Current Microbiology (2020) 77:1334–1340
https://doi.org/10.1007/s00284-020-01885-9

Nitratireductor mangrovi sp. nov., a Nitrate-Reducing Bacterium Isolated from Mangrove Soil

Yanghui Ye[1] · Cen Yan[1] · Yanfang Nie[2] · Jianwei Zhang[3] · Zhe Zhao[2] · Ran Zhang[2] · Chuan Xiao[4] · Min Wu[1]

Received: 16 October 2019 / Accepted: 10 January 2020 / Published online: 2 March 2020

Abstract

A Gram-stain-negative, non-motile and short-rod-shaped bacterium, designated as strain SY7^T, was isolated from rhizosphere soil of the mangrove *Kandelia obovata* of Fugong village, in Zhangzhou, China. The isolate grew at 10–45 °C (optimum 30 °C), pH 6.0–10.0 (optimum pH 7.0) and 0–8% NaCl (optimum 3%, w/v). The 16S rRNA gene sequence and phylogenetic analysis revealed that strain SY7^T located within the radiation of genus *Nitratireductor* and showed the highest sequence similarity of 97.23% to *Nitratireductor pacificus* MCCC 1A01024^T. The DNA G+C content was 64.9%. In silico DNA–DNA hybridization and average nucleotide identity values between strain SY7^T with reference strains of *N. pacificus* MCCC 1A01024^T, *N. basaltis* KCTC 22119^T and *N. aquibiodomus* DSM 15645^T were 16.7%, 14.3%, 14.7% and 75.2%, 72.6%, 73.5%, respectively. The major isoprenoid quinone was Q-10. The dominant fatty acids were 11-methyl $C_{18:1}$ $\omega 7c$, iso-$C_{17:0}$, $C_{19:0}$ $\omega 8c$ cyclo and summed feature 8 ($C_{18:1}$ $\omega 6c$/$C_{18:1}$ $\omega 7c$), a profile that almost matched the other members of the genus *Nitratireductor*. The predominant polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol. On the basis of the phenotypic, phylogenetic and chemotaxonomic analysis, strain SY7^T represents a novel species of the genus *Nitratireductor*, for which the name *Nitratireductor mangrovi* sp. nov., is proposed. The type strain is SY7^T (= KCTC 72110^T = MCCC 1K03723^T).

Introduction

The genus *Nitratireductor* belongs to the family *Phyllobacteriaceae* of class *Alphaproteobacteria* and was originally described in 2004 by Labbé et al. [1]. At the time of writing, there are 8 species of the genus *Nitratireductor* with validly published names have been proposed according to LPSN (www.bacterio.net/nitratireductor.html). Most members of the genus *Nitratireductor* share some common features, such as being Gram-negative, catalase- and oxidase-positive, non-spore forming, rod shaped morphology, white-pigmented, nitrate reduction activity, rod shaped morphology, containing ubiquinone-10 (Q-10) as major isoprenoid quinone [1–8]. In this study, we described a bacterium strain SY7^T, which isolated from a rhizosphere soil sample of mangrove forest. The isolate is considered to represent a novel species of the genus *Nitratireductor*, for which the name *Nitratireductor mangrovi* sp. nov., is proposed by using the polyphasic taxonomical approaches, including phenotypic, genotypic and chemotaxonomic analysis.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain SY7^T is MN239498. Whole Genome Shotgun project of strain SY7^T has been deposited at DDBJ/ENA/GenBank under the accession CP042301.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00284-020-01885-9) contains supplementary material, which is available to authorized users.

