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NOTE

Pontibacter jeungdoensis sp. nov., Isolated from a Solar Saltern in Korea

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A Gram-staining-negative, rod-shaped and red-pigmented bacterial strain, HMD3125^T, was isolated from a solar saltern in Jeungdo, Republic of Korea. A phylogenetic tree based on 16S rRNA gene sequences showed that strain HMD3125^T formed a lineage within the genus *Pontibacter* and was similar to *Pontibacter salisaro* (96.1%) and *P. korlensis* (95.3%). The major fatty acids of strain HMD3125^T were summed feature 4 (comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B; 30.4%), iso-$C_{15:0}$ (20.4%) and iso-$C_{17:0}$ 3OH (17.2%). The polar lipid profile of HMD3125^T consisted of the phosphatidylethanolamine, four unidentified polar lipids, unidentified phospholipid, unidentified aminolipid and unidentified aminophospholipid. Strain HMD3125^T contained MK-7 as the predominant menaquinone and *sym*-homospermidine as the major polyamine. The DNA G+C content of strain HMD3125^T was 45.6 mol%. Strain HMD3125^T assigned as a novel species in the genus *Pontibacter*, for which the name *Pontibacter jeungdoensis* sp. nov. is proposed. The type strain is HMD3125^T (=KCTC 23156^T =CECT 7710^T).

Keywords: *Pontibacter jeungdoensis*, 16S rRNA gene sequence, taxonomy

The genus *Pontibacter* is in the Family *Cytophagaceae* and Phylum *Bacteroidetes*. *Pontibacter* was first described by Nedashkovskaya *et al.* (2005) isolated from an unidentified sea anemone. The genus *Pontibacter* is comprised of *Pontibacter actiniarum* (Nedashkovskaya *et al.*, 2005), *P. akesuensisi* (Zhou *et al.*, 2007), *P. niistensis* (Dastager *et al.*, 2010), *P. lucknowensis* (Dwivedi *et al.*, 2012), *P. korlensis* (Zhang *et al.*, 2008), *P. populi* (Xu *et al.*, 2012), *P. roseus* (Suresh *et al.*, 2006; Wang *et al.*, 2010), *P. saemangeumensis* (Kang *et al.*, 2012), *P. salisaro* (Joung *et al.*, 2011), and *P. xinjiangensis* (Wang *et al.*, 2010). The genus *Pontibacter* have been isolated from an unidentified sea anemone, forest soil, desert soil, and a saltern.

We isolated a red-pigmented strain during a study on the microbial diversity of a tablet solar saltern from Jeungdo, Jeollanam-do, Republic of Korea (34° 59′ 47″ N 126° 10′ 02″ E). The strain was isolated on R2A (Difco, USA) for 48 h at 30°C using the standard dilution plating technique from solar saltern water. The isolate was routinely cultured on R2A and MA (marine agar; Difco) and preserved at -80°C as suspensions in water with 20% glycerol (w/v).

The almost-complete sequence of the 16S rRNA genes was obtained from strain HMD3125^T as described previously (Cho and Giovannoni, 2003). Phylogenetic neighbors were identified, and pairwise 16S rRNA gene sequence similarities were calculated using the EzBioCloud server (Chun *et al.*, 2007). Sequence comparisons showed that strain HMD3125^T was closely related to *P. salisaro* (96.1%), *P. saemangeumensis* (95.6%), *P. korlensis* (95.3%), *P. roseus* (95.2%), *P. actiniarum* (95.1%), and *P. akesuensis* (94.8%). Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbor-joining methods (Saitou and Nei, 1987). Robustness of the maximum-likelihood and neighbor-joining tree topologies was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1,000 resamplings of the sequences and phylogenetic tree was constructed using MEGA version 5 (Tamura *et al.*, 2011). The phylogenetic trees generated in this study (Fig. 1) that the solar saltern strain HMD3125^T belonged to the genus *Pontibacter*. The phylogenetic tree suggested that strain HMD3125^T should be assigned to the genus *Pontibacter* as a novel species.

