Halobacterium piscisalsi Yachai et al. 2008 is a later heterotypic synonym of Halobacterium salinarum Elazari-Volcani 1957

Hiroaki Minegishi,1 Akinobu Echigo,1 Yasuhiro Shimane,1,2 Masahiro Kamekura,3 Somboon Tanasupawat,4 Wonnop Visessanguan5 and Ron Usami1,2

1Bio-Nano Electronics Research Centre, Toyo University, 2100 Kujirai, Kawagoe, Saitama 350-8585, Japan
2Graduate School of Interdisciplinary New Science, Toyo University, 2100 Kujirai, Kawagoe, Saitama 350-8585, Japan
3Halophiles Research Institute, 677-1 Shimizu, Noda, Chiba 278-0043, Japan
4Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
5National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Klong 1, Klong Luang, Pathumthani 12120, Thailand

Halobacterium piscisalsi was proposed by Yachai et al. (2008), with a single strain, HPC1-2T (=BCC 24372T =JCM 14661T =PCU 302T), which was isolated from fermented fish (pla-ra) in Thailand. According to Yachai et al. (2008), the strain was closely related to Halobacterium salinarum based on 16S rRNA gene sequence comparisons and could be differentiated by low DNA–DNA relatedness values and different biochemical profiles compared with other species of the genus. The reanalysis of the 16S rRNA gene sequences and the DNA–DNA relatedness among H. piscisalsi JCM 14661T and H. salinarum strains JCM 8978T, R1 and NRC-1 revealed that they all had exactly the same 16S rRNA gene sequence and shared more than 70 % DNA–DNA relatedness. In addition, the full-length DNA-dependent RNA polymerase subunit B (RpoB) protein sequence of H. piscisalsi JCM 14661T (607 amino acids) was the same as that of H. salinarum JCM 8978T and showed 94.7 and 96.7 % similarities with those of Halobacterium noricense JCM 15102T and Halobacterium jilantaiense JCM 13558T, respectively. Despite the different biochemical properties described by Yachai et al. (2008), the characteristic phenotypic properties of H. piscisalsi agreed with those in the description of H. salinarum emended by Gruber et al. (2004). Therefore, H. piscisalsi Yachai et al. (2008) should be regarded as a later heterotypic synonym of H. salinarum Elazari-Volcani 1957.

Halobacterium piscisalsi was proposed as a novel species of the genus Halobacterium by Yachai et al. (2008) with a sole strain, designated HPC1-2T (=BCC 24372T =JCM 14661T =PCU 302T). The genus contained three species at the time of the proposal: Halobacterium salinarum (the type species of the genus), Halobacterium noricense and Halobacterium jilantaiense (Ventosa & Oren, 1996; Gruber et al., 2004; Yang et al., 2006). Strain HPC1-2T was isolated from pla-ra, a salt-fermented fish product of Thailand.

According to Yachai et al. (2008), levels of 16S rRNA gene sequence similarity between strain HPC1-2T (GenBank accession no. AB285020) and H. salinarum DSM 3754T (Ventosa & Oren, 1996; AJ496185), H. jilantaiense JCM 13558T (Yang et al., 2006; DQ256409, AB477970) and H. noricense A1T (Gruber et al., 2004; AJ548827) were 99.2, 97.8 and 96.6 %, respectively. However, it was distinguished from these three species on the basis of the low levels of DNA–DNA relatedness, showing 36.5 ± 2.5 % sequence similarity with H. salinarum DSM 3754T and 19.3 ± 2.1 % sequence similarity with H. jilantaiense JCM 13558T. Strain HPC1-2T could also be differentiated from other species of the genus by differences in their physiological and biochemical profiles (Yachai et al., 2008).
The 16S rRNA gene sequence is a still useful semantophoretic molecule for the inference of phylogenetic relationships between members of the family Halobacteriaceae, although a number of halobacterial strains have proved to have different gene copies in their genomes. The inference of the phylogenetic positions of every haloarchaeal species should be based on the comparison of high-quality 16S rRNA gene sequences, whereas the one used in the original description of *H. piscisalsi* (AB285020) contained ten ambiguous nucleotides and four extra nucleotides between nt 970 and nt 1032 (AJ496185 numbering) compared with the gene sequences of three other species of the genus *Halobacterium*. As such, the full-length 16S rRNA gene sequence (AB545861, 1473 bp) of *Halobacterium piscisalsi* JCM 14661T (derived from strain HCP1-2T) was determined in the present study using the primer set H16S For: 5'- CCCTCGCGSTMCSCGG3' - and 23S Rev: 5'- GCTTWTCGCGCTTG3'. The 16S rRNA gene sequence obtained with this primer set was exactly the same as that of *H. salinarum* strains DSM 3754T (AJ496185), NRC-1 (AE004437) and R1 (AM774415). These data prompted us to re-evaluate the taxonomic status of strain HPC1-2T in relation to the species *H. salinarum* (Oren & Ventosa, 2010).