✉ Chuan Xiao
xiaochuanapple@163.com

✉ Min Wu
wumin@zju.edu.cn

[1] Ocean College, Zhejiang University, Zhoushan 316000, People's Republic of China

[2] College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

[3] Cardiovascular Health Department, Astrazeneca Trading Co., Ltd., Wuxi 214028, People's Republic of China

[4] College of Landscape Architecture and Life Science/Institute of Special Plants, Chongqing University of Arts and Sciences, Chongqing 402160, People's Republic of China

Materials and Methods

Isolation and Cultivation

Strain SY7^T was isolated from a soil sample collected from rhizosphere soil of the mangrove *Kandelia obovata* of Fugong village (117° 57′ N 24° 24′ E), in Zhangzhou, China, and stored at 4 °C until use. Serially diluted (ten-fold dilutions each) samples were made and spread on MA plates by the traditional dilution-plating method, and then incubated at 30 °C. After 15 days of incubation, a white-pigmented colony was picked and named as SY7^T. After repeated plate streaking on the same medium, pure strains were obtained from individual colonies and preserved at − 80 °C as suspension with 25% (v/v) glycerol for further used [9]. Type strains *N. pacificus* MCCC 1A01024^T, *N. basaltis* KCTC 22119^T and *N. aquibiodomus* DSM 15645^T were obtained from the Marine Culture Collection of China (MCCC), Korean Collection for Type Cultures (KCTC) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), repectively. All the strains were cultured under the same conditions for the experiments.

16S rRNA Gene Sequence and Phylogenetic Analysis

The 16S rRNA gene sequence of strain SY7^T was amplificated with universal primers 27F [5′-AGAGTTTGATCC TGGCTCAG-3′] and 1492R [5′-ACGGCTACCTTGTTA CGACTT-3′] by the method as described by Lane [10]. PCR products were ligated to vector pMD 19-T (TaKaRa) and cloned into *E. coli* DH5*a* for sequencing, and the almost-complete 16S rRNA gene sequence was obtained. The sequence was submitted to NCBI (https://www.blast.ncbi.nlm.nih.gov/Blast.cgi) (Accession No: MN239498) and compared with the closely related taxa provided by the EzTaxon-e server (https://eztaxon-e.ezbiocloud.net) [11, 12]. The multiple sequences were aligned with Clustal W [13]. Phylogenetic trees were reconstructed with MEGA 5.0 using neighbor-joining [14], maximum-parsimony [15] and maximum-likelihood [16] methods with Kimura two-parameter model. Bootstrap method (1000 resample datasets) was used to evaluate the trees topology [17, 18]. The type strains of *N. pacificus* MCCC 1A01024^T, *N. basaltis* KCTC 22119^T and *N. aquibiodomus* DSM 15645^T were selected as reference strains.

Genome Sequence and Analysis

The genome sequence of strain SY7^T was sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Beijing Genomics Institute). The *denovo* assembly of the reads was performed using ABySS 1.5.2 [19]. The assembly k-value was tested from 32 to 64 to find the optimal *k*-value using abyss-pe script. The quality of microbial genome was assessed using Check M [20]. The open reading frames (ORFs) were predicted and annotated by Rapid Annotation using Subsystem Technology (RAST) server online [21]. The genomes of reference type strains of *N. pacificus* MCCC 1A01024^T (AMRM00000000), *N. basaltis* KCTC 22119^T (JMQM00000000) and *N. aquibiodomus* DSM 15645^T (BAMP00000000) were downloaded from the NCBI database. In silico DNA–DNA hybridization (*is*DDH) values were calculated by genome to genome distance calculator (GGDC) [22]. The average nucleotide identity (ANI) values were calculated using the OrthoANIu algorithm of the Chun lab's online Average Nucleotide Identity calculator [23]. The DNA G+C content of strain SY7^T was calculated from the genome sequence.