Cell morphology was examined by light microscopy and transmission electron microscope. Gram staining was determined using the Gram Stain kit (bioMérieux, France), according to the manufacturer's instructions. Cellular pigments were extracted with acetone/methanol (1:1, v/v), and their absorption spectra were determined using a scanning UV/visible spectrophotometer (UV 6101A; Shimadzu, Japan). The presence of flexirubin-type pigments was investigated using the bathochromatic shift test with a 20% (w/v) KOH solution (Bernardet *et al.*, 2002). Oxidase and catalase activities were tested by determining the oxidation of 1% (w/v) tetramethyl-ρ-phenylenediamine (Sigma, USA) and oxygen bubbles in a 3% (v/v) aqueous hydrogen peroxide solution. Anaerobic growth was tested on R2A at 30°C using a GasPak EZ Anaerobic Container System (BD, USA), according to the

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The GenBank accession number for the 16S rRNA gene sequence of strain HMD3125^T is GU339183.

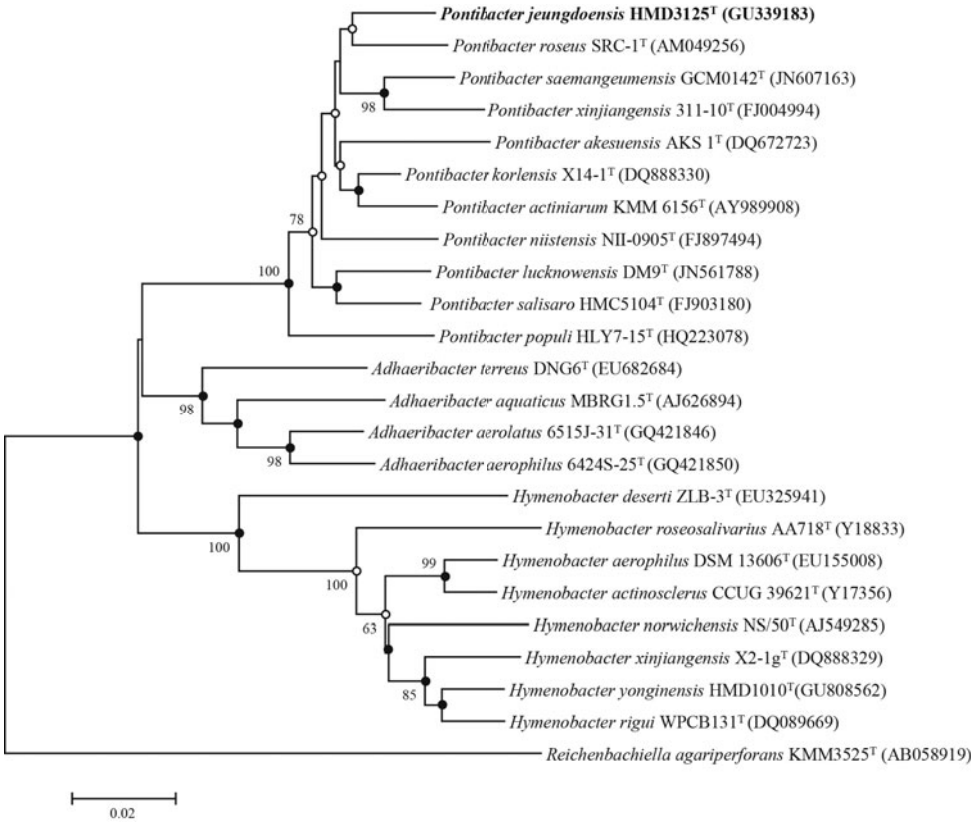

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain HMD3125^T and representative members of the family *Cytophagaceae*. Bootstrap percentages (>50%) from both neighbor joining (above nodes) analyses are shown. Filled and open circles indicate nodes recovered by all three treeing methods or by two treeing methods (NJ and ML, NJ and MP, and ML and MP), respectively. *Reichenbachiella agariperforans* KMM3525^T was used as an outgroup. Bar, 0.02 substitution per an nucleotide position.

