# Supplementary Materials

# *Haloferax profundi* sp. nov. and *Haloferax marisrubri* sp. nov., Isolated from the Discovery Deep Brine-Seawater Interface in the Red Sea

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**Supplementary Figures.**

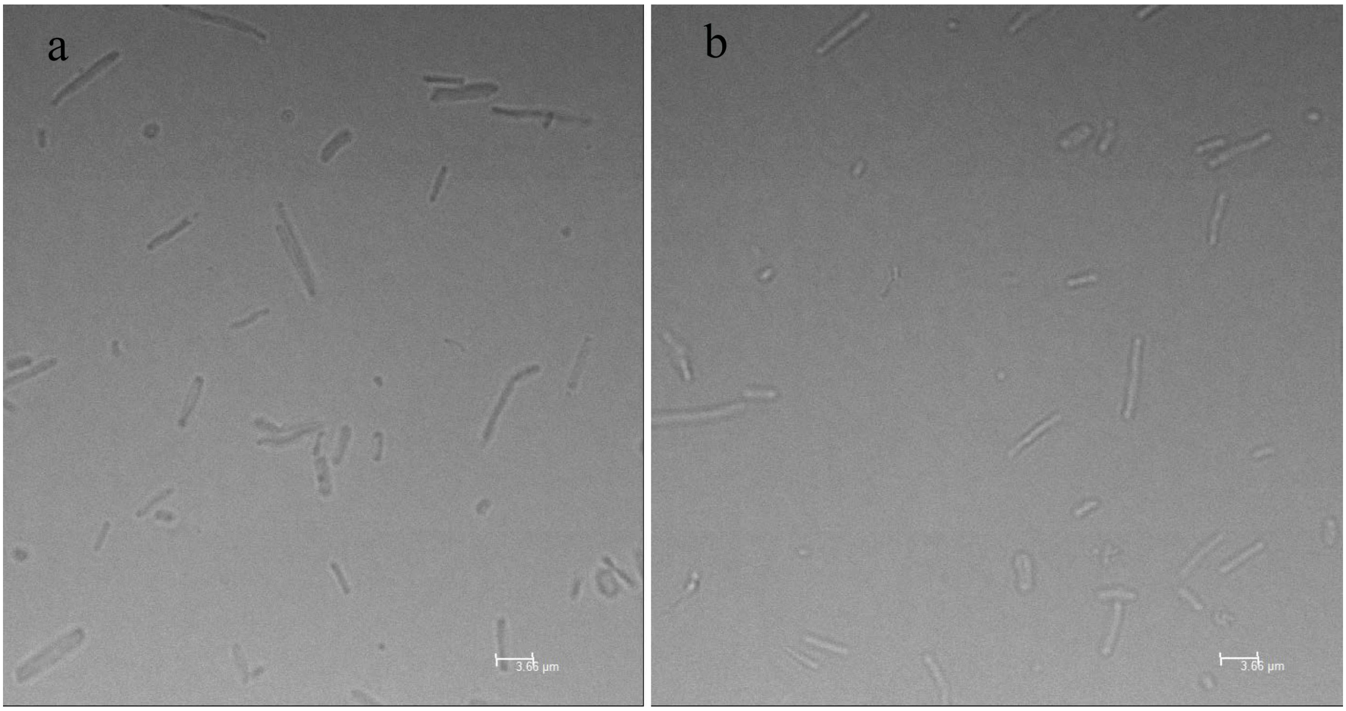

**Figure S1.** Phase-contrast micrograph of strain SB29$^T$ (a) and SB3$^T$ (b) after 72 h growth in liquid MR2A medium at 37 ℃, illustrating the pleomorphic nature of cells of strains SB29$^T$ and SB3$^T$. Scale bars are shown on the images.