Morphology, Physiology, Biochemistry and Chemotaxonomy

Cell morphology was determined by using optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) as described by Chen et al. [24]. Motility was tested by hanging drop method and semi-solid agar method [25]. The pH range for growth (pH 5–10, with intervals of 0.5 pH units) was investigated in MB medium by using the appropriate biological buffers: citrate/phosphate (pH 5.0 and 5.5), MOPS (Sigma) (pH 6.0–8.0), boric acid/borax (pH 8.5 and 9.0) and borax/NaOH (pH 9.5 and 10.0). The temperature range for growth was investigated at 4, 10, 15, 20, 25, 28, 30, 32, 35, 37, 40, 45 and 50 °C in MB medium. Growth at NaCl concentration range (0–12%, w/v, at intervals of 0.5%) was investigated in NaCl-free MB medium (according to MB formula, but without NaCl). Gram-staining was performed by the following method outlined by Claus et al. [26]. Oxidase and catalase activities were examined by the addition of 1% (w/v) tetramethyl-*p*-phenylene diamine and 3% (w/v) H_2O_2 solution, respectively [27]. Other physiological and biochemical activities tests were processed in API 20NE, API ZYM (bioMérieux) and GENIII MicroPlates (Biolog) according to the manufacturers' instructions. Unless otherwise indicated, the tests of physiological and biochemical activities between strain SY7^T and all reference strains were cultured in MB medium at 30 °C.

Whole-cell fatty acids of strain SY7^T were analyzed according to Sasser M [28]. For the preparation of cellular fatty acid methyl esters (FAMEs), cells of strain SY7^T, *N. pacificus* MCCC 1A01024^T, *N. basaltis* KCTC 22119^T and *N. aquibiodomus* DSM 15645^T were harvested and lyophiled after cultured on MA for 3 days at 30 °C. Identification and quantification of the FAMEs were performed by

the Sherlock Microbial Identification System (MIDI) with the standard MIS Library Generation software version 4.5 (Microbial ID) [29, 30]. Cells of strain SY7^T cultured in MB medium for 5 days at 30 °C which used for respiratory quinones and polar lipids analysis. Isoprenoid quinones were extracted according to the procedure described by Minnikin et al. [31], and analyzed by using HPLC–MS (Agilent 1200 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer) [32]. Polar lipids of the isolate were extracted from 3.0 g freeze-dried cells, then separated by two-dimensional TLC on silica gel 60F_{254} plates (Merck) and identified as previously described [31, 33, 34].

Results and Discussion

16S rRNA Gene Sequence and Phylogenetic Analysis

The PCR-based 16S rRNA gene sequence and genome-based 16S rRNA gene sequence were identical. And the 16S rRNA gene sequence (1411 bp) of strain SY7^T indicated that the novel isolate belonged to the genus *Nitratireductor* and showed the highest sequence similarities to *N. pacificus* MCCC 1A01024^T (97.23%), *N. basaltis* KCTC 22119^T (96.83%) and *N. aquibiodomus* DSM 15645^T (96.52%). In all phylogenetic trees (Fig. 1, Fig. S1), strain SY7^T belonged to the cluster of the genus *Nitratireductor* and formed an independent lineage with moderate bootstrap support.

Genome Sequence and Analysis

The draft genome sequence of strain SY7^T generated 584 Mb of clean data. The genome completeness of strain SY7^T was 98.72% with 0.82% contamination, which considered as good reference genome for deeper analysis (≥ 95% completeness, ≤ 5% contamination). The draft genome sequence of strains SY7^T has a genome size of 4,838,603 bp and yielded 70 contigs after assembly. N50 value of strains SY7^T was 217,092 bp with the largest contig of 346,613 bp. As a result, the quality of the genome was high enough for taxonomical analysis [35]. The genome in silico DNA–DNA hybridization (GGDC) values of strain SY7^T and *Nitratireductor pacificus* MCCC 1A01024^T, *Nitratireductor basaltis* KCTC 22119^T and *Nitratireductor aquibiodomus* DSM 15645^T were 16.7%, 14.3% and 14.7%, respectively. The average nucleotide identity (ANI) values of strain SY7^T and *Nitratireductor pacificus* MCCC 1A01024^T, *Nitratireductor basaltis* KCTC 22119^T and *Nitratireductor aquibiodomus* DSM 15645^T were 75.2%, 72.6% and 73.5%, respectively. These results were lower than the 70% threshold value for GGDC and 95–96% for ANI proposed for the delineation of bacterial species, which indicating that strain SY7^T was distinguished from the reference strains of the genus

