolysed starch and casein, but not cellulose, chitin or tyrosine. Other phenotypic features of strain 311-10^T are presented and compared with those of related strains in Table 1.

Isolate 311-10^T contained MK-7 as the predominant isoprenoid quinone. It had fatty acid profiles very similar to those of *Pontibacter* species, having a predominance of branched-chain saturated and unsaturated fatty acids, with iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 (containing C_{16:1}ω6c and/or C_{16:1}ω7c) and summed feature 4 (comprising anteiso-C_{17:1} B and/or iso-C_{17:1} I) as the major constituents. A comparison of fatty acid profiles among related species is shown in Table 2. The DNA G+C content of strain 311-10^T was 47.8 mol%, which is in agreement with those of members of the genus *Pontibacter*.

The almost-complete 16S rRNA gene sequence (1448 nt) of strain 311-10^T was determined and compared with those of representatives of the phylum 'Bacteroidetes'. Strain 311-10^T displayed 16S rRNA gene sequence similarity to *Pontibacter* species (93.9–95.6 %) and [*E.*] *roseus* SRC-1^T (94.7 %). A neighbour-joining tree (Fig. 1) showed that strain 311-10^T was related to the three *Pontibacter* species and [*E.*] *roseus* SRC-1^T with a high bootstrap value of 100 %, and formed the deepest branch outside all type strains of the genus *Pontibacter* and [*E.*] *roseus* SRC-1^T. Within the coherent cluster, [*E.*] *roseus* SRC-1^T formed another compact cluster distinct from that for members of the genus *Pontibacter*. This coherent cluster was also found in the tree generated using the maximum-parsimony algorithm (shown in Supplementary Fig. S1, available in IJSEM Online).

Table 1. Phenotypic characteristics of strain 311-10^T and type strains of related species

Taxa: 1, strain 311-10^T; 2, *P. actiniarum* KMM 6156^T; 3, *P. akesuensis* AKS 1^T; 4, *P. korlensis* X14-1^T; 5, [*E.*] *roseus* SRC-1^T. All data are from the present study. All strains are positive for gelatin hydrolysis and catalase, but negative for nitrate reduction, production of indole and H₂S, glucose acidification, arginine dihydrolase and urease. +, Positive; –, negative.

Characteristic	1	2	3	4	5
Oxidase	–	+	+	+	+
Gliding motility	–	+	–	+	–
Hydrolysis of:					
Starch	+	–	+	+	+
Casein	+	–	–	–	+
Aesculin	+	+	+	+	–
Growth at:					
4 °C	+	–	+	–	+
42 °C	–	+	–	+	–
Salinity range (%) for growth	0–5	0–10	0–4	0–8	0–8
Carbon-source oxidation (Biolog GN2):					
Cellobiose	+	–	+	+	+
D-Fructose	+	–	–	+	+
D-Galactose	+	–	–	+	+
α-D-Glucose	+	–	–	+	+
α-D-Lactose	+	–	–	+	+
Sucrose	+	–	–	+	+
Maltose	+	–	+	+	+
D-Mannitol	+	–	+	–	–
α-Ketobutyric acid	–	+	–	–	–
L-Histidine	–	–	+	–	–
L-Pyrogutamic acid	–	–	+	–	–
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	+	+	+	+	–

The phenotypic and chemotaxonomic results of the present study support the phylogenetic analysis, which suggests that strain 311-10^T belongs to a novel species of the genus *Pontibacter* and that it is necessary to re-evaluate the taxonomic status of [*E.*] *roseus* SRC-1^T and to determine whether transfer into the genus *Pontibacter* is warranted. Accordingly, *P. actiniarum* KMM 6156^T and [*E.*] *roseus* DSM 17521^T were obtained from the Collection of Marine Microorganisms (KMM) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), respectively. It was found that the phenotypic characteristics of [*E.*] *roseus* DSM 17521^T were consistent with those of members of the genus *Pontibacter*, including the ability to grow on

Table 2. Cellular fatty acid compositions (%) of strain 311-10^T and type strains of related species

Taxa: 1, strain 311-10^T; 2, *P. actiniarum* KMM 6156^T; 3, *P. akesuensis* AKS 1^T; 4, *P. korlensis* X14-1^T; 5, *[E.] roseus* SRC-1^T. ND, Not detected.

