Halomonas salipaludis sp. nov., isolated from the saline-alkali wetland soil

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Abstract

Strain WRN001^T, a Gram–staining–negative, strictly aerobic, non–motile bacterium was isolated from the natural saline-alkali wetland soil of Binhai new district, Tianjin, China (38°46'N, 117°13'E). Cells of strain WRN001^T were 0.3-0.5 µm in width and 1.5-2.5 µm in length, and the growth occurred optimally at 33-37 °C, pH 7.5-8.0, and in the presence of 8-10% (w/v) NaCl. Based on 16S rRNA gene sequence analysis, the isolate could be affiliated to the genus *Halomonas*, and the highest 16S rRNA gene sequence similarity of strain WRN001^T to its closest relative *Halomonas qiaohouensis* YIM QH88^T was 97.47%. The size of the genome as presented here was 5,475,884 bp with a G+C content of 63.8 mol %. The major respiratory quinone of strain WRN001^T was Q-9, and the dominant fatty acids were summed feature 8, summed feature 3, C_{10:0}, C_{12:0}, C_{12:0} 3-OH, C_{16:0}, and C_{17:0} cyclo. The major polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), two phospholipids (PL), aminolipid (AL), and three unidentified lipids (L). These data mentioned above combined with the low DDH values between strain WRN001^T and the close relative, *Halomonas alkalitolerans* 1513^T (42.20%) and base on comparisons with currently available genomes, the highest average nucleotide identity (ANIm) value was 91.39% to *Halomonas alkalitolerans* 1513^T (GenBank accession No. GCA_001971685.1). Therefore, we propose a novel species in the genus *Halomonas* to accommodate this novel isolate: *Halomonas salipaludis* sp. nov. (type strain WRN001^T = KCTC 52853^T = ACCC 19974^T).

Introduction

The *Halomonas* as a large genus was firstly described by Vreeland et al. (1980), with *Halomonas elongata* as the type species, and most of the species of the genus *Halomonas* were isolated from saline habitats, such as sea sediments, salt lakes, brines, salty foods, deep sea hydrothermal vent environments, as well as saline sand and soils (Arenas et al. 2009; Cao et al. 2013; Dou et al. 2015; Guan et al. 2010; Guzmán et al. 2010; Kaye et al. 2004; Kim et al. 2010; Lee et al. 2005; Reddy et al. 2003; Romano et al. 1996; Wang et al. 2012; Wang et al. 2014; Xu et al. 2007). At the time of writing, the genus *Halomonas* includes 96 species with validly published names (<http://www.bacterio.net/halomonas.html>). Some of them have been recognized for potential use in biotechnology, such as in the production of bioactive compounds (Wang et al. 2006), extreme-enzymes (Kumar et al. 2012), exopolysaccharide (Kumar et al. 2012; Martínezcheca et al. 2005; Poli et al. 2013), and hydrolytic enzymes (Sánchez-Porro et al. 2003). In this paper, we described the isolation, identification and physio-biochemical characteristics of novel strain WRN001^T and proposed the name *Halomonas salipaludis* for this bacterium.

Materials And Methods

Isolation and Culture Conditions

Strain WRN001^T was isolated from the natural saline-alkali wetland soil of Binhai new district, Tianjin, China (38°46'N, 117°13'E) in June 2015. Soils were transferred to the laboratory with ice. The *in-situ* temperature, salinity and pH of the samples were measured as 30 °C, 4.0 %-13.5 % and 7.8-9.3, respectively. To isolate halophilic heterotrophic microorganisms, 1.0 g of soil was placed in sterile 30 ml glass tube for enrichment using DifcoTM marine 2216 amended with final concentration of 10.0% NaCl for 3 days and subsequently purified into single colonies.

