ORIGINAL PAPER



Pontibacter locisalis Sy30T sp. nov. isolated from soil collected from an abandoned saltern

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Received: 26 June 2015/Accepted: 4 January 2016/Published online: 29 January 2016 © Springer International Publishing Switzerland 2016

Abstract A novel Gram-stain negative, red, rodshaped, non-motile and aerobic bacterial strain, designated Sy30^T, was isolated from dry soils of an abandoned marine saltern at Weihai, China. 16S rRNA sequence analysis indicated that strain Sy30^T belongs to the genus *Pontibacter* in the family *Cytophagaceae*, with sequence similarities ranging from 93.3 to 96.4 % with other type species of the genus *Pontibacter*. The predominant cellular fatty acids were iso-C_{15:0} and summed feature 4 (iso-C_{17:1} I and/or anteiso-C_{17:1} B). The major menaquinone was MK-7. The major polyamine was *sym*-homospermidine. The DNA G+C content was 47.7 mol%. The major polar lipids consisted of phosphatidylethanolamine, phosphoaminolipid and two unidentified polar lipid. Based on

Electronic supplementary material The online version of this article (doi:10.1007/s10482-016-0646-0) contains supplementary material, which is available to authorized users.

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phenotypic, chemotaxonomic and phylogenetic analysis, strain Sy30^T represents a novel species of the genus *Pontibacter* in the family *Cytophagaceae*, phylum *Bacteroidetes*, for which the name *Pontibacter locisalis* sp. nov. is proposed. The type strain is Sy30^T (=KCTC 42498^T = CICC AB 2015060^T).

Keywords *Pontibacter locisalis* · 16S rRNA gene sequence · Polyphasic taxonomy

Introduction

The genus *Pontibacter* belongs to the family *Cytopha*gaceae within the phylum Bacteroidetes, and was first described by Nedashkovskaya et al. (2005). In the genus, menaquinone (MK-7) is the major quinone, and most members possess phosphatidylethanolamine as one of the major polar lipids (Xu et al. 2012; Dwivedi et al. 2013; Kang et al. 2013; Singh et al. 2013; Subhash et al. 2013; Joung et al. 2013; Zhang et al. 2013; Singh et al. 2014; Srinivasan et al. 2014; Subhash et al. 2014; Cao et al. 2014; Dai et al. 2014; Xu et al. 2014; Singh et al. 2015; Mahato et al. 2015). Currently, the genus Pontibacter comprises 20 validly named species. These species have been isolated from diverse environments, including soil of solar salterns, soil of hexachlorocyclohexane (HCH) dump sites, sediment containing discarded HCH isomer waste, forest soil, rhizospheric soil, desert soil, sea water and a drainage system. 'Pontibacter salisaro', 'Pontibacter jeungdoensis' and Pontibacter odishensis were originally isolated from soil of solar salterns (Joung et al. 2011, 2013; Subhash et al. 2013). Here, we report the taxonomic characteristics of a novel Pontibacter species from dry soil of a abandoned marine solar saltern at Weihai, China.

Materials and methods

Bacterial strains isolation and cultivation

Strain Sy30^T was isolated from dry soil of an abandoned marine solar saltern at Weihai, China (122°0'21.6"E, 36°69'52.8"N) during October 2013. One gram of soil was suspended in10 ml sterile water, serially diluted to 10^{-4} dilution, and 100 µl dilution was spread on 2216E agar (Hopebio) plate. Single colonies were isolated after incubation at 28 °C for 1 week, and a red strain was obtained and designated as strain Sy30^T. The routine cultivation of the strain and phenotypic tests was carried on 2216E agar. The reference strains, Pontibacter xinjiangensis CCTCC AB 207200^T and Pontibacter korlensis CCTCC AB 206081^T, were kindly provided by China Center for type Culture Collection (CCTCC). The type species of Pontibacter, Pontibacter actiniarum KCTC 12367^T was purchased from Korean Collection for Type Cultures (KCTC). All strains were cultured under the comparable conditions for physiological tests and chemotaxonomic characterisations (except polar lipid analysis) and preserved at -80 °C in sterile distilled water supplemented with 1 % NaCl (w/v) and 15 % (v/v) glycerol.

