Salinirussus salinus gen. nov., sp. nov., isolated from a marine solar saltern

Heng-Lin Cui,* Zhen-Zhen Lü, Yang Li and Yao Zhou

Abstract

A halophilic archaeal strain, YGH44\(^T\), was isolated from the Yinggehai marine solar saltern in Hainan Province of China. Cells were rod-shaped, stained Gram-negative and formed red-pigmented colonies on agar plates. Optimal growth was obtained with 3.4 M NaCl (range: 2.6–4.8 M), 0.5 M MgCl\(_2\) (range: 0.005–1.0 M), at 37 °C (range: 25–55 °C) and at pH 7.0 (range: pH 5.0–9.0). The cells lysed in distilled water, and the minimal NaCl concentration to prevent cell lysis was 1.7 M. Phylogenetic tree reconstructions based on 16S rRNA genes and rpoB\(^E\) genes revealed that strain YGH44\(^T\) was distinct from the related genera, Halovenus, Halapricum, Halorientalis, Halorhabdus and Halosimplex of the order Halobacterales. The major polar lipids of the strain were phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and three unidentified glycolipids. The DNA G+C content of strain YGH44\(^T\) was 69.0 mol%. The phenotypic, chemotaxonomic and phylogenetic properties suggested that strain YGH44\(^T\) (=CGMCC 1.12234\(^T\)=JCM 18646\(^T\)) represents a novel species of a new genus within the order Halobacterales, for which the name Salinirussus salinus gen. nov., sp. nov. is proposed.

Marine solar salterns are a kind of artificial ecosystem for the production of sea salt from seawater. They contain three kinds of pond, an evaporation pond, a concentration pond and a crystallizer pond, in which characteristic salt-adapted microbial communities are found along the salinity gradient [1]. Diverse halophilic archaea and bacteria are always found to be present in the crystallizer ponds where halite precipitates [2–5]. Halophilic archaea are the most diverse group of the domain Archaea and may consist of the members of the class Halobacteria, halophilic methanogenic archaea and the uncultured ‘Nanohaloarchaea’ [6, 7]. The members of the class Halobacteria are the most well-known halophilic archaea other than the halophilic methanogenic archaea [7]. Currently, the class Halobacteria is divided into three orders, Halobacteriales, Halofacales and Natrionalales [8]. In 2010, during our survey on halophilic archaeal diversity of Yinggehai marine solar saltern, the biggest marine solar saltern of Hainan Island in China, a halophilic archaeal strain, YGH44\(^T\), was isolated and found to be most closely related to the members of the order Halobacterales, as judged from 16S rRNA gene sequence analysis. In this study, a novel genus is proposed to accommodate strain YGH44\(^T\) based on phenotypic characteristics and phylogenetic analyses.

Strain YGH44\(^T\) was isolated from a brine sample taken in 2010 from the Yinggehai marine solar saltern, Hainan Province, China (18° 31’ 52” N 108° 43’ 39” E; elevation, sea level). The pH of the brine was pH 7.2, and the salinity was 226 g l\(^{-1}\). The brine was serially diluted and inoculated onto neutral haloarchaeal medium (NHM) containing the following ingredients (g l\(^{-1}\)): yeast extract (Angel Yeast) 0.05, fish peptone (Sinopharm Chemical Reagent) 0.25, sodium pyruvate 1.0, KCl 5.4, K\(_2\)HPO\(_4\) 0.3, CaCl\(_2\) 0.29, NH\(_4\)Cl 0.27, MgSO\(_4\) 26.8, MgCl\(_2\) 6H\(_2\)O 23.0, NaCl 184.0 and agar powder 15, adjusted to pH 7.0 with 1 M NaOH solution. The agar plates were incubated aerobically at 37 °C for 1 month. The reddish colonies were picked and were successively re-streaked on NHM agar plates at least three times to obtain pure colonies. The strain isolated was preserved at −20 °C as a suspension in NHM broth supplemented with glycerol (150 g l\(^{-1}\)).

Characterization of strain YGH44\(^T\) was performed according to the proposed minimal standards for description of new taxa [9] and as described previously by Cui et al. [10]. The NaCl range for growth was tested by incubating the strain at NaCl concentrations of 0.9, 1.4, 1.7, 2.1, 2.6, 3.1, 3.4, 3.9, 4.3, 4.8 and 5.1 M. The MgCl\(_2\) concentration for growth was determined in media containing final MgCl\(_2\) concentrations of 0, 0.005, 0.01, 0.03, 0.05, 0.1, 0.3, 0.5, 0.7 and 1.0 M. The pH range for growth was determined at pH 5.0–10.0 (with intervals of 0.5 pH units) using the following
buffers: MES (pH 5.5–6.7), PIPES (pH 6.1–7.5), MOPS (pH 6.5–7.9), HEPES (pH 6.8–8.2), Tricine (pH 7.4–8.8) and CHES (pH 8.6–10.0) at a concentration of 25 mM. The temperature range for growth was determined by incubating the strain at 10, 15, 20, 25, 30, 37, 40, 42, 45, 50, 55 and 60 °C. Anaerobic growth on nitrate and formation of gas from nitrate were tested in screw-topped tubes (with Durham tubes) filled completely with liquid NHM medium supplemented with NaNO₃ (5 g l⁻¹). Antimicrobial sensitivity tests were determined by spreading cell suspensions on NHM agar plates and then applying discs impregnated with antimicrobial agents.