Morphological, Physiological and Biochemical Characterization

Cell size, morphology and motility of strain WRN001^T were established by using a Leica microscope equipped with phase-contrast optics (Leica DM 6000 B) during exponential growth phase. Cell morphology was also assessed by transmission electron microscopy (TEM), i.e., cells were harvested from exponentially growing culture, and the cells were negatively stained with 0.5 % uranyl acetate and the grids were examined at the microscope (Tecnai Spirit, FEI, Hillsboro, OR, USA). Gram staining was performed using BD Gram staining kits according to the manufacturer's instructions. Oxidase activity was tested using the oxidase reagent kit (bioMérieux) according to the manufacturer's instructions. Catalase activity was determined by pouring a 3.0% H₂O₂ solution onto bacterial colonies and observing bubble production. Reduction of nitrate and hydrolysis of starch, casein, gelatin, and Tween 80 were analyzed according to the methods of Smibert and Krieg (1994) and Dong and Cai (2001). The optimal growth temperature of strain WRN001^T was determined after incubation on DifcoTM marine 2216 agar (8.0% NaCl) and shaking in DifcoTM marine 2216 liquid medium (8.0% NaCl) at 4, 10, 15, 20, 25, 30, 33, 37, 40, 45, and 50 °C (at pH 7.5). Bacterial growth was measured as increase in turbidity at 600 nm, using a DU 800 spectrophotometer (Beckman Coulter). NaCl tolerance was tested on Luria-Bertani (LB) agar and in LB liquid medium amended with 0.0-25.0% NaCl (w/v). Similarly, the pH range for growth was measured by adjusting the final pH to 5.0, 5.5, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0 (at 8.0% NaCl, 33 °C) with the appropriate buffers (Na₂HPO₄/NaH₂PO₄ for pH 5.0-7.0 and Na₂CO₃/NaHCO₃ for pH 8.0-12.0). Anaerobic growth was determined through measuring the OD_{600 nm} at 33 °C with 8.0% NaCl (w/v) in the tubes with the butyl rubber stopper and screw cap.

For all physiological experiments, we selected the *Halomonas qiaohouensis* YIM QH88^T, *Halomonas socia* NY-011^T, and the closely related strain *Halomonas pantelleriensis* AAP^T as reference organisms. Unless otherwise stated, all the strains mentioned above were incubated at 30 °C in DifcoTM marine 2216 medium amended with final concentration of 8.0% NaCl for strain WRN001^T and final concentration of 10.0% NaCl for reference organisms.

Carbon source utilization and enzyme activities were tested using the Biolog GEN III MicroPlates, API 20E, API 20NE and API ZYM (bioMérieux, Biolog Inc.) according to the manufacturer's instructions, with the exception that salinity was adjusted to 8.0% (w/v) NaCl. Susceptibility to antibiotics was assessed on

Difco™ marine 2216 agar (8.0% NaCl) using the disc–diffusion plate method (Fraser and Jorgensen 1997) with discs containing ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), kanamycin (30 µg), penicillin G (10 µg), streptomycin (10 µg), tetracycline (30 µg), vancomycin (30 µg), gentamicin (10 µg), or polymyxin B (30 µg). Antibiotics–containing discs were placed on the Difco™ marine 2216 agar (8.0% NaCl) plate surfaces, and the bacterial cultures (200 µL) that were spread on the plate were checked for clearing zones after 3 d at 33 °C.

Chemotaxonomic Characterization

Cells of strain WRN001^T and the reference strains were harvested during the late exponential growth phase in Difco™ marine 2216 liquid medium (8.0% NaCl for strain WRN001^T and 10.0% NaCl for reference strains) at 33 °C for characterization of respiratory quinones, cellular fatty acids, and polar lipids. Respiratory quinones were extracted with chloroform/methanol (2:1) (v/v) from lyophilized cells (300 mg) and purified using high performance liquid chromatography (HPLC) (Minnikin DE 1984). The fatty acids were identified and quantified by the Sherlock Microbial Identification System with standard MIS Library Generation Software (VERSION 6.0 and Date 4, Microbial ID Inc., Newark, DE, USA) and a 6890N gas chromatograph (Agilent) according to the method of Sasser (1990). Polar lipids were extracted from 200 mg of freeze–dried cell material using a chloroform: methanol: aqueous NaCl mixture (0.3%, w/v) with the ratio of 1:2:0.8 (v/v/v), modified after Bligh and Dyer (Bligh and Dyer 1959), recovered into the chloroform phase by adjusting the mixture to a ratio of 1:1:0.9 (v/v/v), and separated by two–dimensional silica gel thin–layer chromatography. The first dimension was developed in a chloroform: methanol: water (65:25:4, v/v/v) mixture and the second was developed in a chloroform: methanol: acetic acid: water (80:12:15:4, v/v/v/v) mixture. Total lipid material was detected using molybdatophosphoric acid and specific functional groups detected using spray reagents specific for defined functional groups (Tindall BJ 2007). The respiratory quinones and cellular fatty acids were determined by the identification service of the China Center of Agricultural Culture Collection in Beijing, China, and the polar lipid analysis was performed by the Identification Service of the DSMZ, Braunschweig, Germany.