manufacturer's instructions. Growth in the presence of 0, 0.5, and 1.0–10% (1.0% intervals) NaCl concentrations (w/v) was investigated in R2A broth at 30°C. The temperature range and optimum for growth were measured in R2A broth supplemented with 1% NaCl at 4°C, 10–30°C (at 5°C intervals), 37°C and 42°C. The pH range for growth was determined in 1% NaCl R2A broth adjusted to pH 4.0–10.0 (at intervals of 1.0 pH unit) at 30°C. Hydrolysis of casein [3% skimmed milk (Difco), w/v], CM-cellulose [1% (Sigma), w/v], cellulose (1% w/v, filter paper # 1, Whatman, UK) and starch (1%, w/v) were tested using R2A agar as the base medium. Growth was tested on cetrimide (Difco), MacConkey agar (Difco), and DNase test agar (Difco) with DNase. Basic biochemical tests and carbon-source-oxidation tests were performed using API 20NE and API ZYM strips (bioMérieux) and GN2 MicroPlates (Biolog, USA) at 30°C, according to the manufacturer's instructions. For fatty acid analysis, strain HMD3125^T was grown on MA for 48 h at 30°C. Fatty acids were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.1). The fatty acids were analysed by GC (Hewlett Packard 7890) and identified by using the RTSBA6 database of the Microbial Identification System (Sasser, 1990). Polar lipids and isoprenoid quinones were extracted according to Minnikin *et al.* (1984) and isoprenoid quinones analyzed by HPLC as described by Collins (1985). Polar lipids were separated by two-dimensional TLC (coated with silica gel, 10×10 cm; Merck, Germany). Plates were sprayed with various specific reagents for detection of different polar lipids (Minnikin *et al.*, 1984). Polyamines were extracted as described by Scherer and Kneifel (1983). The running solvent was used mixture of ethylacetate and cyclohexane (2:3). Polyamine spots were detected by UV light at 360 nm.

Morphological, cultural, physiological and biochemical characteristics of strain HMD3125^T are listed in Table 1 and in the species description. Strain HMD3125^T exhibited a number of phenotypic similarities with other species in *Pontibacter*, including cell morphology, red pigment, strictly aerobic growth and growth in a broad range of NaCl concentrations. These features of HMD3125^T are typical of members in the genus *Pontibacter*. However, a number of phenotypic characteristics including the carbon utilization pattern using the GN2 MicroPlate and α-chymotrypsin enzyme activities using API ZYM distinguished strain HMD3125^T from other *Pontibacter* species (Table 1). The major fatty acid profile of strain HMD3125^T included summed feature 4 (comprising with iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B; 30.4%), iso-$C_{15:0}$ (20.4%), and iso-$C_{17:0}$ 3OH (17.2%). The complete fatty acid composition of strain HMD3125^T is given in Table 2. Strain HMD3125^T was similar to fatty acid profile of other *Pontibacter* species as iso-$C_{15:0}$, summed feature 4 and iso-$C_{17:0}$ 3OH. The polar lipid profile of HMD3125^T consisted of the compound phosphatidylethanolamine, four unidentified polar lipids, unidentified phospholipid, unidentified aminolipid, and unidentified aminophospholipid (Fig. 2). Polar lipid profile was similar to *Pontibacter* species, but the profile of unidentified aminolipid and phospholipid profile was differenced (Dwivedi *et al.*, 2012; Kang *et al.*, 2012). The major polyamine was *sym*-homospermidine. The DNA G+C

Table 1. Differential characteristics of strain HMD3125^T and recognized *Pontibacter*

Strains: 1, HMD3125^T; 2, *P. salisaro* HMC5104^T; 3, *P. roseus* KACC 14095^T; 4, *P. actiniarum* KCTC 12367^T; 5, *P. akesuensis* KCTC 12758^T; 6, *P. korlensis* KCTC 22337^T; 7. *P. saemangeumensis* GCM0142^T. All data were from this study. +, Positive reaction; –, negative reaction.