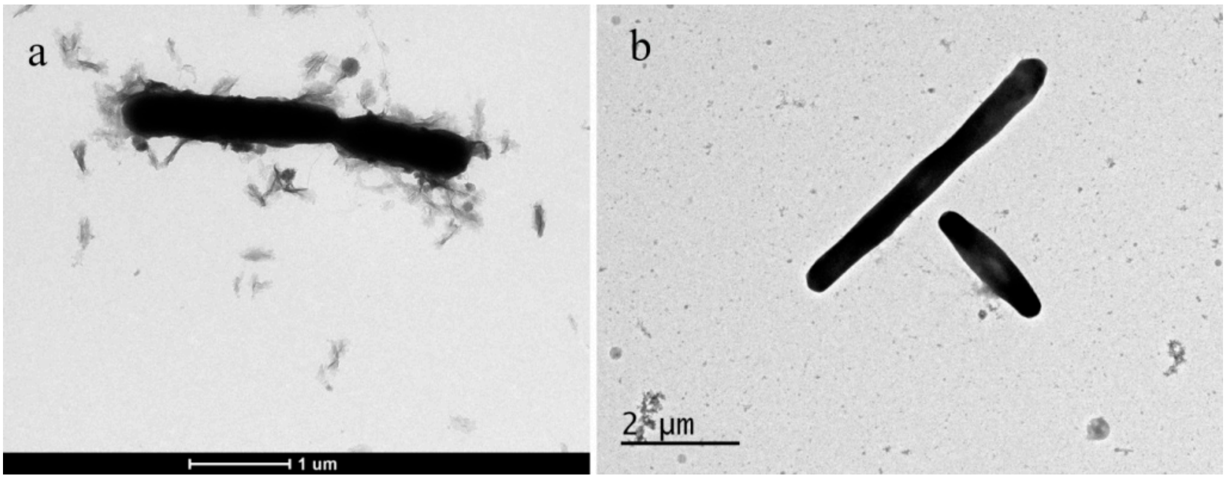

**Figure S2.** Scanning Electron Microscope (SEM) micrograph of strain SB29$^T$ (a) and SB3$^T$ (b) after 72 h of growth in liquid MR2A medium at 37 ºC, scale bar = 1 µm for panel a, scale bar = 2 µm for panel b.