Molecular Characterisation

The 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primer set 27F and 1492R (Lane 1991; Weisburg et al. 1991). The PCR product was purified using the PCR purification kits (MinElute PCR Purification Kit, QIAGEN) and sequenced by Sangon Biotech (Shanghai) Co., Ltd., China. The 16S rRNA gene sequence of strain WRN001^T, as determined in this study, was submitted to GenBank, and the 16S rRNA gene sequences of microorganisms related taxa were obtained from the GenBank database (<http://www.ncbi.nlm.nih.gov/>). Phylogenetic trees were constructed by maximum–likelihood method, and Neighbor–joining (NJ) & maximum parsimony (MP) phylogenetic trees were also

constructed to corroborate the phylogenetic position of the strain WRN001^T in software MEGA 7.0 (Kumar et al. 2016).

Genomic DNA was extracted using a commercial kit (TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0) and subsequently sequenced on the Illumina MiSeq 2000 platform in Shanghai Personal Biotechnology Co., Ltd. China. Filtering and trimming of the genomic raw data were done with PRINSEQ v0.20.4 (Schmieder and Edwards 2011), and the trimmed reads were assembled using SOAPdenovo v1.05 (Li et al. 2008; Li et al. 2010) with default parameters. The genome completeness (100%) was assessed using CheckM (version 1.03) (Parks et al. 2015). Protein-coding open reading frames were predicted by Glimmer (version 3.02) (Delcher et al. 2007). For RNA prediction, rRNAs were predicted by RNAmmer (version 1.2) (Lagesen et al. 2007), and tRNAs were predicted by tRNAscan-SE (version 1.21) (Lowe and Eddy 1997).

Experiments with digital DNA–DNA hybridization (dDDH) between strain WRN001^T and the type strains of the phylogenetically most closely related *Halomonas* species were performed as described by Ezaki et al. (Takayuki et al. 1989). In addition to dDDH, average nucleotide identity (ANI) values between the strain WRN001^T genome and closely related genomic sequences from GenBank were also determined according to Goris et al. (2007), i.e., whole-genome sequences in a pairwise comparison were split into consecutive 1000 bp windows, then sequences were aligned with nucmer in MUMmer version 3.23 (Kurtz et al. 2004) and ANI values were calculated using JSpecies version 1.2.1 (Goris et al. 2007).

Results And Discussion

Morphological, Physiological and Biochemical Characteristics

Colonies of strain WRN001^T on DifcoTM marine 2216 (8.0% NaCl) were circular, wet, smooth, and pigmented light yellow, and around 0.35 mm in diameter after 72 h of incubation at 30 °C. Strain WRN001^T cells were aerobic, Gram–stain–negative, non–motile, rod–shaped, approximately 0.3–0.5 µm in width and 1.5–2.5 µm in length (Supplementary Fig. S1), and cells were oxidase-positive and catalase-negative. Strain WRN001^T grew with 0.5–20.0% NaCl (optimum growth at 8.0–10.0% NaCl), at 10–45 °C (optimum 33–37 °C), and pH 5.5–11.0 (optimum pH 7.5–8.0). The novel isolate WRN001^T can utilize starch, casein and Tween 80, but gelatin was not used as carbon source, and nitrate was not reduced, and strain WRN001^T cannot grow under anaerobic conditions. The physiological and biological characteristics of Strain WRN001^T are summarized in the species description, and a comparison of the selective characteristics of strain WRN001^T and the related type strains was given in Table 1.