Halophilic archaeal polar lipids were extracted using a chloroform/methanol system and analysed using one- and two-dimensional TLC, as described previously [10]. Two specific detection spray reagents, phosphate stain reagent for phospholipids and α-naphthol stain for glycolipids, were used. The general detection reagent, sulfuric acid/ethanol (1:2, v/v), was also used to detect total polar lipids. The presence of phospholipids and glycolipids on the two-dimensional TLC was confirmed by comparing with one-dimensional TLC on which the polar lipid profile of reference strains was developed.

Genomic DNA of strain YGH44ᵀ was extracted and purified using a genomic DNA extraction kit (Beijing ComWin Biotech), and the 16S rRNA gene was amplified with the forward primer 0018F and reverse primer 1518R, then cloned and sequenced as described previously [11]. The rpoB gene was amplified using the primer pair Hrp0B2 1420F and Hrp0A 153R [12], and the PCR product was sequenced using the following primers: Hrp0B2 1420F, Hrp0A 153R and B1-628F (5’-CCNGCNSSVCAGACTTC-3’). The sequences were aligned using the ClustalW program integrated in the MEGA 6 software [13], and the phylogenetic trees were reconstructed using the maximum-likelihood [14], maximum-parsimony [15] and neighbour-joining [16] algorithms in the MEGA 6 software. Sequence similarity was analysed by comparing the 16S rRNA gene sequence of strain YGH44ᵀ with the sequences from the EzTaxon database (http://www.ezbiocloud.net/eztaxon) [17]. The DNA G+C content of strain YGH44ᵀ was determined from the mid-point value (Tₘ) of the thermal denaturation method [18] at 260 nm with a Beckman-Coulter DU800 spectrophotometer equipped with a high-performance temperature controller.

Cells of strain YGH44ᵀ were motile and pleomorphic rods (0.5–1.0×1.0–2.0 µm) when grown in NHM liquid medium (Fig. S1, available in the online Supplementary Material). They stained Gram-negative and the colonies were red-pigmented. Strain YGH44ᵀ was able to grow at 25–50 °C (optimum 37 °C), with 2.6–4.8 M NaCl (optimum 3.4 M NaCl), with 0.005–1.0 M MgCl₂ (optimum 0.5 M MgCl₂) and at pH 5.0–9.0 (optimum pH 7.0). The cells lysed in distilled water, and the minimal NaCl concentration to prevent cell lysis was 1.7 M. The strain was able to grow under anaerobic conditions using nitrate and l-arginine but not DMSO. The formation of H₂S and indole was positive. Strain YGH44ᵀ could hydrolyse starch and gelatin but not casein or Tween 80. It was sensitive to the following antimicrobial compounds (µg per disc, unless otherwise indicated): rifampin (5), nystatin (100) and ciprofloxacin (5). It was resistant to the following antimicrobial compounds: novobiocin (30), bacitracin (0.04 IU per disc), nitrofurantoin (300), norfloxacine (10), trimethoprim (5), erythromycin (15), penicillin G (10 IU per disc), ampicillin (10), chloramphenicol (30), neomycin (30), streptomycin (10), kanamycin (30), tetracycline

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Halovenus aranensis CGMCC 1.11001^T (KJ534549)
Halovenus rubra R28^T (HM159605)
Salinirussus salinus YGH44^T (JQ937358)
Halapricum salinum CBA1105^T (BBM01000002)
Halorhabdus tiamatea SARL4^B (AFNT02000002)
Halorhabdus utahensis DSM 12940^B (NC_013158)
Halorientalis brevis YC89^T (JQ237120)
Halorientalis persicus IBRC-M 10043^B (FOCX01000004)
Halorientalis regularis TNN28^T (GQ282621)
Halosimplex carlsbadense JCM 11222^T (HQ263561)
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Halomicrobium mukohataei DSM 12286^B (NC_013202)
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Haloarcula vallismortis CGMCC 1.2048^B (EF645688)
Halomicroarcula pellucida BNERC 31^T (AB766179)
Halomicroarcula pellucida BNERC 31^T (AB766180)
Natronomonas pharaonis DSM 2160^T (NC_007426)
Natrialba asiatica JCM 9576^T (AB663455)
Halofex volcanii DS 2^T (NC_013967)