Phenotypic characterization

Gram reaction was determined as described by Smibert and Krieg (1994). Cell morphology and size of the isolate and reference strains were examined by light microscopy (Ci-L; Nikon) after incubation on 2216E agar at 30 °C for 3 days. Hydrolysis of gelatin, aesculin, Tween 80, agar, starch and casein were determined as described by Cowan and Steel (1965). Alginate hydrolysis activity was detected by flooding the 2216E agar plate containing 1.0 % sodium alginate with a diluted Lugol solution, according to (Akagawa and Yamasato 1989) and Schlesner et al. (1990). Catalase and oxidase activities were tested by bubble formation in 3.0 % (v/v) H_2O_2 solution and the bioMerieux oxidase reagent kit, respectively. Growth at 4, 10, 15, 20, 25, 28, 30, 33, 35, 37, 40, 42 and 45 $\,^{\circ}\mathrm{C}$ were measured in 2216E liquid medium (Hopebio), and the pH range for growth was determined in 2216E liquid medium adjusted to pH 5.5-9.5 with 20 mM MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0 and 9.5). Tolerance to NaCl was tested as described previously (Du et al. 2014). Gliding motility tests were performed by preparing a light suspension of cells in seawater and then placing a drop on quarterstrength 2216E liquid medium solidified with 1.0 % agarose. After 16 h incubation at 10 °C, the inoculated area was covered with a glass coverslip and observed by oil-immersion phase-contrast microscopy (AX70; Olympus) (Bowman 2000). The presence of flexirubin-type pigments was identified by KOH test (Fautz and Reichenbach, 1980). Growth under anaerobic conditions was determined after incubation in 2216E liquid medium with or without 0.1 % (w/v) NaNO₃ in an anaerobic chamber for 2 weeks at 30 °C. Antibiotic sensitivity was investigated on 2216E agar plates by using discs (Tianhe) containing different antibiotics for 3 days at 30 °C. Assimilation of different carbohydrates was tested in basal media supplemented with a final concentration of 1.0 % (w/v) of the tested carbon sources (Xu et al. 2010). Other biochemical tests were carried out using API 20E and API ZYM strips (bioMerieux) according to the manufacturer's instructions, with the modification of adjusting the NaCl concentration to 2.0 % (w/v).

Phylogenetic analysis of 16S rRNA gene sequence

Genomic DNA extraction, PCR amplification and 16S rRNA gene sequencing were carried out as described previously (Liu et al. 2014). Similarity of 16S rRNA gene was calculated by using NCBI BLAST and the EzTaxon-e server (Kim et al. 2012). The phylogenetic trees were constructed using the neighbour-joining (NJ) and maximum-likelihood (ML) algorithms in the program MEGA version 6.0 (Tamura et al. 2013). Distances were calculated by the Kimura two-parameter model, and the robustness was evaluated by bootstrap analyses based on 1000 replications.

Chemotaxonomic characterization

Cells of strain Sy30^T were harvested at the lateexponential growth phase in 2216E liquid medium at 30 °C for characterization of isoprenoid quinones, cellular fatty acids and polar lipids. Extraction, methylation and analysis of the fatty acids were according to the standard Microbial Identification (MIDI) system (Sasser 1990). Polyamines were extracted from late exponentially growing cells in 2216E liquid medium according to Busse and Auling (1988) and analysed by one-dimensional TLC. A mixture of ethylacetate and cyclohexane (2:3) was used as the running solvent. Polyamine spots were visualized under UV light after the TLC plate was allowed to air dry. Polyamines were identified by comparing Rf values by using commercially prepared standards. Respiratory quinones were extracted and detected by HPLC as described by Hiraishi et al. (1996). Polar lipid analysis of strain Sy30^T was carried out by the Identification Service of the DSMZ, Braunschweig, Germany, DNA G+C content of strain Sy30^T was determined using HPLC according to Mesbah et al. (1989).

Results and discussion

Phenotypic characteristics

Strain Sy30^T was found to be Gram-stain negative, non-motile, aerobic and rod-shaped with the size of $0.4-0.6 \ \mu m$ in width and $2.0-7.0 \ \mu m$ in length. Colonies were observed to be red, non-luminescent, and circular with entire edges and 1.0-2.0 mm in diameter after 72 h incubation on 2216E agar at 30 °C. Growth occurs at 15-40 °C (optimum, 30-33 °C) and pH 6.5-9.0 (optimum, 7.0-8.0) and in presence 0.5–5.0 % of NaCl (optimum, 2.0–2.5 %). No growth in salt-free medium, which differed from related species. Strain Sy30^T and three reference strains were negative for indole production; and all strains could hydrolyse alginate, gelatin, but not cellulose, tween 80 and urea. Flexirubin-type pigments were not detected in any strains tested. However, strain Sy30^T differed from its close relatives, Pontibacter xinjiangensis CCTCC AB 207200^T and Pontibacter korlensis CCTCC AB 206081^T, in that it neither utilized D-mannose nor produced α -galactosidase. Other phenotypic features of strain Sy30^T are presented and compared with those of related strains in Table 1. The type strain is sensitive to nalidixic acid, TMP oxygen pyrimidine, tetracycline, streptomycin ampicillin, penicillin, ceftriaxone, linkomicin, rifampicin, clindamycin, neomycinand, chloramphenicol, erythromycin, norfloxacin, vancomycin, acetylspiramycin, cefotaxime, ofloxacin, but resistant to kanamycin, gentamicin and tobramycin.