Cells were sensitive to chloramphenicol (30 µg), erythromycin (15 µg), streptomycin (10 µg), vancomycin (30 µg) and polymyxin B (30 µg), but resistant to ampicillin (10 µg), kanamycin (30 µg), penicillin G (10 µg), tetracycline (30 µg) and gentamicin (10 µg). However, the closest relative *Halomonas qiaohouensis*

YIM QH88^T were sensitive to chloramphenicol (30 µg), tetracycline (30 µg), gentamicin (10 µg), vancomycin (30 µg), streptomycin (10 µg), polymyxin B (30 µg) and kanamycin (30 µg), but resistant to penicillin G (10 µg), ampicillin (10 µg), and erythromycin (15 µg). The differential antibiotic characteristics were also listed in Table 1.

Chemotaxonomic Characteristics

Q-9 was identified as the major respiratory quinone of strain WRN001^T, which is consistent with the case of *Halomonas qiaohouensis* YIM QH88^T, *Halomonas socia* NY-011^T, and most other members of the genus *Halomonas*. Strain WRN001^T had the following cellular fatty acids: summed feature 8 (C_{18:1} ω6c and/or C_{18:1} ω7c) (55.68%), summed feature 3 (C_{16:1} ω6c and/or C_{16:1} ω7c) (9.62%), C_{10:0} (2.95%), C_{12:0} (3.64%), C_{12:0} 3-OH (6.02%), C_{16:0} (16.36%), C_{17:0} cyclo (1.60%) and C_{19:0} cyclo ω8c (2.43%). C_{18:1} (54.2%), C_{18:0} (11.2%), C_{16:0} (8.6%), 11-methyl C_{18:1} ω7c (7.7%), C_{19:0} cyclo ω8c (3.3%), and C_{12:1} 3-OH (3.5%), which is consistent with those of the selected reference species: *Halomonas pantelleriensis* AAP^T, *Halomonas qiaohouensis* YIM QH88^T and *Halomonas socia* NY-011^T (Table 2).

Strain WRN001^T contained the following major polar lipids: Diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), amino lipid (AL), phospholipid (PL), and an unidentified lipid (L). The polar lipids of the strain WRN001^T and its close relatives *Halomonas qiaohouensis* YIM QH88^T and *Halomonas socia* NY-011^T are very similar, however, their differences are mainly at Aminolipid (AL), Aminoglycolipid (GNL) and Lipid (L) (Supplementary Fig. S2).

Molecular Characteristics

Strain WRN001^T has only one copy of the 16S rRNA gene (1528 bp). Based on the 16S rRNA phylogenetic analysis, strain WRN001^T was clearly affiliated with the *Halomonas* clade in the family *Halomonadaceae* (Fig. 1, Supplementary Fig. S3 and S4). The 16S rRNA gene of strain WRN001^T was most closely related to *Halomonas qiaohouensis* YIM QH88^T (97.47%), *Halomonas socia* NY-011^T (96.65%), and *Halomonas pantelleriensis* AAP^T (95.96%).

The genome assembly of strain WRN001^T (accession number NSKB000000000) as presented here is 5,475,884 bp in size. It has a G+C content of 63.8% and consists of 33 contigs with a 200-fold coverage. We predicted a total of 4,940 ORFs, 65 tRNAs, and 10 rRNAs. And the dDDH values between strain WRN001^T and its close relatives, *Halomonas alkalitolerans* 1513^T, *Halomonas pantelleriensis* AAP^T and *Halomonas shengliensis* CGMCC 1.6444^T, were 42.20%, 35.10%, and 23.30%, respectively, which adding evidence that strain WRN001^T represents a novel species of the genus *Halomonas*, based on the DNA-DNA hybridization as a standard method for species definition and the recommended minimum relatedness value for strains of the same species is 70% (Graham 1991; Wayne 1987).

Taxonomic Conclusion

In this study, we isolated and described the novel strain WRN001^T from the natural saline-alkali wetland soil. Phylogenetic, phenotypic, and genetic analyses indicate that the strain WRN001^T represents a novel species of the genus *Halomonas*, for which we propose the name *Halomonas salipaludis* sp. nov.