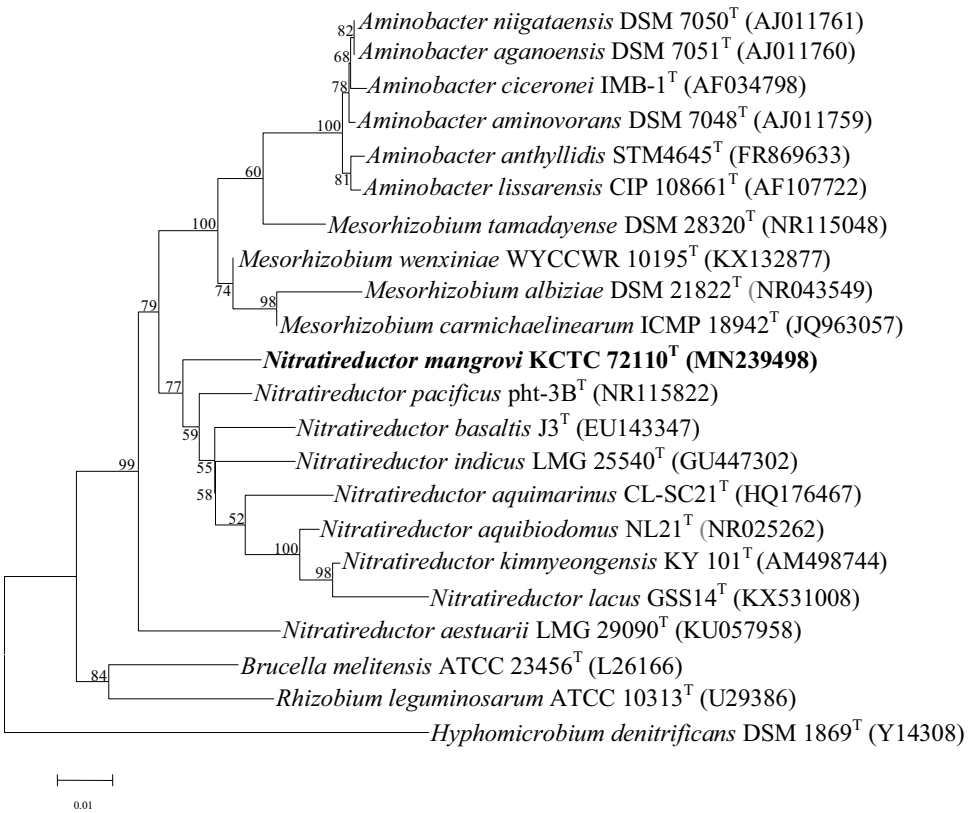

Fig. 1 Neighbor-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship between strain SY7^T and other related species of the genus *Nitratireductor*. *Brucella melitensis* ATCC 23456^T, *Rhizobium leguminosarum* ATCC 10313^T and *Hyphomicrobium denitrificans* DSM 1869^T were used as outgroup. Bootstrap values were expressed as a percentage of 1000 replicates and only those at or above 50% were given at the branch points. Bar, 0.01 substitutions per nucleotide position

Nitratireductor [35, 36]. The genomic DNA G+C content of strain SY7^T was 64.9%, different from those of *Nitratireductor pacificus* MCCC 1A01024^T (63 mol%) [2], *Nitratireductor basaltis* KCTC 22119^T (56.7 mol%) [3], *Nitratireductor aquibiodomus* DSM 15645^T (57 mol%) [1].

Morphology, Physiology, Biochemicstry and Chemotaxonomy

Strain SY7^T formed smooth, circular, convex and creamy colonies with a diameter of 0.5–1.0 mm after 5 days of incubation at 30 °C on MA. The isolate was Gram-negative, positive for catalase, oxidase and nitrate reduction, which were in accordance with the characteristics of the genus *Nitratireductor*. Strain SY7^T could be distinguished from its relatives by their phenotypic, physiological and biochemical characteristics. For example, cells of strain SY7^T were short-rod-shaped without flagellum (Fig. 2), positive for hydrolysis of urea, assimilation of D-turanose, D-gluconic acid, negative for utilization of α-chymotrypsin, α-glucosidase, *N*-acetyl-glucosamine. Detailed characteristics between strain SY7^T and the closely related species are shown in Table 1.