*H. piscisalsi* JCM 14661T (=HCP1-2T) was obtained from JCM. In order to confirm its provenance, we also obtained a descendant culture of HPC1-2T from the Biotech Culture Collection, BCC 24372T. The following relevant strains were included in this study: *H. salinarum* JCM 8978T ( =NCIMB 764T), *H. salinarum* NRC-1 (=JCM 11081 =ATCC 700922), *H. salinarum* R1 (=JCM 9409 =NCIMB 2080 =ATCC 29341), *H. noricense* JCM 15102T (=NCIMB 13967T =strain A1T) and *H. jilantaiense* JCM 13558T (=strain NG4T). Purified DNA samples of these strains were prepared from 20 ml cultures in JCM No.168 medium containing (1-1) 5 g Casamino acids, 5 g yeast extract, 1 g sodium glutamate, 3 g trisodium citrate, 20 g MgSO4·7H2O, 2 g KCl, 200 g NaCl, 36 mg FeCl3·4H2O and 0.36 mg MnCl2·4H2O (pH 7). DNA–DNA relatedness among these strains was determined by using the fluorometric method of Ezaki et al. (1989) using FluoroNunc Modules/Plates (catalogue no. 475515, Thermo Fisher Scientific) (n=4). The probe DNA was labelled with photobiotin and hybridized with fixed DNA in the microplate wells at 55 °C for 2 h in the presence of 50 % formamide. The hybrid DNA was then quantitatively detected with streptavidin-β-D-galactosidase and 4-methylumbelliferyl β-D-galactopyranoside (Sigma) as a fluorogenic substrate. The DNA–DNA relatedness values among *H. piscisalsi* JCM 14661T and the three strains of *H. salinarum*, JCM 8978T, NRC-1 and R1, were 83, 84 and 77 %, respectively, and 85 %, 82 % and 77 %, reciprocally. Two other descendant strains of the *H. piscisalsi* type strains (HPC1-2T and BCC 24372T) also showed high relatedness to the type strain of *H. salinarum* JCM 8978T (83-88 %, including reciprocal values; Table S1, available in IJSEM Online). These values were well above the threshold value of 70 % DNA–DNA relatedness generally accepted for the delineation of the bacterial species (Wayne et al., 1987; Stackebrandt & Ebers, 2006). Likewise, the relatedness values between *H. salinarum* strains JCM 8978T and NRC-1 were 89–90 % (including reciprocal values), between *H. salinarum* JCM 8978T and strain R1, were 71–72 % and between NRC-1 and R1 were 88–89 %. Thus, *H. salinarum* JCM 8978T and *H. piscisalsi* JCM 14661T, as well as *H. salinarum* strains NRC-1 and R1, should be included in a single genospecies. On the other hand, *H. salinarum* JCM 8978T and *H. noricense* JCM 15102T showed 24 % DNA–DNA relatedness, while *H. salinarum* JCM 8978T and *H. jilantaiense* JCM 13558T showed 43–44 % DNA–DNA relatedness and *H. noricense* JCM 15102T and *H. jilantaiense* JCM 13558T showed 31–33 % DNA–DNA relatedness. These values supported the separated taxonomic status of these two species from *H. salinarum*.

In order to confirm these results, we conducted a comparison of full-length RNA polymerase subunit B (*rpoB*) gene sequences among these strains. Our previous work on *rpoB* sequencing of 85 strains in 27 genera of the family Halobacteriaceae demonstrated that the gene (1812 bp) is useful as a supplementary tool in determining the phylogenetic position of new isolates (Minegishi et al., 2010). The *rpoB* gene sequence (1827 bp) similarities between *H. salinarum* JCM 8978T and *H. noricense* JCM 15102T, *H. jilantaiense* JCM 13558T, and *H. piscisalsi* JCM 14661T were 89.1 % (200 nt difference), 91.4 % (157 nt difference) and 99.7 % (5 nt difference), respectively; the translated RpoB protein (608 amino acids) sequence similarities were 94.7 % (32 aa difference), 96.7 % (20 aa difference) and 100 %, respectively (Table S2). These data support the proposal that the type strains of *H. piscisalsi* and *H. salinarum* could be included in a single species.

According to Yachai et al. (2008), the two strains of *H. piscisalsi* and *H. salinarum* can be differentiated by several biochemical properties such as utilization of lactose and melizitose as carbon sources and production of esterase (C4) and esterase lipase (C8) as estimated by API ZYM; however, they share similar phenotypic properties as a whole, including their phospholipid and menaquinone compositions. Actually, the characteristic phenotypic properties of the *H. piscisalsi* strain agree well with those in the description of *H. salinarum* emended by Gruber et al. (2004).

Therefore, in the light of the refined 16S rRNA gene sequence and DNA–DNA hybridization data, as well as the comparable *rpoB* gene sequences and the phenotypic profiles, *H. piscisalsi* Yachai et al. (2008) should be considered as a later heterotypic synonym of *H. salinarum* Elazari-Volcani 1957.

All authors of the paper by Yachai et al. (2008) have expressed that the data on DNA–DNA relatedness were erroneous and consented to the conclusion of this manuscript.
References