Fatty acid	1	2	3	4	5
iso-C _{13:0} 3-OH	1.38	1.44	0.95	0.36	1.73
iso-C _{15:1} F	1.40	1.42	1.05	ND	1.43
anteiso C _{15:1} A	0.43	0.51	0.28	ND	0.48
iso-C _{15:0}	16.19	24.17	16.10	15.01	13.97
anteiso C _{15:0}	1.48	0.29	1.17	0.31	1.11
C _{15:1} ω6c	0.81	0.41	1.47	1.06	0.26
iso-C _{16:1} H	1.30	0.34	4.70	1.25	1.84
iso-C _{16:0}	1.27	0.46	5.19	1.09	4.00
C _{16:1} ω5c	6.94	1.42	2.51	0.93	2.05
C _{16:0}	3.14	2.25	4.80	3.18	2.87
iso-C _{15:0} 3-OH	2.40	3.38	3.16	2.63	2.41
iso-C _{17:0}	1.17	3.00	2.73	1.88	3.51
C _{17:1} ω6c	2.94	1.25	4.63	6.17	2.17
C _{17:0}	0.41	0.33	0.46	0.51	0.35
iso-C _{16:0} 3-OH	0.62	0.28	0.63	0.37	0.70
iso-C _{18:1} H	0.88	ND	0.53	0.31	0.80
C _{18:1} ω9c	1.39	1.11	1.29	1.82	1.49
C _{18:1} ω5c	0.88	0.21	ND	ND	ND
C _{18:0}	3.92	3.06	3.46	3.58	3.79
iso-C _{17:0} 3-OH	8.43	9.21	8.82	9.58	14.69
Summed features*:					
1	1.83	2.67	2.75	1.73	1.90
3	14.51	10.28	4.62	10.28	0.35
4	21.91	30.51	25.76	33.39	34.87
5	1.64	1.17	ND	1.58	ND
8	1.35	0.56	0.90	1.15	0.75

*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 and/or C_{18:1}ω6c.

MA, pink-pigmented colonies and similar tolerances to salinity and temperature. Phylogenetic analysis also indicated that *[E.] roseus* SRC-1^T is related closely to *Pontibacter* species. The creation of the genus *[Effluviibacter]* was mainly due to the following. (i) Cells of *[E.] roseus* SRC-1^T were non-motile. (ii) The fatty acid composition differed from those of *Pontibacter* species, with *[E.] roseus* SRC-1^T containing significant quantities of iso-C_{16:0} (5.9%) and iso-C_{18:1} H (3.1%), which were absent in *P. actiniarum* KMM 6156^T. One of the predominant fatty acids (14.7%) of *P. actiniarum* KMM 6156^T was summed feature 3 (containing iso-C_{15:0} 2-OH, C_{16:1}ω7c and/or C_{16:1}ω7t), which was absent in *[E.] roseus* SRC-1^T (Suresh *et al.*, 2006). (iii) The DNA G+C content was 59.5 mol%, which is significantly higher than those of *Pontibacter* species. However, in this study, after fatty acids were determined under the same conditions, a number of different results were obtained. *[E.] roseus* DSM 17521^T

contains summed feature 3 (containing C_{16:1}ω7c and/or C_{16:1}ω6c), and some *Pontibacter* species (e.g. *P. akesuensis* AKS 1^T and *P. korlensis* X14-1^T) also contain iso-C_{16:0} and iso-C_{18:1} H. Therefore, *[E.] roseus* DSM 17521^T shares a similar fatty acid pattern with *Pontibacter* species (Table 2). In addition, gliding motility was variable among *Pontibacter* species, so the non-motile feature of *[E.] roseus* SRC-1^T was not enough to support a separate genus. We re-examined the DNA G+C content of *[E.] roseus* DSM 17521^T in this study in triplicate experiments and demonstrated it to be 52.0–52.3 mol%, which is similar to those of *Pontibacter* species.

The 16S rRNA gene sequence similarities among the novel isolate, type strains of the genus *Pontibacter* and *[E.] roseus* SRC-1^T, together with similar phenotypic, chemotaxonomic and genotypic characteristics, suggest that *[E.] roseus* should be reclassified in the genus *Pontibacter* as *Pontibacter roseus* comb. nov., with the type strain SRC-1^T (=MTCC 7260^T=DSM 17521^T), and that strain 311-10^T (=CCTCC AB 207200^T=NRRL B-51335^T) represents a novel species within the genus *Pontibacter*, for which the name *Pontibacter xinjiangensis* sp. nov. is proposed.