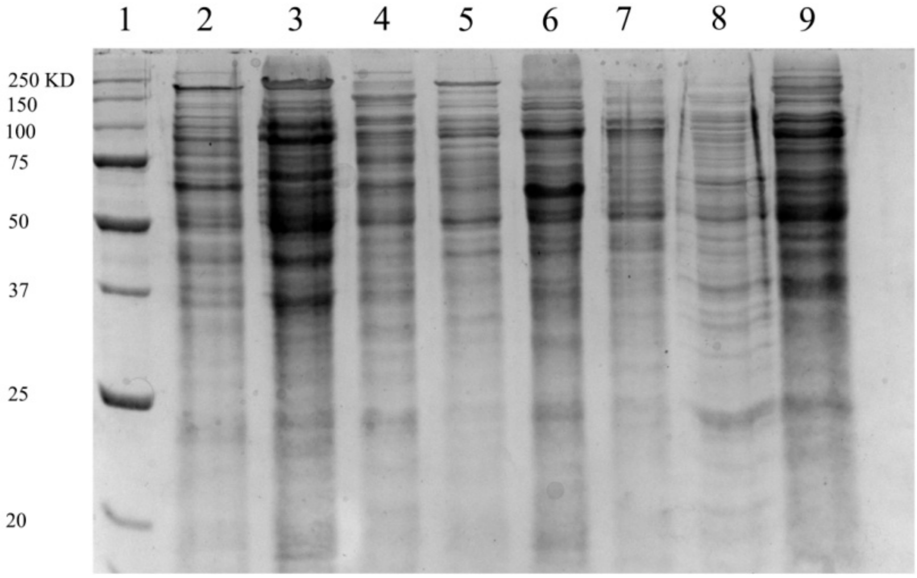

**Figure S3.** Whole-cell proteins from various halophilic archaeal strains and isolates SB29$^T$ and SB3$^T$, following separation by SDS-PAGE. Following cell lysis, approximately 15 μg of protein was applied to each lane. Proteins were stained with Coomassie brilliant blue. Lanes: 1, molecular mass marker; 2, Strain SB29$^T$; 3, Strain SB3$^T$; 4, *Hfx. alexandrinus* JCM 10717$^T$; 5, *Hfx. larsenii* JCM 13917$^T$; 6, *Hfx. lucentense* DSM14919$^T$; 7, *Hfx. prahovense* DSM 18310$^T$; 8, *Hfx. sulfurifontis* DSM 16227$^T$; 9. *Hfx. elongans* JCM 14791$^T$.