Description of *Halomonas salipaludis* sp. nov.

Halomonas salipaludis (sa.li.pa.lu'dis. L. masc. n. *sal*, *salis* salt; L. fem. n. *palus*, *paludism* swamp, marsh; N.L. gen. n. *salipaludis* of a salt marsh).

Cells are aerobic, Gram-stain-negative, nonmotile, and oxidase-positive and catalase-negative with 0.3–0.5×1.5–2.5 µm. Colonies are circular, wet, smooth, and light yellow. Growth occurs at 0.5–20.0% NaCl (optimum growth at 8.0–10.0% NaCl), at 10–45°C (optimum 33–37°C), and pH 5.5–11.0 (optimum pH 7.5–8.0). Do not produce indole. Starch, casein, and Tween 80 can be hydrolyzed, but gelatin was not hydrolyzed. Nitrate was not reduced to nitrite. Positive for the following enzymatic activities: α-chymotrypsi, esterase (C₄), esterase lipase (C₈), lipase (C₁₄), valinearyl amidase, β-glucosidase, α-mannosidase, α-glucosidase and N-acetyl-glucosaminidase, and negative for leucine arylamidase, α-galactosidase, β-fucosidase, cystinearyl amidase, β-galactosidase, alkaline phosphatase, β-glucuronidase, Naphthol-AS-BI-phosphohydrolase and acid phosphatase. Based on the Biolog GNIII, API 20 NE and API 20 E, the following compounds are utilized as sole carbon and energy sources: D-glucose, esculin ferric citrate, L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose, adipic acid, malic acid, trisodium citrate, starch, esculin, and Tween 80, however, L-tryptophane, L-arginine, urea, gelatin, 4-nitrophenyl-β-D-galactopyranosid, D-mannose, potassium gluconate, capric acid, phenylacetic acid and gelatin are not. The cells of strain WRN001^T are sensitive to chloramphenicol, erythromycin, streptomycin, vancomycin and polymyxin B, but resistant to ampicillin, kanamycin, penicillin G, tetracycline, and gentamicin. The major respiratory quinone of strain WRN001^T is Q-9, and the dominant fatty acids are summed feature 8, summed feature 3, C_{10:0}, C_{12:0}, C_{12:0} 3-OH, C_{16:0}, C_{17:0} cyclo, C_{19:0} cyclo ω 8c. The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), two phospholipids (PL), aminolipid (AL), and three unidentified lipids (L).

The type strain WRN001^T (= KCTC 52853^T = ACCC19974^T) was isolated from the natural saline-alkali wetland soil of Binhai new district, Tianjin, China (38°46'N, 117°13'E), and the genomic DNA is a single circular chromosome (5,475,884 bp) with a G + C content of 63.8%.

Declarations

Funding

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Availability of data and material

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene of *Halomonas salipaludis* sp. nov. strain WRN001^T is MF782428. The whole genome was deposited at GenBank/EMBL/DDBJ under the accession number NSKB000000000 for strain WRN001^T. Transmission electron micrographs (TEM) of cells of strain WRN001^T, thin-layer chromatograms of the polar lipids extracted from strain WRN001^T and closely related species, additional phylogenetic trees, and the table containing the average nucleotide identity (ANI), and dDDH values to closely related genomes are available as Supplementary Materials.

Code availability

Not applicable.

Authors' contributions

JX contributed to performing the experiments and writing the initial draft. QG provided samples of experiment and participated in the isolation and cultivation of strains. GZ and JZ contributed to the guidance of experimental operations. LT and JL contributed to the morphological analyzes. HF, XW and HL performed genome analysis. GZ and JZ contributed to reagents, instrumentation, and the financial support for this work.

Ethics approval

Not applicable.

Consent to participate

All authors approved the manuscript.

Consent for publication

Written informed consent for publication was obtained from all participants.

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Tables

Table 1 Differences in physiological and biochemical characteristics between strain WRN001^T and closely related strains.