The major fatty acids of strain SY7^T were 11-methyl $C_{18:1}$ $\omega 7c$ (8.0%), iso-$C_{17:0}$ (10.3%), $C_{19:0}$ $\omega 8c$ cyclo (22.6%) and summed feature 8 ($C_{18:1}$ $\omega 6c$/$C_{18:1}$ $\omega 7c$) (22.4%). Detailed fatty acids profiles of strain SY7^T and other closely related strains shown in Table 2. The fatty acids profiles of strain SY7^T were similar to those of the reference strains, such as larger amounts of $C_{19:0}$ $\omega 8c$ cyclo and summed feature 8 ($C_{18:1}$ $\omega 6c$/$C_{18:1}$ $\omega 7c$). But strain SY7^T contained more iso-$C_{17:0}$ (10.3%), 11-methyl $C_{18:1}$ $\omega 7c$ (8.0%), iso-$C_{15:0}$ (4.0%), anteiso-$C_{15:0}$ (3.9%) and $C_{8:0}$ 3-OH (3.2%). Respiratory quinone analysis revealed that the sole quinone of strain SY7^T was ubiquinone-10 (Q-10), same as the other species of the genus *Nitratireductor* [6–8]. The isolate contained phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG) as major polar lipids, which was in agreement with data published previously for other species of the genus *Nitratireductor* [7, 8]. In addition, strain SY7 was also detected with an unidentified phospholipid (PL) and two unidentified lipid (L) (Fig. S2).

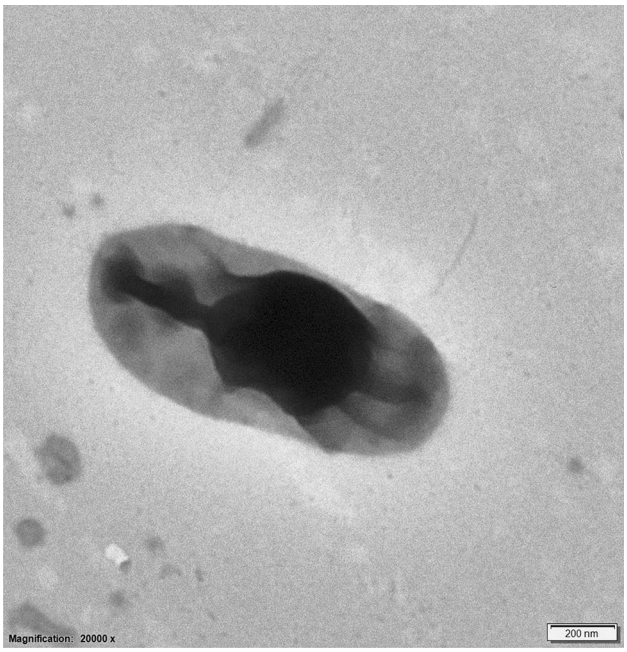

Fig. 2 Transmission electron micrographs showing the cell morphology of strain SY7^T after 5 days of incubation on MA at 30 °C. Bar, 200 nm

Taxonomic Conclusion

On the basis of the polyphasic taxonomical analysis as presented above, strain SY7^T, which isolated from a rhizosphere soil sample of the mangrove forest, is considered to represent a novel species of the genus *Nitratireductor*, for which the name *Nitratireductor mangrovi* sp. nov., with strain SY7^T as the type strain, is proposed.

Description of *Nitratireductor mangrovi* sp. nov.

Nitratireductor mangrovi (man.gro'vi. N.L. gen. n. *mangrovi* of a mangrove, referring to the isolation of the type strain from mangrove soil).