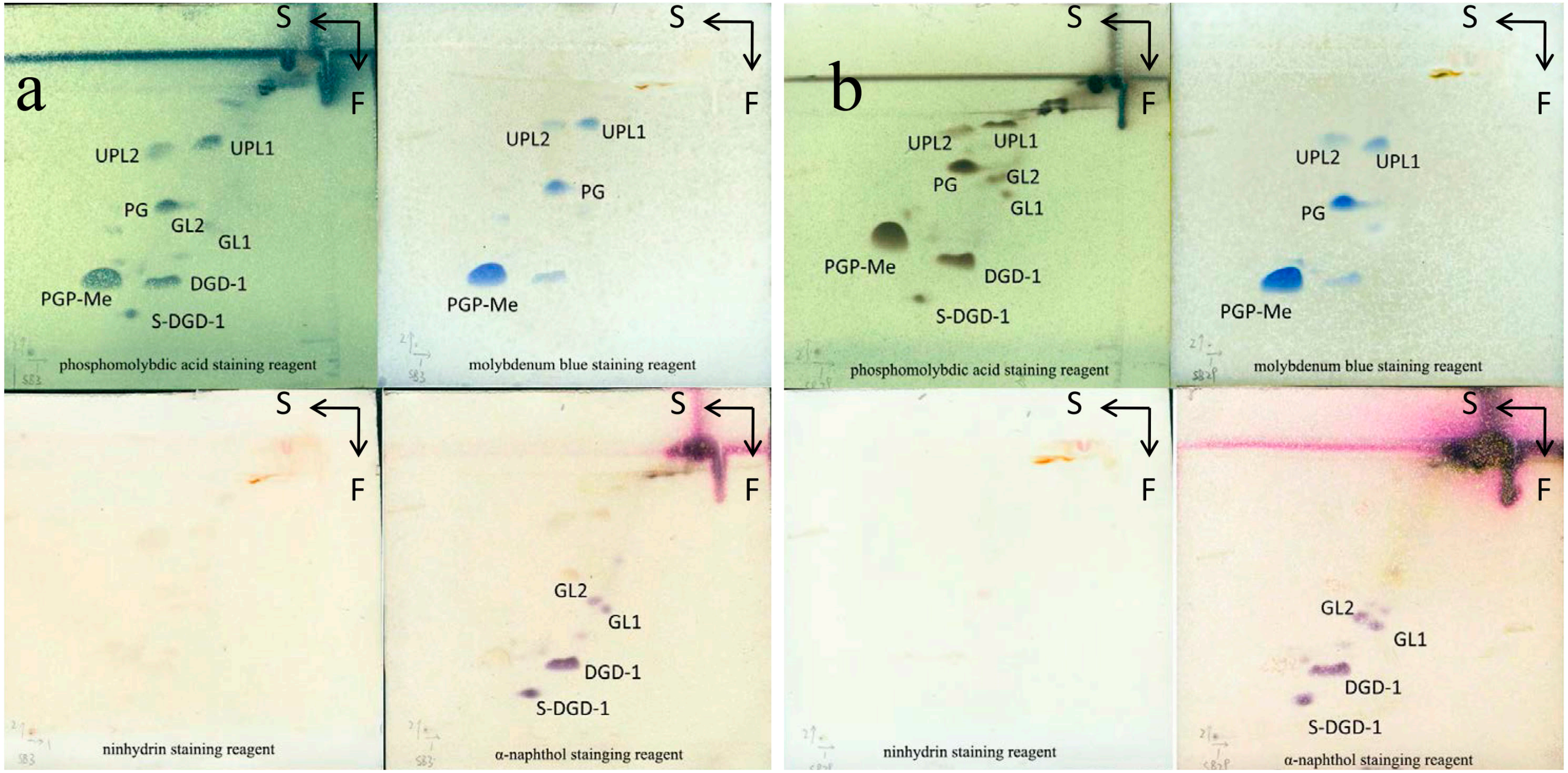

**Figure S4.** Polar lipids profiles for strain SB3$^T$ (a) and SB29$^T$ (b) include PG, PGP-Me, S-DGD-1, DGD-1, UPL1 and UPL2; and an unidentified GL, which support the placement of both strains into the genus *Haloferax*. The TLC plates were stained with 10% phosphomolybdic acid in 95% ethanol for detection of total lipids, 0.2 % ninhydrin in 1-butanol for aminolipids, molybdenum blue reagent (Sigma) for phospholipids, and a-naphthol staining reagent (Sigma) for cholines. Abbreviations: PG, phosphatidyl glycerol; PGP-Me, phosphatidyl glycerol phosphate-methlester; S-DGD-1, sulfated mannosyl glucosyl diether; DGD-1, mannosyl glucosyl diether; UPL1 and UPL2, two unidentified phospholipids; GL, glycolipid; F, first dimension of TLC; S, second dimension of TLC.