Characteristics	1	2	3	4	5	6	7
Growth at 15°C	-	+	+	+	+	-	+
Growth at 42°C	+	-	-	+	+	+	-
Growth without NaCl	+	+	-	-	-	-	+
MacConkey agar	-	-	+	+	-	-	-
Enzyme activity (API ZYM):							
Cystinearylamidase	+	+	-	+	-	+	-
α-Chymotrypsin	-	+	-	-	+	-	-
α-Galactosidase	-	-	-	-	-	+	+
β-Glucuronidase	-	-	-	-	-	+	-
β-Glucosidase	-	-	-	-	-	+	+
Degradation of:							
DNA growth on	-	-	-	-	-	+	-
Utilization of (GN2 Microplate):							
Glycogen	-	+	+	-	+	+	+
Tween 40	-	+	-	-	-	-	-
Tween 80	-	+	-	-	-	-	-
L-Arabinose	-	+	-	-	-	-	+
D-Galactose	-	+	-	-	-	+	+
α-D-Lactose	-	-	-	+	+	+	+
Maltose	-	+	+	-	-	-	+
D-Mannose	-	+	-	-	-	-	+
D-Raffinose	-	+	+	-	-	+	+
L-Rhamnose	-	+	-	+	-	+	-
D-Sorbitol	-	+	+	-	-	+	+
Turanose	-	+	+	-	-	+	+
Xylitol	-	+	-	-	+	+	-
Citric acid	-	-	-	-	-	-	-
Succinamic acid	+	+	+	+	-	+	-
L-Asparagine	-	-	+	+	+	+	+
L-Aspartic acid	-	-	+	+	+	+	+
D-Serine	-	+	-	+	-	+	-
Phenylethylamine	-	-	-	+	+	-	-
Putrescine	-	-	-	-	+	+	-
DNA G+C content (mol %)	45.6	46.0	52.3[a]	48.7[a]	51.4[a]	48.2[a]	48.9

[a] Data from Wang *et al.* (2010), Nedashkovskaya *et al.* (2005), Zhou *et al.* (2007), and Zhang *et al.* (2008).

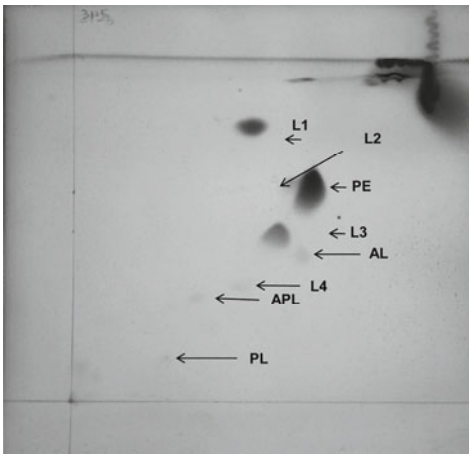

Fig. 2. Polar lipid of the HMD3125^T by Two-dimensional TLC separation. PE, Phosphatidylethanolamine; APL, unidentified aminophospholipid; AL, unidentified aminolipid; PL, unidentified phospholipid; L, unidentified polar lipids.

content of strain HMD3125^T was 45.6 mol%. Phenotypic all data distinguished between strain HMD3125^T and other *Pontibacter* group. And phenotypic all data confirmed the phylogenetic affiliation of strain HMD3125^T to the genus *Pontibacter*. Therefore, HMD3125^T should be classified as a novel species, for which the name *Pontibacter jeungdoensis* is proposed.

Description of *Pontibacter jeungdoensis* sp. nov.

Pontibacter jeungdoensis (*Jeung do en*. N.L. masc. adj. *jeung-doensis* of Jeungdo, a Korean island, from where the type strain was isolated).

Cells are Gram-staining negative, non-motile and aerobic rods. Cell size are 0.8–0.9 μm diameter and 1.4–1.7 μm length. Colonies on R2A are convex, circular and smooth with entire margins, red in color and approximately 5 mm in diameter after 2 days at 25°C. Good growth occurred on MA, TSA, and R2A agars. No growth occurred on cetrimide or MacConkey agars. Growth occured in the presence of

Table 2. Cellular fatty acid profiles of strains HMD3125^T and recognized *Pontibacter*

Strains: 1, HMD3125^T; 2, *P. salisaro* HMC5104^T; 3, *P. roseus* KACC 14095^T; 4, *P. actiniarum* KCTC 12367^T; 5, *P. akesuensis* KCTC 12758^T; 6, *P. korlensis* KCTC 22337^T; 7, *P. saemangeumensis* GCM0142^T. All data were from this study. All strains were grown on MA at 30°C for 2 days. Only the fatty acids amounting to at least 1.0% of the total fatty acids in at least one strain are shown. tr, trace (<1%); nd, no detected.