Cells are Gram-negative, non-motile, non-spore-forming, short-rod-shaped, approximately 0.4–0.7 μm in width and 1.0–1.5 μm in length. Colonies are 0.5–1.0 mm in diameter, smooth, circular, convex, creamy after incubation on MA for 5 days at 30 °C. The pH, temperature and NaCl concentration ranges for growth are determined to pH 6.0–10.0 (optimum pH 7.0), 10–45 °C (optimum 30 °C) and 0–8% NaCl (optimum 3.0%, w/v), respectively. Positive for oxidase, catalase, nitrate reduction, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase and naphthol-AS-Bi-phosphatase, hydrolysis of esculin, β-galactosidase, urea and D-glucose, assimilation of D-maltose, D-trehalose, D-cellobiose, sucrose, *N*-acetyl-D-glucosamine, D-mannose, glycyl-L-proline, L-alanine, L-glutamic acid, L-pyroglutamic acid, L-lactic acid, citric acid, α-ketoglutaric acid, Tween 40, γ-amino-butyric acid, acetoacetic acid, acetic acid, D-turanose, α-D-glucose, D-fructose, D-gluconic acid, β-methyl-D-glucoside, quinic acid and DL-fucose, 1% sodium lactate, tetrazolium blue, lithium chloride, potassium tellurite and aztreonam. The predominant fatty acids are 11-methyl $C_{18:1}$ $\omega 7c$, iso-$C_{17:0}$, $C_{19:0}$ $\omega 8c$ cyclo and summed feature 8 ($C_{18:1}$ $\omega 6c$/$C_{18:1}$ $\omega 7c$). The isoprenoid

Table 1 Differential phenotypic and biochemical characteristics of strain SY7^T and its closely related species

Characteristics	1	2	3	4
Cell shape	Short rods	Rods	Rods	Cocci, rods
Cell dimensions (μm)				
Width	0.4–0.7	0.8	1	0.6
Length	1.0–1.5	1.5	2.3	0.6–2.0
Motility	−	+	+	−
Temperature range for growth (optimum) (°C)	10–45 (30)	10–41 (25–30)[1]	10–40 (30–35)[2]	15–45 (35)[3]
PH range for growth (optimum)	6.0–10.0 (7.0)	NA	6.5–9.0 (7.0)[2]	5.5–10.0 (7.0–8.1)[3]
NaCl range for growth (optimum) (%, w/v)	0–8.0 (3.0)	0–7.0 (3.0)[1]	0–5.0 (1.0)[2]	0–8.0 (3.0)[3]
Tests of API ZYM				
Lipase (C14)	−	+	−	−
Cystine arylamidase	+	+	+	−
α-Chymotrypsin	−	+	+	W
α-Galactosidase	−	w	−	+
β-Galactosidase	−	w	−	+
α-Glucosidase	−	+	+	W
β-Glucosidase	−	+	−	−
N-acetyl-*β*-glucosaminidase	−	+	+	−
Tests of API 20NE				
Hydrolysis of urea	+	−	−	−
D-Glucose	+	−	+	+
D-Mannose	−	−	+	-
D-Mannitol	−	−	w	+
N-acetyl-glucosamine	−	+	+	+
Potassium gluconate	−	−	−	+
Capric acid	−	−	−	+
Adipic acid	−	w	−	+
Trisodium citrate	−	+	+	-
Tests of GENIII MicroPlates				
Dextrin	−	w	+	w
D-Turanose	+	−	−	−
β-Methyl-D-glucoside	+	−	−	−
N-acetyl-D-galactosamine	−	−	+	−
α-D-Glucose	+	−	−	+
D-Fructose	+	+	−	+
D-Galactose	−	−	+	w
DL-Fucose	W	−	+	w
myo-inositol	−	−	+	−
Glycerol	−	+	−	+
L-Histidine	−	−	+	−
L-Serine	−	w	+	+
D-Gluconic acid	+	−	−	−
Quinicacid	+	−	−	−
β-Hydroxy-DL-butyric acid	−	+	w	+
Nalidixic acid	−	+	+	+
Troleandomycin	−	+	+	+
Rifamycin	−	+	+	+
Minocycline	−	−	−	+
Lincomycin	−	+	+	+
Vancomycin	−	−	−	+

