The Journal of Microbiology (2011) Vol. 49, No. 2, pp. 290-293
DOI 10.1007/s12275-011-0093-6

NOTE

Pontibacter salisaro sp. nov., Isolated from a Clay Tablet Solar Saltern in Korea

Yochan Joung[2], Haneul Kim[1], Tae-Seok Ahn[2], and Kiseong Joh[1]*

[1]*Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Youngin 449-791, Republic of Korea*
[2]*Departement of Environmental Science, Kangwon National University, Chuncheon 200-701, Republic of Korea*

(Received March 9, 2010 / Accepted November 12, 2010)

A Gram-negative, aerobic, rod-shaped, and red-pigmented bacterial strain, HMC5104^T, was isolated from a solar saltern, found in Jeungdo, Republic of Korea (34°59′47″N 126°10′02″E). The major fatty acids were summed feature 4 (comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B; 37.2%), iso-$C_{15:0}$ (20.4%), and iso-$C_{17:0}$ 3OH (15.3%). The DNA G+C content was 46.0 mol%. The major isoprenoid quinone was menaquinone-7 (MK-7). A phylogenetic tree based on 16S rRNA gene sequences showed that strain HMC5104^T formed a lineage within the genus *Pontibacter*, and was closely related to *Pontibacter korlensis* (95.9%), *P. roseus* (94.9%), and *P. actiniarum* (94.3%). Similarities to all other *Pontibacter* species were between 95.9-93.9%. On the basis of the evidence presented in this study, strain HMC5104^T represents a novel species of the genus *Pontibacter*, for which the name *Pontibacter salisaro* sp. nov. is proposed. The type strain is HMC5104^T (=KCTC 22712^T =NBRC 105731^T).

Keywords: *Pontibacter salisaro* sp. nov., saltern

The genus *Pontibacter*, first described by Nedashkovskaya *et al.* (2005), is a member of the Family *Cytophagacea*. Members of the genus *Pontibacter* are Gram-negative, strictly aerobic, red-pigmented, and have menaquione-7 (MK-7) as their main respiratory quinone. The genus *Pontibacter* consisted of 5 validly published species, at the time of writing: *Pontibacter actiniarum*, *P. akesuensis*, *P. korlensis*, *P. roseus*, and *P. xinjiangensis* (Nedashkovskaya *et al.*, 2005; Suresh *et al.*, 2006; Zhou *et al.*, 2007; Zhang *et al.*, 2008; Wang *et al.*, 2010).

In the course of a study on the microbial diversity of a solar saltern in Jeungdo, Jeollanam-do, Republic of Korea, we isolated a red-pigmented bacterial strain. Using the standard dilution plating technique, the solar slatern samples were incubated on tryptic soy agar (TSA; Difco, USA), for 48 h at 30°C. The isolate was routinely cultured on TSA and R2A (Difco), and the culture was suspended in aqueous glycerol (20%, w/v) for storage at -80°C.

Almost-complete sequences of the 16S rRNA genes were obtained for strain HMC5104^T, described previously (Cho and Giovannoni, 2003). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were performed using the EzTaxon server [http://147.47.212.35:8080/index.jsp; (Chun *et al.*, 2007)]. The phylogenetic relationships between strain HMC5104^T and the type strains of *Pontibacter* species were defined by MEGA4 (software description) (Tamura *et al.*, 2007). Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou and Nei, 1987) algorithms. The robustness of the topologies for the maximum parsimony, maximum-likelihood, and neighbour-joining trees were evaluated by means of bootstrap analysis (Felsenstein, 1985); based on 1,000 resamplings of the sequences, respectively. All of the phylogenetic trees generated in this study (Fig. 1), including maximum parsimony, maximum-likelihood, and neighbour-joining, indicated that the solar saltern strain HMC5104^T belonged to the genus *Pontibacter*. Strain HMC5104^T exhibited 95.9, 94.9, and 94.3% 16S rRNA sequence similarities with *P. korlensis*, *P. roseus*, and *P. actiniarum*, respestviely. Similarities to all other *Pontibacter* species were within a range of 95.9-93.9%. This phylogenetic analyses suggested that the strain should be assigned to the Genus *Pontibacter* as the representative of a novel species.