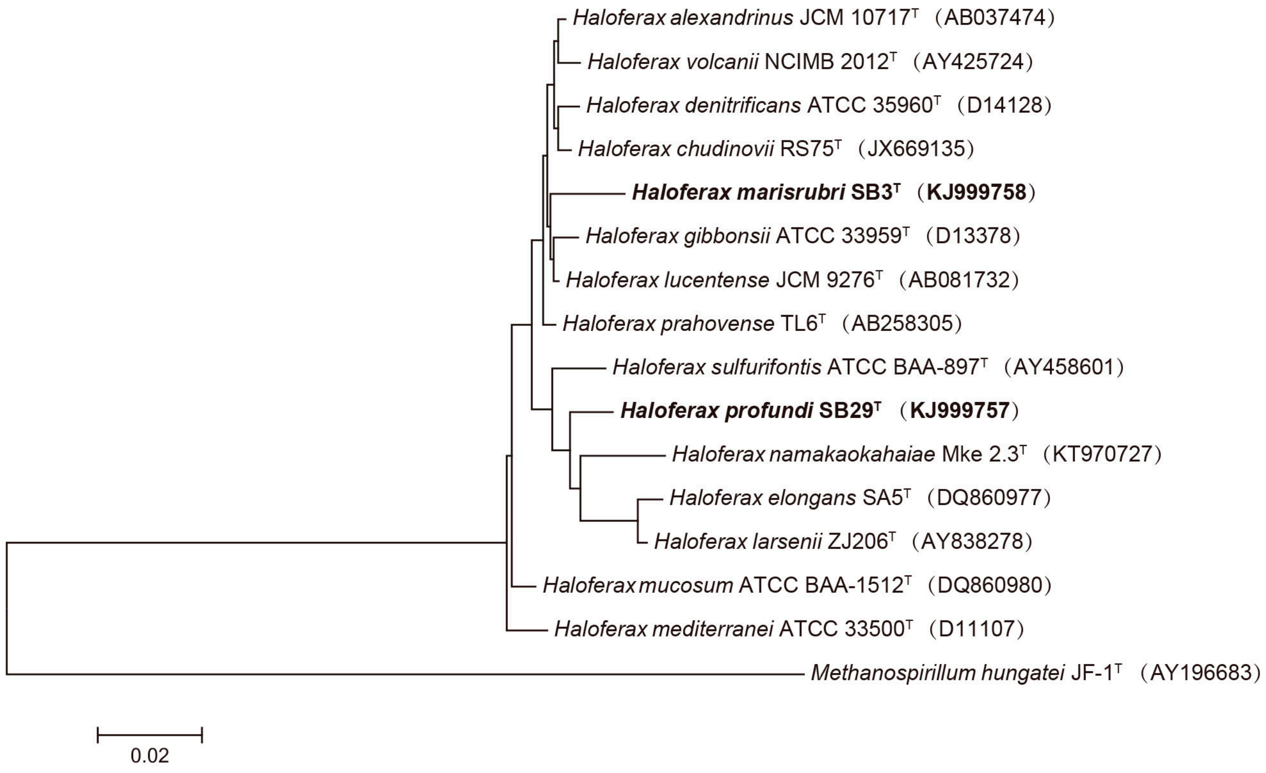

**Figure S5.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain SB3$^T$ and SB29$^T$ (bold) and related taxa. Scale bar indicates 0.01 substitutions per nucleotide position. *Methanospirillum hungatei* JF-1$^T$ (AY196683) was used as outgroup. GenBank accession numbers are indicated for each strain in parentheses.

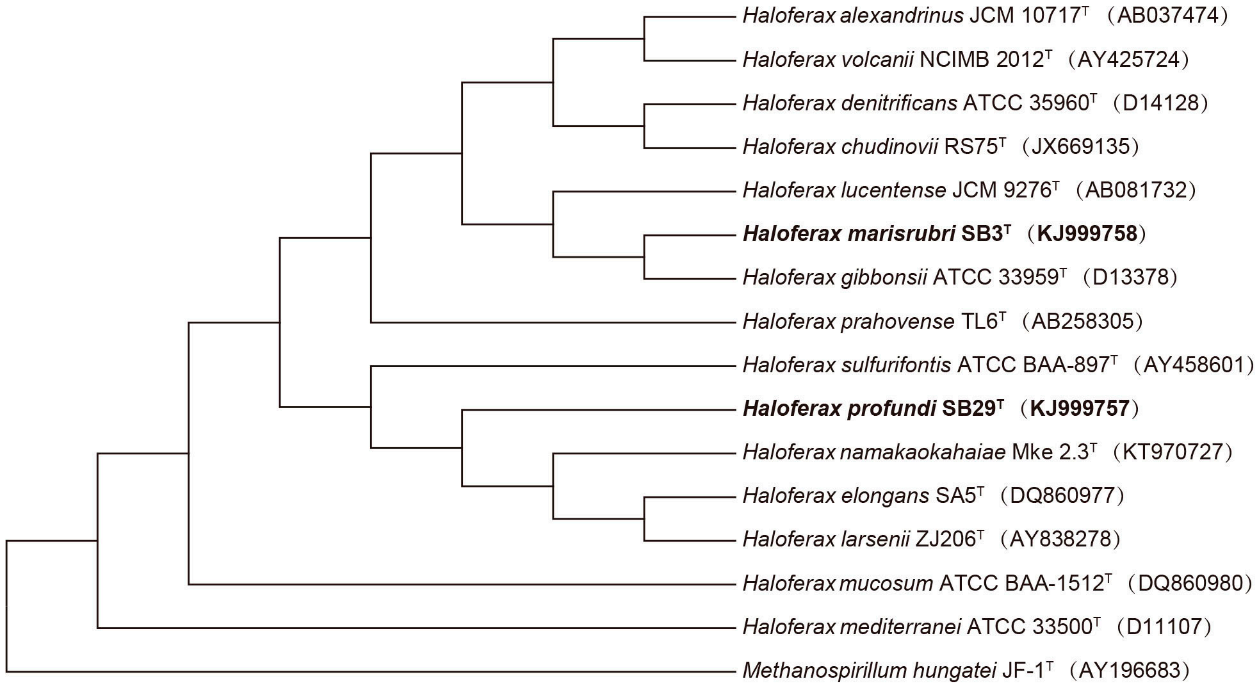

**Figure S6.** Maximum parsimony phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain SB3$^T$ and SB29$^T$ (both in bold) and related taxa. *Methanospirillum hungatei* JF-1$^T$ (AY196683) was used as outgroup. GenBank accession numbers are indicated for each strain in parentheses.