Fig. 1. Maximum-likelihood phylogenetic tree reconstructions based on 16S rRNA gene (a) and rpoB gene (b) sequences, showing the relationships between strain YGH44^T and related members within the class Halobacteria. Bootstrap values (percentages) are based on 1000 replicates and are shown for branches with more than 50% bootstrap support. Bars, expected substitutions per nucleotide position.
The main phenotypic characteristics differentiating strain YGH44\(^T\) from the related members of the family Halobacteria were as follows: cell shape, utilization of sugars and amino acids, hydrolysis of starch, gelatin and Tween 80, presence of phosphatidylglycerol sulfate (PGS) (Table 1). More detailed phenotypic features of YGH44\(^T\) are given in the species description.

The major polar lipids of strain YGH44\(^T\) were phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and three unidentified glycolipids (Fig. S2). The phospholipid profile set strain YGH44\(^T\) apart from the members of the genus Halorhabdus, which contain PGS [19]. The glycolipid composition differentiated strain YGH44\(^T\) from the members of the genus Halorientalis, which contain sulfated mannosyl glucosyl diether (S-DGD-1) [20], the members of the genus Halorhabdus, which contain galactosyl mannosyl glucosyl diether (TGD) and sulfated galactosyl mannosyl glucosyl diether (STGD) [21], and the members of the genus Halosimplex, which contain disulfated mannosyl glucosyl diether (S-2DGD) [22]. The DNA G+C content of strain YGH44\(^T\) was 69.0 mol%, which is higher than those of members of the genera Halovenus (66.0 mol%), Halovenus (56.3–63.1 mol%), Halorientalis (61.3–62.8 mol%), Halorhabdus (61.2–64.0 mol%) and Halosimplex (62.5–64.4 mol%).

Strain YGH44\(^T\) had one kind of 16S rRNA gene sequence (1471 nt, JQ937538) and was closely related to members of the genera Halovenus (89.9–94.1%), Halapricum (93.6%), Halorientalis (91.8–93.8%), Halorhabdus (93.1–93.3%) and Halosimplex (88.4–91.0%). Phylogenetic tree reconstruction using the maximum-likelihood algorithm revealed that strain YGH44\(^T\) formed a distinct clade, separating from the genera Halovenus and Halapricum (Fig. 1a). The phylogenetic position was also confirmed in other trees generated using the maximum-parsimony and neighbour-joining algorithms (Figs S3a and S4a).

The rpoB\(^\prime\) gene of strain YGH44\(^T\) was sequenced and found to be 1833 bp in full length. It was closely similar to that of the related members of the genera Halosimplex (86.9–87.8%), Halapricum (85.4%), Halorhabdus (83.5–84.6%), Halorientalis (83.6–84.1%) and Halovenus (82.4%). In reconstructed phylogenetic trees, strain YGH44\(^T\) formed a monophyletic clade separated from the genus Halosimplex (Fig. 1b). The phylogenetic position was also confirmed in trees generated using the maximum-parsimony and neighbour-joining algorithms (Figs S3b and S4b).

The phenotypic, chemotaxonomic and phylogenetic properties suggested that strain YGH44\(^T\) represents a novel species in a new genus within the class Halobacteria, for which the name Salinirussus salinus gen. nov., sp. nov. is proposed.

**DESCRIPTION OF SALINIRUSSUS SALINUS SP. NOV.**

Salinirussus salinus (sa.li.ni. rus.sus. L. fem. pl. n. salinae salterns, salt works; L. masc. adj. russus red; N.L. masc. n. Salinirus-sus red salt organism from salt works).

Cells are Gram-stain-negative, motile and rod-shaped under optimal growth conditions. Cells lyse in distilled water. Colonies on agar plates are red, moist and round. Catalase test and the oxidase test are positive. Growth occurs at 25–55 °C, with 2.6–4.8 M NaCl, with 0–1.0 M MgCl\(_2\) and at pH 5.0–9.0. At least 0.005 M MgCl\(_2\) is required for growth. Sugars are metabolized, in some cases with the formation of acids. The polar lipids are phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and three unidentified glycolipids. The type species is Salinirussus salinus. Recommended three-letter abbreviation: Srs.
Halomicrobium marine solar saltern. a new member of the family Halobacteriaceae description of new taxa in the order Bacterio1997;47:233–240.


Gupta RS, Naushad S, Baker S. Phylogenomic analyses and molecular signatures for the class Halobacteria and its two major clades: a proposal for division of the class Halobacteria into an emended order Halobacteriales and two new orders, Halorhabdaceae ord. nov. and Natrialbales ord. nov., containing the novel families Halorhabdaceae fam. nov. and Natrialbaceae fam. nov. Int J Syst Evol Microbiol 2015;65:1050–1069.


References