Characteristics	1	2	3	4
Source	Salt mine soil	Hard sand ^a	Salt mine soil ^b	Saline soil ^c
Colony color	Cream to yellow	Cream to pink _a	Cream-colored ^b	Cream-colored ^c
NaCl range (optimum)	0.5-20 (8-10)	1.25-15 (10) ^a	0.5-20 (2-6) ^b	0.5-26 (8) ^c
Nitrate reduction	-	+	+	-
Oxidase	+	+	+	-
Hydrolysis of				
Tween 80	+	-	-	-
Urea	-	+	-	-
Pectin	+	-	-	-
Utilization of				
L-Arabinose	+	-	+	-
D-Galactose	+	-	-	(+)
D-Glucose	+	+	-	+
D-Mannose	-	+	+	-
D-Maltose	+	+	-	+
D-Trehalose	+	+	-	+
D-Cellobiose	-	-	+	-
D-Turanose	-	-	-	+
α-D-Lactose	-	-	+	-
β-Methyl-D-Glucoside	-	-	+	-
D-Salicin	+	+	-	-
D-Fructose	+	-	-	+
D-Sorbitol	+	-	+	(+)
D-Mannitol	+	-	-	+
L-Alanine	+	-	-	-
L-Arginine	-	+	-	-
L-Aspartic Acid	+	-	-	-

L-Glutamic Acid	+	-	+	+
L-Pyroglutamic Acid	+	-	-	-
L-Serine	+	-	+	+
D-Malic Acid	+	-	+	+
Acetic Acid	+	-	+	+
Propionic Acid	-	+	-	-
Glucuronamide	+	-	+	+
Inosine	+	-	+	+
Glycerol	+	+	-	-
Troleandomycin	+	-	(+)	+
Dextrin	-	(+)	-	+
Raffinose	-	-	-	-
Starch	+	-	-	-
Acetate	+	+	-	-
Lactose	-	-	+	-
Antibiotic susceptibility				
Chloramphenicol	+	+	+	-
Penicillin G	-	-	-	+
Tetracycline	-	+	+	-
Ampicillin	-	+	-	+
Gentamicin	-	-	+	+
Vancomycin	+	-	+	+
Quinones	Q-9	ND ^a	Q-9 ^b	Q-9 ^c
DNA G+C content (mol%)	63.8	65.0 ^a	64.6 ^b	62.7 ^c

Taxa: 1, WRN001^T; 2, *Halomonas pantelleriensis* AAP^T; 3, *Halomonas qiaohouensis* YIM QH88^T; 4, *Halomonas socia* NY-011^T. + indicates positive, (+) indicates weakly positive, – indicates negative, ND indicates not determined.

^a Data from (Romano et al. 1996), ^b Data from (Wang et al. 2014), ^c Data from (Cao et al. 2013), and all other data was obtained from this study.

Table 2 Cellular fatty acid compositions (%) of strain WRN001T and related species of the genus *Halomonas*.

Fatty acid	1	2	3	4
C _{16:0}	16.36	15.83	13.61	12.03
C _{12:0} 3-OH	6.02	6.13	7.05	6.60
C _{12:0}	3.64	3.64	4.26	3.53
C _{10:0}	2.95	2.75	3.03	2.88
C _{19:0} cyclo ω 8 <i>c</i>	2.43	10.86	15.16	1.26
Summed Feature 3 ^a	9.62	4.61	3.46	8.10
Summed Feature 8 ^a	55.68	49.45	47.10	63.17

Taxa: 1, WRN001^T; 2, *Halomonas pantelleriensis* AAP^T; 3, *Halomonas qiaohouensis* YIM QH88^T; 4, *Halomonas socia* NY-011^T. The compositions of the fatty acids that less than 1.0 % in all strains were not listed in the Table 2. All the data were taken 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 3 contains C_{16:1} ω 6*c* and/or C_{16:1} ω 7*c*; summed feature 8 comprised C_{18:1} ω 6*c* and/or C_{18:1} ω 7*c*.

Figures

Figure 1

Maximum likelihood (ML) phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain WRN001T and related taxa. Bootstrap values > 50 % (1000 replicates) are shown. Scale bar, 0.01 substitutions per nucleotide position. *Zymobacter palmae* T109T (D14555) was used as an outgroup. GenBank accession numbers are indicated for each strain.

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