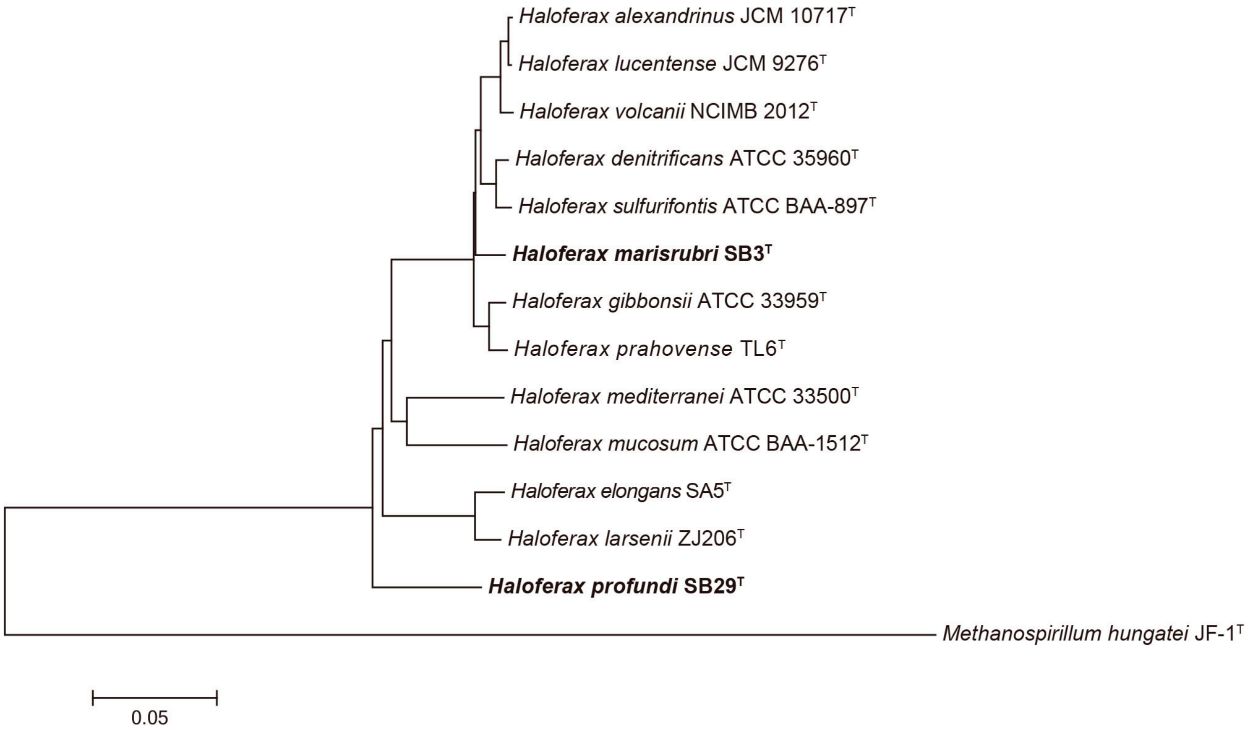

**Figure S7.** Phylogenomic tree based on the concatenation of the 31 single-copy core genes from all 11 available genomes of type strains for *Haloferax* spp. were inferred with Neighbor-Joining (NJ) method and rooted by *Methanospirillum hungatei* JF-1$^{T}$. The genes were katG2, top6A, ntpB, atpA, ftsZ_2, aroB, fusA, uvrA, Ion, gatA, ndk, rplB, ftsZ_1, pdxS, nikR_2, guaA_2, rpoB_1, rpsK, rpsJ_1, tufB_1, rps12P, rps28e, DUF2073, rps2P, rpoK, rpl40e, rps3p, Mcm2 and three hypothetical proteins (PRK00939, PRK04016, PRK04243; https://www.ncbi.nlm.nih.gov/cdd) as determined in Roary (see methods section).