Molecular phylogenetic analysis

Based on the almost full-length 16S rRNA gene sequence (1446 bp), strain Sy30^T showed high similarity to *P. xinjiangensis* 311-10^T (96.4 %), *Pontibacter korlensis* X14-1^T (96.1 %), *Pontibacter yuliensis* H9X^T (95.8 %), and *Pontibacter saemangeumensis* GCM0142^T (95.5 %), while all other similarities were 93.3–95.3 %. As shown in Fig. 1, phylogenetic analysis showed that strain Sy30^T formed a clade with *P. xinjiangensis* 311-10^T (69 % bootstrap support) within the genus *Pontibacter*. The same topology was retrieved in the ML tree. The 16S rRNA gene sequence similarities and phylogenetic location suggested that the novel strain belongs to the genus *Pontibacter*.

Chemotaxonomic characterization

The major fatty acids (>10 %) of strain $Sy30^{T}$ were iso- $C_{15:0}$, and summed feature 4 (iso- $C_{17:1}$ I and/or anteiso- $C_{17:1}$ B), which were similar to the related *Pontibacter* type strains (Table S1, available in the online Supplementary Material), except for the proportion of major components and the types of minor components. In addition, strain Sy30^T was distinguished from the close phylogenetic neighbour, P. xinjiangensis CCTCC AB 207200^T, by absence of the major fatty acid of summed feature $3(C_{16:1}\omega7c \text{ and/or iso-}C_{15:0} 2\text{-OH})$. The DNA G+C content of strain $Sy30^{T}$ was 47.7 mol%, a value that fell within the range of those reported for other Pontibacter species. The polyamine pattern showed the presence of sym-homospermidine as the major polyamine, in accordance with that of Pontibacter ramchanderi LP43^T, Pontibacter lucknowensis DM9^T, P. jeungdoensis HMD3125^T, Pontibacter soli HYL7-26^T, Pontibacter indicus LP100^T and Pontibacter chinhatensis LP51^T (Dwivedi et al. 2013; Singh et al. 2013; Joung et al. 2013; Dai et al. 2014; Singh et al. 2014; Singh et al. 2015). The predominant menaquinone was MK-7, a characteristic feature of the genus Pontibacter. The

Table 1 Different characteristics of strain Sy 30^{T} and type strains of the close relatives in the genus <i>Pontibacter</i> <i>Strain 1 P. locisalis</i> Sy 30^{T} , <i>2 P. xinjiangensis</i> CCTCC AB 207200 ^T , <i>3 P. korlensis</i> CCTCC AB 206081 ^T , <i>4 P.</i> actiniarum KCTC 12367 ^T , <i>5 P. yuliensis</i> H9X ^T (Cao et al. 2014), <i>6 P.</i> <i>diazotrophicus</i> H4X ^T (Xu et al. 2014), <i>7 P.</i> <i>saemangeumensis</i> GCM0142 ^T (Kang et al. 2013), <i>8 P. humi</i> SWU8 ^T (Srinivasan et al. 2014). Data were obtained in this study unless indicated otherwise. +, positive, -, negative, <i>w</i> weak, <i>ND</i> no data * Data from Wang et al. (2010), Nedashkovskaya et al. (2005) and Zhang et al. (2008)	Characteristic	1	2	3	4	5	6	7	8
	Colony color	Red	Pink	Pink	Pink	Pink	Red	Pink	Pink
	Motility	-	_	+	+	+	+	-	_
	Growth at/with								
	4 °C	-	+	-	_	+	+	+	_
	pH 10	-	+	+	_	+	-	+	_
	0 % NaCl (w/v)	-	+	+	+	+	+	+	+
	Nitrate reduction	-	+	-	_	-	-	-	_
	Hydrolysis of								
	Oxide	-	w	+	+	w	+	+	+
	Casein	-	+	-	_	+	+	+	+
	Gelatin	+	+	+	+	-	-	-	-
	Enzyme activity								
	α-Chymotrypsin	_	+	-	_	-	-	-	-
	α-Galactosidase	_	+	+	_	-	-	+	-
	β-Galactosidase	-	w	W	+	-	-	+	+
	α-Glucosidase	-	_	+	+	-	-	+	+
	β-Glucosidase	-	_	+	_	-	-	+	-
	Carbon-source utilization								
	D-Mannose	-	+	+	+	-	+	+	-
	D-Arabitol	+	_	+	+	ND	ND	+	-
	D-Melibiose	+	+	+	_	_	_	+	+
	DNA G+C content (mol%)	47.7	48.2*	48.2–48.9*	48.7*	55.2	51.4	48.9	48.5

polar lipid profile consisted of major amounts of phosphatidylethanolamine (PE), two unidentified polar lipids (L), a phosphoaminolipid (PN), and minor amounts of two unidentified polar lipids and two phospholipids (PL) (Fig. S1, available in the online supplementary material). The presence of major amount of PN distinguished strain $Sy30^{T}$ from the close phylogenetic neighbour, *P. xinjiangensis* CCTCC AB 207200^T (Kang et al. 2013), while the profile included PE as one of the major polar lipids in line with other members of the genus *Pontibacter*.