Fatty acids	1	2	3	4	5	6	7
iso-$C_{15:0}$	20.4	20.4	15.1	28.7	17.0	20.2	16.2
anteiso-$C_{15:0}$	1.1	1.5	4.1	tr	5.2	tr	2.5
$C_{15:1}$ *ω6c*	tr	tr	tr	tr	tr	tr	2.9
iso-$C_{16:1}$ H	1.4	1.2	5.9	tr	2.0	tr	3.5
iso-$C_{16:0}$	1.1	1.9	5.5	tr	1.3	tr	1.1
$C_{16:1}$ *ω5c*	1.1	1.0	1.0	nd	1.1	1.2	4.1
$C_{16:0}$	tr	tr	nd	1.2	tr	tr	tr
iso-$C_{15:0}$ 3OH	8.0	3.9	2.4	3.8	2.7	6.3	1.2
iso-$C_{17:0}$	3.0	2.8	1.8	2.4	1.2	5.5	1.5
anteiso-$C_{17:0}$	1.4	tr	2.0	tr	1.6	tr	tr
$C_{17:1}$ *ω6c*	2.8	3.6	3.7	1.5	4.5	2.2	4.3
iso-$C_{16:0}$ 3OH	nd	tr	1.2	tr	nd	tr	tr
$C_{18:1}$ *ω9c*	4.2	2.3	1.0	1.9	2.2	1.9	2.1
iso-$C_{17:0}$ 3OH	17.2	15.3	nd	nd	7.2	14.5	3.9
Summed feature[a]							
1	2.0	tr	1.7	2.7	1.8	tr	nd
3	1.4	tr	nd	13.6	tr	nd	6.2
4	30.4	37.2	42.1	29.9	46.2	30.7	36.8
8	nd	nd	nd	nd	tr	2.5	1.2
9	2.1	1.9	nd	1.2	1.0	tr	nd

[a] Summed feature are groups of two or three fatty acids that cannot be separated by GLC using the Midi system. Summed feature 1 comprised $C_{13:0}$ 3OH and/or iso-$C_{15:1}$ H, summed feature 3 comprised $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*, summed feature 4 comprised anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I, summed feature 8 comprised $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c* and summed feature 9 comprised iso-$C_{17:1}$ *ω9c* and/or 10-methyl $C_{16:0}$.

0–5% (w/v) NaCl (optimum, 0.5–1%), pH 7–8 (optimum, pH 8), and 15–37°C (optimum, 30°C). Oxidase and catalase activities are present, but DNase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, β-galactosidase, and L-phenylalanine deaminase activities are absent. CM-Cellulose, casein and starch are not hydrolyzed. Esculin is utilized, but citrate and gelatin are not utilized. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucinearyl amidase, valine arylamidase, cysteine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and *N*-acetyl-β-glucosaminidase activities are present in the API ZYM gallery, but lipase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, and α-fucosidase activities are absent. Succinic acid mono-methyl-ester and succinic acid are utilized as sole carbon sources on GN2 MicroPlates. Other carbon sources are not utilized. The polar lipid profile of HMD3125^T consists of the compound phosphatidylethanolamine, four unidentified polar lipids, unidentified phospholipid, unidentified aminolipid, and unidentified aminophospholipid. The major fatty acids (>10.0%) are summed feature 4, iso-$C_{15:0}$, and iso-$C_{17:0}$ 3OH. The major polyamine is *sym*-homospermidine. The complete fatty acid content is given in Table 2. The DNA G+C content is 45.6 mol%.

The type strain, HMD3125^T (=KCTC 23156^T =CECT 7710^T), was isolated from a clay tablet solar saltern in Jeungdo, Jeollanam-do, Republic of Korea (34° 59′ 47″ N 126° 10′ 02″ E).

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