**Table S1.** Average nucleotide identity (ANIm) and digital DNA-DNA hybridization (dDDH) values (%) between the genomes of strain SB3$^T$ (GenBank accession: LOPW00000000) , and closely related genomes in GenBank.

| Most closely related genomes in GenBank | GenBank assembly accession | ANIm | DDH |
|---|---|---|---|
| *Haloferax gibbonsii* ATCC 33959$^T$ | GCA_000336775.1 | 92.2 | 49.2 |
| *Haloferax mediterranei* ATCC 33500$^T$ | GCA_000306765.2 | 81.8 | 26.0 |
| *Haloferax denitrificans* ATCC 35960$^T$ | GCA_000337795.1 | 92.6 | 51.1 |
| *Haloferax sulfurifontis* ATCC BAA-897$^T$ | GCA_000337835.1 | 91.9 | 48.7 |
| *Haloferax mucosum* ATCC BAA-1512$^T$ | GCA_000337815.1 | 81.8 | 25.9 |
| *Haloferax lucentense* JCM 9276$^T$ | GCA_000336795.1 | 92.0 | 49.2 |
| *Haloferax alexandrinus* JCM 10717$^T$ | GCA_000336735.1 | 92.0 | 49.4 |
| *Haloferax volcanii* NCIMB 2012$^T$ | GCA_000025685.1 | 91.9 | 48.8 |
| *Haloferax elongans* SA5$^T$ | GCA_000336755.1 | 80.3 | 24.3 |
| *Haloferax profundi* SB29$^T$ | GCA_001469865.1 | 81.1 | 25.1 |
| *Haloferax prahovense* TL6$^T$ | GCA_000336815.1 | 92.2 | 50.0 |
| *Haloferax larsenii* ZJ206 $^T$ | GCA_000336955.1 | 80.5 | 24.5 |

**Table S2.** Average nucleotide identity (ANIm) and digital DNA-DNA hybridization (dDDH) values (%) between the genomes of strain SB29$^T$ (GenBank accession no.: LOPV00000000), and closely related genomes in GenBank. .

| Most closely related genomes in GenBank | GenBank assembly accession | ANIm | DDH |
|---|---|---|---|
| *Haloferax gibbonsii* ATCC 33959$^T$ | GCA_000336775.1 | 80.8 | 25.0 |
| *Haloferax mediterranei* ATCC 33500$^T$ | GCA_000306765.2 | 80.7 | 25.1 |
| *Haloferax denitrificans* ATCC 35960$^T$ | GCA_000337795.1 | 80.8 | 25.1 |
| *Haloferax sulfurifontis* ATCC BAA-897$^T$ | GCA_000337835.1 | 82.6 | 28.1 |
| *Haloferax mucosum* ATCC BAA-1512$^T$ | GCA_000337815.1 | 80.2 | 25.6 |
| *Haloferax lucentense* JCM 9276$^T$ | GCA_000336795.1 | 80.7 | 25.0 |
| *Haloferax alexandrinus* JCM 10717$^T$ | GCA_000336735.1 | 80.7 | 24.8 |
| *Haloferax volcanii* NCIMB 2012$^T$ | GCA_000025685.1 | 80.7 | 24.9 |
| *Haloferax elongans* SA5$^T$ | GCA_000336755.1 | 80.1 | 24.3 |
| *Haloferax marisrubri* SB3$^T$ | GCA_001469875.2 | 81.0 | 25.1 |
| *Haloferax prahovense* TL6$^T$ | GCA_000336815.1 | 80.8 | 25.0 |
| *Haloferax larsenii* ZJ206 $^T$ | GCA_000336955.1 | 81.4 | 25.5 |