Taxonomic conclusion

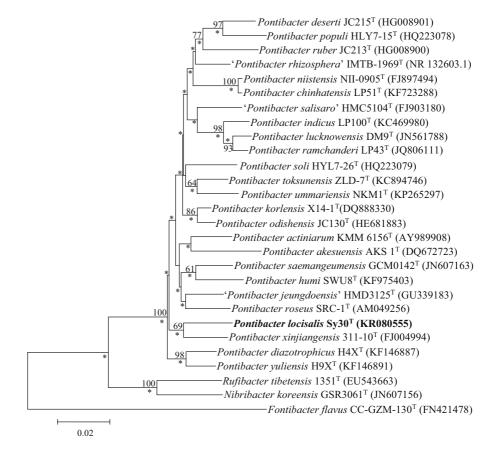
Based on phylogenetic analysis, strain $Sy30^{T}$ could be assigned to the genus *Pontibacter*. However, the strain could be distinguished from the close relatives by several phenotypic characteristics (Table 1). Considering the common chemotaxonomic characterization, strain $Sy30^{T}$ is considered to represent a novel species of the genus *Pontibacter*, for which the name *Pontibacter locisalis* sp. nov. is proposed. The type strain is $Sy30^{T}$ (=KCTC 42498^T = CICC AB 2015060^T).

Description of *Pontibacter locisalis* sp. nov.

Pontibacter locisalis (lo.ci.salis. L. n. locus place, locality; L. gen. n. salis of salt; N.L. gen. n. *locisalis* from a place of salt)

Cells are Gram-stain negative, non-motile, aerobic and rod-shaped with the size of 0.4-0.6 µm in width and 2.0-7.0 µm in length. Colonies are red, non-luminescent, and circular with entire edges on 2216E agar. Flexirubin-type pigments are absent. Growth occurs at 15-40 °C (optimum, 30-33 °C) and pH 6.5-9.0 (optimum, 7.0-8.0) and in presence 0.5-5.0 % of NaCl (optimum, 2.0-2.5 %). The type strain requires NaCl for growth. Can hydrolyze gelatin, aesculin, alginate, starch, but not casein, cellulose and agar. Nitrate reduction, lysine decarboxylase, ornithine decarboxylase, H₂S production, citrate utilization, Voges-Proskauer reaction, indole production, glucose acidification, arginine dihydrolase and urease are negative. Positive for catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-

Fig. 1 Neighbour-joining phylogenetic tree based on 16S RNA gene sequences of strain Sy30^T and close relatives. *Fontibacter flavus* was used as an outgroup. Bootstrap values (1000 replications) above 50 % are showed at *nodes*. *Asterisks* indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. *Bar* 0.02 substitutions per nucleotide position



BI-phosphohydrolase and N-acetyl-β-glucosaminidase, but negative for oxidase, lipase (C14), α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. Assimilates sucrose, D-lactose, D-glucose, Dgalactose, D-fucose, L-rhamnose, D-melibiose, D-arabitol, D-trehalose, but not D-mannose, L-alanine and sodium lactate. The predominant fatty acids are iso-C_{15:0}, and summed feature 4 (iso-C_{17:1} I and/or anteiso-C_{17:1} B). The major menaquinone is MK-7. The major polyamine is sym-homospermidine. The DNA G+C content of the type strain is 47.7 mol%. The polar lipids comprise major amounts of phosphatidylethanolamine, a phosphoaminolipid and two unidentified polar lipids, and minor amounts of two unidentified polar lipids and two phospholipids.

The type strain Sy30^T (=KCTC 42498^T=CICC AB 2015060^T), was isolated from dry soil of an abandoned marine solar saltern at Weihai, China. The GenBank accession number for the 16S rRNA gene sequence of strain Sy30^T is KR080555.

Acknowledgments This work was supported by the National Natural Science Foundation of China (31370057, 31370108), National Science and Technology Major Project of China (2013ZX10004217) and 2013 Shandong Provincial Second Group Projects on Resource Platforms for Marine Economic and Innovative Development Regions: Marine Microorganisms Preservation Platform (2150299). We thank China Center for type Culture Collection (CCTCC) for kindly providing the type strains, *Pontibacter xinjiangensis* CCTCC AB 207200^T and *Pontibacter korlensis* CCTCC AB 206081^T.

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