Cell morphology was examined by light microscopy, and the presence of flagella was determined by motility test medium (Difco). Gram staining was performe using the Gram Stain Kit (bioMérieux, USA) 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, USA). The presence of flexirubin-type pigments was investigated using the bath chromatic shift test, with a 20% (w/v) KOH solution (Bernardet *et al*., 2002). Catalase and oxidase tests were performed according to standard methods (MacFaddin, 1980). The pH range for growth was determined in modified R2A broth (containing yeast extract 0.5 g, peptone 0.5 g, casamino acids 0.5 g, dextrose 0.5 g, starch 0.5 g, sodium pyruvate 0.3 g, dipotassium phosthate 0.3 g, and magnesium sulfate 0.05 g; per 1,000 ml distilled water), which was adjusted to various pH values from 4.0 to 10.0 (at intervals of 1.0 pH). Growth in the presence of 0.5%, 1.0-5.0% (at intervals of 1.0%), and 10.0% NaCl (w/v) was

* For correspondence. E-mail: ksjoh@hufs.ac.kr; Tel: +82-31-330-4271; Fax: +82-31-330-4566

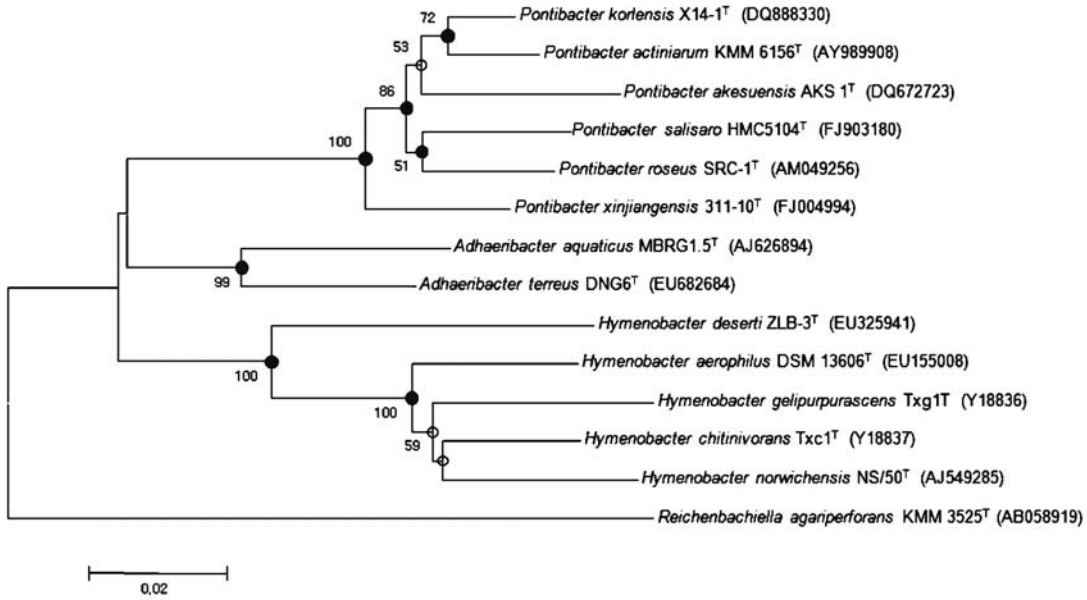

Fig. 1. Neighbour-joining phylogenetic tree. Phylogenetic position of strain HMC5104^T within the genus *Pontibacter*, based on 16S rRNA gene sequences. Bootstrap percentages (>50%) from both neighbour joining (above nodes) is shown. Filled and open circles indicate nodes recovered by all three treeing methods (neighbor joining, maximum-likelihood and maximum parsimony) and by two treeing methods (NJ and ML or NJ and MP), respectively. *R. agariperforans* KMM3525^T (AB058919) was used as outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics of strain HMC5104^T and species of the genus *Pontibacter*
Strains: 1, HMC5104^T; 2, *Pontibacter roseus* KACC140954^T; 3, *Pontibacter actiniarum* KCTC12367^T; 4, *Pontibacter akesuensis* KCTC12758^T; 5, *Pontibacter korlensis* KCTC22337^T; 6, *Pontibacter xingiangensis* (Wang *et al*., 2010). All data was obtained from this study; except the DNA G+C content of the 4 reference strains (Nedashkovskaya *et al.*, 2005; Suresh *et al.*, 2006; Zhou *et al.*, 2007; Zhang *et al.*, 2008). +, Positive; –, negative reaction; nt, not tested.