1 data taken from Ou et al. [6], *2* data taken from Yu et al. [7], *3* data taken from Jang et al. [8]. Strains: *1* SY7^T, *2 Nitratireductor pacificus* MCCC 1A01024^T, *3 Nitratireductor aquibiodomus* DSM 15645^T, *4 Nitratireductor basaltis* KCTC 22119^T. All strains were positive for catalase, oxidase, nitrate reduction. The other common characteristics of strain SY7^T and its closely related species showing in Table S2. + positive, - negative, *w* weakly positive, *NA* no data available. Unless indicated otherwise, all data were obtained from this study

Table 2 Fatty acids profiles of strain SY7^T and type strains of phylogenetically related species

Fatty acids	1	2	3	4
$C_{10:0}$	4.7	1.3	3.0	tr
$C_{16:0}$	3.7	3.2	2.6	2.4
$C_{18:0}$	3.7	4.6	4.0	3.7
$C_{14:1}$ $\omega 5c$	2.8	tr	1.8	–
$C_{15:1}$ $\omega 8c$	2.2	tr	1.4	–
11-methyl $C_{18:1}$ $\omega 7c$	8.0	–	–	2.2
$C_{18:1}$ $\omega 9c$	–	1.7	–	–
C19:0 $\omega 8c$ cyclo	22.6	19.8	15.5	4.6
iso-$C_{14:0}$	1.8	tr	tr	–
iso-$C_{15:0}$	4.0	tr	–	Tr
iso-$C_{17:0}$	10.3	3.4	1.3	2.7
Anteiso-$C_{15:0}$	3.9	–	–	Tr
$C_{8:0}$ 3-OH	3.2	Tr	1.9	Tr
$C_{10:0}$ 2-OH	1.1	–	–	–
$C_{11:0}$ 2-OH	2.3	–	–	–
iso-$C_{15:0}$ 3-OH	–	Tr	1.4	Tr
Summed feature 1[a]	2.5	tr	1.4	Tr
Summed feature 3[a]	–	1.0	tr	1.5
Summed feature 8[a]	22.4	58.4	64.2	80.2

Strains: *1* SY7^T, *2 Nitratireductor pacificus* MCCC 1A01024^T, *3 Nitratireductor aquibiodomus* DSM 15645^T, *4 Nitratireductor basaltis* KCTC 22119^T, *tr* trace (less than 1%), – no data available. All data were obtained from this study

[a]Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 consists of iso-$C_{13:0}$ 3-OH/iso-$C_{15:1}$ H; Summed feature 3 consists of $C_{16:1}$ $\omega 6c$/$C_{16:1}$ $\omega 7c$; summed feature 8 consists of $C_{18:1}$ $\omega 6c$/$C_{18:1}$ $\omega 7c$

quinone is Q-10. The major polar lipids are phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), and moderate amounts of an unidentified phospholipid (PL), two unidentified lipids (L) are also detected. The DNA G+C content of strain SY7^T is 64.9%.

The type strain is SY7^T (= KCTC 72110^T = MCCC 1K03723^T), and was isolated from rhizosphere soil of the mangrove *Kandelia obovata* of Fugong village in Zhangzhou, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SY7^T is MN239498. Whole Genome Shotgun project of strain SY7^T has been deposited at DDBJ/ENA/GenBank under the accession number CP042301.

Acknowledgement This work was supported by grants from the Science and Technology Basic Resources Investigation Program of China (2017FY100300), the National Natural Science Foundation of China (No. 31700566) and the Siyuan Foundation (308000-521501/008).