Emended description of the genus *Pontibacter* Nedashkovskaya *et al.* 2005

Pontibacter (Pon.ti.bac'ter. L. n. *pontus* the sea; N.L. masc. n. *bacter* from Gr. neut. n. *baktron* rod; N.L. masc. n. *Pontibacter* a marine bacterium).

Main characteristics are as those given for the genus description by Nedashkovskaya *et al.* (2005), with the following emendments. Certain member species are capable of gliding, whereas others are non-motile. Oxidase-positive or oxidase-negative. The type species is *Pontibacter actiniarum*.

Description of *Pontibacter roseus* comb. nov.

Pontibacter roseus (ro'se.us. L. masc. adj. *roseus* rose-coloured, pink).

Basonym: *Effluviibacter roseus* Suresh *et al.* 2006.

The DNA G+C content of the type strain is 52.0–52.3 mol%. The predominant whole-cell fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 4 (comprising anteiso-C_{17:1} B and/or iso-C_{17:1} I). The rest of the description is identical to that given for *[Effluviibacter] roseus* by Suresh *et al.* (2006).

The type strain is SRC-1^T (=MTCC 7260^T=DSM 17521^T), which was isolated from muddy water from an occasional drainage system in Chandigarh, India.

Description of *Pontibacter xinjiangensis* sp. nov.

Pontibacter xinjiangensis (xin.jiang.en'sis. N.L. masc. adj. *xinjiangensis* pertaining to Xinjiang, an autonomous region in north-west China).

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain 311-10^T and representative members of the phylum 'Bacteroidetes'. The tree is based on a comparison of 16S rRNA gene sequences that are $\geq 90\%$ complete. Numbers at nodes indicate bootstrap values (%). Bar, 0.02 changes per nucleotide position.

Cells are Gram-negative, aerobic, rod-shaped and non-motile ($0.4\text{--}0.6 \times 1.2\text{--}2.0\ \mu\text{m}$). Colonies on $0.3 \times \text{MA}$ are circular, convex, shiny, pink and smooth. Growth occurs at $4\text{--}37\ ^\circ\text{C}$ (optimum $30\ ^\circ\text{C}$) and pH $6.0\text{--}10.0$. NaCl concentrations for growth are $0\text{--}5\%$ (w/v). Oxidase-negative and catalase-positive. Hydrolyses gelatin, aesculin, starch and casein, but not tyrosine, chitin or cellulose. Nitrate reduction, H_2S production, citrate utilization, indole production, glucose acidification, arginine dihydrolase and urease are negative. According to API ZYM, the type strain is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl- β -glucosaminidase, but negative for lipase (C14), β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase. In Biolog GN2 microplates, the type strain is positive for dextrin, glycogen, cellobiose, D-fructose, D-galactose, α -D-glucose, α -D-lactose, maltose, D-mannitol, D-mannose, raffinose, sucrose, methyl β -D-glucoside, L-rhamnose, trehalose, L-leucine, D-gluconic acid, L-glutamic acid, α -D-glucose 1-phosphate and D-glucose 6-phosphate. Does not utilize α -cyclodextrin, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, D-arabinose, i-erythritol, L-fucose, melibiose, D-psicose, D-sorbitol, xylitol, pyruvic acid methyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-glucuronic acid, α -, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, malonic acid, propionic acid, quinic acid, sebacic acid, succinamic acid, D-alanine, L-histidine, L-pyroglutamic acid, D-serine, γ -aminobutyric acid, urocanic acid or 2-aminoethanol. The major fatty acids of the type strain are iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 (containing C_{16:1} ω 7c and/or C_{16:1} ω 6c) and summed feature 4 (comprising anteiso-C_{17:1} B and/or iso-C_{17:1} I). MK-7 is the predominant menaquinone. The G+C content of the genomic DNA of the type strain is 47.8 mol%.

The type strain, 311-10^T (=CCTCC AB 207200^T=NRRL B-51335^T), was isolated from a soil sample from Xinjiang, China.

Acknowledgements

This work was supported by the R&D Infrastructure and Facility Development Program from the Ministry of Science and Technology of the People's Republic of China (grant no. 2005DKA21208).

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