Characteristics	1	2	3	4	5	6
Habitats	saltern	muddy water	soil	marine	soil	soil
Colony color	red	pink	orange	orange	orange	pink
Growth at (°C)						
4	-	+	-	-	-	+
15	-	+	+	+	-	+
42	-	-	+	+	+	-
Growth on 8.0% NaCl	+	+	-	+	+	-
Enzyme activity : (API kit)						
Arginine dehydrolase	+	-	+	+	+	-
Urease	+	-	+	+	+	-
Indole production	-	+	-	-	-	-
Gelatinase	-	+	-	-	-	nt
Utilization of : (GN2 Microplate)						
α-D-Glucose	-	+	-	-	+	+
m-Inositol	+	-	+	+	+	nt
Lactulose	+	-	+	+	+	nt
D-Raffinose	-	+	-	-	+	+
D-Sorbitol	-	-	-	-	+	nt
Acetic acid	+	-	+	+	+	-
D-Alanine	+	-	+	-	-	-
L-Alanine	+	-	+	-	-	nt
L-Alanyl-glycine	+	+	+	+	-	nt
L-Ornithine	+	+	+	-	-	nt
L-Proline	-	+	-	+	+	nt
DNA G+C content (mol/%)	46.0	52.0	48.7	51.4	48.2	47.8

Table 2. Cellular fatty acid profiles of strain HMC5104^T and species of the genus *Pontibacter*
Strains: 1, HMC5104^T; 2, *Pontibacter roseus* KACC140954^T; 3, *Pontibacter actiniarum* KCTC12367^T; 4, *Pontibacter akesuensis* KCTC12758^T; 5, *Pontibacter korlensis* KCTC22337^T; 6, *Pontibacter xingiangensis* (Wang *et al*., 2010). All data was obtained from this study; except *P. xingiangensis*. All strains were grown on TSA at 30°C for 2 days. Only the fatty acids amounting for 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
unknown 13.565	tr	tr	2.2	3.3	6.2	nd
iso-$C_{15:0}$	20.4	18.0	35.5	19.7	25.9	16.2
anteiso $C_{15:0}$	1.5	4.0	tr	tr	tr	1.5
iso-$C_{16:1}$ H	1.2	1.4	tr	1.6	tr	1.3
iso-$C_{16:0}$	1.9	1.4	tr	tr	tr	1.3
$C_{16:1}$ ω5*c*	1.0	tr	1.1	1.3	1.5	6.9
iso-$C_{15:0}$ 3OH	3.9	2.9	3.8	2.9	4.1	2.4
iso-$C_{17:1}$ ω9*c*	tr	tr	2.1	1.3	1.0	nd
iso-$C_{17:0}$	2.8	1.9	1.4	3.5	6.8	1.2
$C_{17:1}$ ω6*c*	3.6	5.0	tr	5.4	2.2	2.9
$C_{18:1}$ ω9*c*	2.3	1.7	tr	tr	tr	1.4
iso $C_{17:0}$ 3OH	15.3	10.3	4.7	6.8	5.7	8.4
Summed Feature 1[a]	tr	1.1	3.4	2.7	tr	1.8
Summed Feature 3	tr	tr	4.5	2.6	2.0	14.5
Summed Feature 4	37.2	44.0	33.5	39.5	37.9	21.9
Summed Feature 9	1.9	1.4	nd	nd	nd	nd

[a] Summed Features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 1 comprised iso-$C_{15:1}$ H and/or $C_{13:0}$ 3OH, summed feature 3 comprised iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ ω7*c*, summed feature 4 comprised iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B and summed feature 9 comprised iso-$C_{17:1}$ ω9c and/or 10-methyl $C_{16:0}$.

also tested in modified R2A broth. The temperature range of strain viability and optimum for growth were assessed on R2A at 4°C, 10-30°C (at 5°C intervals), 37°C and 42°C. Hydrolysis of casein [3% (v/v) skimmed milk (Difco)], CM-cellulose [1.0% (w/v) CM-cellulose (Sigma, USA)] and starch (1.0%, w/v) were tested, using R2A as the basal medium. MacConkey agar (Difco) and DNase test agar (Difco) were used for growth and DNase assays, respectively. Basic biochemical tests and carbon-source-oxidation tests were performed using API 20E and API ZYM strips (bioMérieux), and GN2 MicroPlates (Biolog Inc., USA); according to the manufacturer's instructions. The G+C content was determined using HPLC analysis of hydrolysed DNA, according to Tamaoka (1986). The fatty acid methyl esters (FAMEs) were obtained from cells by saponification, methylation, and extraction. Analysis by gas chromatography was performed with MIDI software (Microbial ID), and peaks were automatically integrated and identified by the Microbial identification software package. Isoprenoid quinones were isolated according to Minnikin *et al.* (1984), and analysed by HPLC as described by Collins (1984).