References

1. Labbé N, Parent S, Villemur R (2004) *Nitratireductor aquibiodomus* gen. nov. sp. nov., a novel alphaproteo bacterium from the marine denitrification system of the Montreal Biodome (Canada). Int J Syst Evol Microbiol 54:269–273
2. Lai Q, Yu Z, Yuan J et al (2011) *Nitratireductor indicus* sp. nov., isolated from deep-sea water. Int J Syst Evol Microbiol 61:295–298
3. Kim KH, Roh SW, Chang HW et al (2009) *Nitratireductor basaltis* sp. nov., isolated from black beach sand. Int J Syst Evol Microbiol 59:135–138
4. Lai Q, Yu Z, Wang J et al (2011) *Nitratireductor pacificus* sp. nov., isolated from a pyrene-degrading consortium. Int J Syst Evol Microbiol 61:1386–1391
5. Kang HS, Yang HL, Lee SD (2009) *Nitratireductor kimnyeongensis* sp. nov., isolated from seaweed. Int J Syst Evol Microbiol 59:1036–1039
6. Ou D, Huang H, Bai R et al (2017) *Nitratireductor aestuarii* sp. nov., a marine alphaproteo bacterium isolated from an estuary. Int J Syst Evol Microbiol 67:1637–1642
7. Yu Z, Zhuang L, Pan J et al (2016) *Nitratireductor lacus* sp. nov., isolated from Yuncheng Salt Lake. China. Int J Syst Evol Microbiol 66:4963–4967
8. Jang GI, Hwang CY, Cho BC (2011) *Nitratireductor aquimarinus* sp. nov., isolated from a culture of the diatom Skeletonemacostatum and emended description of the genus *Nitratireductor*. Int J Syst Evol Microbiol 61:2676–2681
9. Ye YY, Chen C, Ren YH et al (2019) *Pseudomonas mangrovi* sp. nov., isolated from mangrove soil. Int J Syst Evol Microbiol 69:377–383
10. Lane DJ (1991) 16S/23S rRNA sequencing. Nucleic Acid Tech Bact Syst 2:125–175
11. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402
12. Yoon SH, Ha SM, Kwon S et al (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617
13. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
14. Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. J Mol Biol Evol 4:406–425
15. Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. J Syst Zool 20:406–416
16. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376
17. Tamura K, Peterson D, Peterson N et al (2011) MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. J Mol Biol Evol 28:2731–2739
18. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120
19. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ et al (2009) ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123
20. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW (2015) Check M: assessing the quality of microbial genomes recovered from isolates, single cells, and meta genomes. Genome Res 25:1043–1055

21. OverbeekR OR, Pusch GD, Olsen GJ, Davis JJ et al (2014) The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 42:D206–D214
22. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60–60
23. Lee I, Kim YO, Park SC, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Micro Biol 66:1100–1103
24. Chen C, Anwar N, Wu C et al (2018) *Halomonas endophytica* sp. nov., isolated from liquid in the stems of Populuseuphratica. Int J Syst Evol Microbiol 68:1633–1638
25. Reddy CA (2007) Methods for general and molecular bacteriology. American Society for Microbiology, Washington DC
26. Claus D (1992) A standardized gram staining procedure. World J Microbiol Biotechnol 8:451–452
27. Cappuccino JG, Sherman N (2002) Microbiology: a laboratory manual, 6th edn. Benjamin Cummings, Menlo Park
28. Paisley R (1996) MIS whole cell fatty acid analysis by gas chromatography training manual. MIDI, Newark
29. Miller LT (1982) Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. J Clin Microbiol 16:584–586
30. Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Inc., Newark
31. Minnikin DE, O'Donnell AG, Goodfellow M et al (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Micro Biol Methods 2:233–241
32. Yu XY, Yu XD, Fu GY et al (2018) *Marinicaulis flavus* gen. nov. sp. nov., a novel stalked bacterium of the family *Parvularculaceae*. Int J Syst Evol Microbiol 68:2061–2067
33. Tindall BJ (1990) A comparative study of the lipid composition of *Halobacterium saccharovorum*, from various sources. Syst Appl Microbiol 13:128–130
34. Chen C, Su Y, Tao TY et al (2017) *Maripseudobacter aurantiacus* gen. nov. sp. nov., a novel genus of the family *Flavobacteriaceae* isolated from sedimentation basin. Int J Syst Evol Microbiol 67:778–783
35. Chun J, Oren A, Ventosa A et al (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466
36. Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. PNAS 106:19126–19131