Morphological, cultural, physiological, and biochemical characteristics of strain HMC5104^T have been listed in Table 1 and in the species description. HMC5104^T exhibited a number of phenotypic similarities to species of the genus *Pontibacter*, including similar cell morphology, red-colored pigments, and strictly aerobic growth. These features of HMC5104^T are typical characteristics of members of the genus *Pontibacter*; however, several characteristics of HMC5104^T clearly differentiated this strain from the type strains of *Pontibacter* genus (Table 1). The DNA G+C content of strain HMC5104^T was 46.0 mol%; which is, lower than the values of the other strains of the genus *Pontibacter* (48.7-59.5 mol%).

The fatty acid profile of the sample of the strain HMC5104^T comprised of summed feature 4 (comprising of iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B; 37.2%), iso-$C_{15:0}$ (20.4%), and iso-$C_{17:0}$ 3OH (15.3%). This fatty acid profile was similar to other species of the genus *Pontibacter*, in terms of summed feature 4 and iso $C_{15:0}$; but the relative proportions of iso $C_{17:0}$ 3OH, $C_{17:1}$ ω6*c* and anteiso $C_{15:0}$ differed to what is found in other *Pontibacter* species. The major isoprenoid quinone in strain HMC5104^T was menaquinone-7 (MK-7), which is similar to those of other *Pontibacter* species. Therefore, the strain HMC5104^T should be classified, in the genus *Pontibacter*, as a member of novel species, for which the name *Pontibacter salisaro* sp. nov. is proposed.

Description of *Pontibacter salisaro* sp. nov.

Pontibacter salisaro (*salis. aro.* NL. N. *salis* salt aro NL. N. *aro* farm; saltern salt farm).

Cells are Gram-negative, non-motile, strictly aerobic, and rod shaped; and were found to be 0.7-0.8 μm in diameter and 1.5-1.7 μm in length. Good growth occurs on MA, TSA, and R2A agar. No growth occurs on cetrimide agar and MacConkey agar. Colonies on TSA are convex, circular, smooth (with entire margins), red in colour, and approximately 5 mm in diameter; after 2 days at 30°C. No flexirubin-type pigments are formed. Growth occurs in the presence of 0-10% (w/v) NaCl (optimum, 0.5-1%), between pH 8-9 (optimum, pH 9), and between 20-37°C (optimum, 30°C).

Oxidase, catalase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase activities are present. β-Galactosidase and L-phenylalanine deaminase activities are absent. Casein and starch are hydrolysed and utilized, but tyrosine and citrate are not. Aesculin and gelatin are hydrolysed.

In the API ZYM gallery, alkaline phosphatase, esterase (C4), ester lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and *N*-acetyl-β-glucosaminidase activities are present; but lipase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-xannosidase, and α-fucosidase activities are absent. The following compounds are utilized as sole carbon sources in GN2 microplates: *N*-acetyl-D-galactosamine, D-cellobiose, *i*-erythritol, D-fructose, L-fucose, m-inositol, α-D-lactose, lactulose, D-melibiose, β-methyl-D-glucoside, D-psicose, L-rhamnose, sucrose, D-trehalose, pyruvic acid methyl ester, succinic acid mono-methyl-ester, acetic acid, Cis-aconitic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, β-hydroxybutyric acid, itaconic acid, α-keto valeric acid, malonic acid, propionic acid, sebacic acid, succinic acid, bromosuccinicacid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, D-serine, L-serine, L-threonine, D,L-carnitine, urocanic acid, inosine, uridine, phenylethyl-amine, 2-aminoethanol, 2,3-butanediol, glycerol, D,L,α-glycerol phosphate, and α-D-glucose-1-phosphate. The following carbon sources are not utilized: α-cyclodextrin, dextrin, glycogen, tween40, tween80, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannitol, D-mannose, D-raffinose, D-sorbitol, turanose, xylitol, citric acid, formic acid, D-glucuronic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, ρ-hydroxy phenlyacetic acid, α-keto butyric acid, α-keto glutaric acid, D,L-lactic acid, quinic acid, D-saccharic acid, glycyl-L-glutamic acid, L-proline, L-pyroglutamic acid, γ-amino butyric acid, thymidine, putrescine, and D-glucose-6-phosphate.

The major fatty acids are summed feature 4 (comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B), iso-$C_{15:0}$ and iso-$C_{17:0}$ 3OH. The complete fatty acid content is given in Table 2. The DNA G+C content is 46.0 mol%.

The type strain, HMC5104^T (=KCTC 22712^T =NBRC 105731^T), was isolated from a solar saltern in Jeungdo, Jeollanam-do, Republic of Korea.

This research was supported by the project for the survey and excavation of Korean indigenous species by the National Institute of Biological Resources (NIBR), under the Ministry of Environment, Korea.

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