Supplementary Materials and Methods

Thioalkalivibrio sp. 10fs10 genome sequencing

Thioalkalivibrio sp. 10fs10 genomic DNA was extracted by GenElute™ Bacterial Genomic DNA, following producer's instructions. DNA quantification was performed using Qubit® 3.0 (Invitrogen, Thermo Fisher Life Technologies) following producer's instructions. Thioalkalivibrio sp.10fs10 genome was sequenced in St. Petersburg state University, St. Petersburg (Russia). One Mate-Pair (MP) libraries was prepared using FC-132-1001 Nextera® Mate Pair Sample Prep Kit for 1µg DNA input with the following modifications: DNA was sizeselected after the tagmentation using 0.8 % agarose gel and the selected region was 2.5 - 6 kb. The libraries were sequenced in paired-end 2x100 mode on Illumina HiSeq 2500 using PE-402-4002 HiSeq® Rapid PE Cluster Kit v2 and FC-402- 4021 HiSeq® Rapid SBS Kit v2 (200 Cycle). Two Pair-End (PE) libraries were prepared using FC 121-1031 Nextera® DNA library preparation kit on the same amount of DNA and sequenced on MiSeq PE sequencing 2x300 using MS-102-3003 MiSeq® Reagent Kit v3 (600 cycle). Demultiplexed raw data were trimmed and quality filtered using Trimmomatic v0.36 [1] on paired-ends libraries and NxTrim v0.4.2 [2] on mate-paired library. Genome assembly was performed using the approach previously described [3], with some modifications. The draft genome of Thioalkalivibrio sp. isolate 10fs10 was generated performing multiple steps of assembly, using two assemblers: Abyss 2.0.1 [4] and SPAdes 3.10.1 [5]. A total of two fake reads libraries were generated using BBmap (BBMap - Bushnell B. - sourceforge.net/projects/bbmap/) from the first assembly (using minum lenght option of 1.5 Kbp and 10 Kbp respectively). The overlapping fake reads libraries were used to harmonize the two different libraries obtained with different sequencing platforms, to improve de novo assembly performance, as suggested by [6]). To resolve the gaps generated during scaffolding step, GapFiller v1.10 [7] was used. Finally, consensus sequences were processed using Prodigal 2.50 [8] for coding sequence (CDS) prediction. CDS aligned against different databases, such as Swissprot, TrEMBL, Pfam, Tigrfam, using Blast 2.5.0 [9] and HMMER [10]. Blast2go 4.1 [11] was used to align sequences against InterPro and Gene Ontology, BlastKoala [12] was used to annotate proteins in Kegg Orthology (KO) and tRNAscaSE 1.3 [13] was used to find tRNA sequences. Assembled DNA automatic annotations were also performed using RAST platform (http://rast.nmpdr.org) and IMG-ER platform [14]. Genome assembly and annotation project are available at IMG, under IMG-ER submission ID 183849.

Genes involved in bacterial homeostasis in saline and alkaline environments are reported in Table S1

 Table S1: Genes involved in bacterial homeostasis in saline and alkaline environments annotated in

 Thioalkalivibrio sp. 10fs10 genome. IMG-ER and Rast identifiers are reported

| | | IMG identifier | Rast identifier | Product name |
|--------------------|-----------------|-------------------|-----------------------------|---------------------|
| osmolyte synthesis | | | | Sarcosine/ |
| | | | | dimethylglycine |
| | Glycine/betaine | Ga0265412_1001641 | fig 66666666.276697.peg.648 | N-methyltransferase |
| | synthesis | | | Glycine/ |
| | | | | sarcosine |
| | | Ga0265412_1001642 | fig 66666666.276697.peg.649 | N-methyltransferase |
| | | | | Sucrose-phosphate |
| | | Ga0265412_1001186 | fig 6666666.276697.peg.199 | synthase. |

| | Sucrose/sucrose- | | | |
|--------|-------------------|--------------------|------------------------------|----------------------|
| | phosphate | | | |
| | synthesis | Ga0265412_10012349 | fig 66666666.276697.peg.2296 | Sucrose synthase |
| | | Ga0265412_1001303 | fig 66666666.276697.peg.316 | Dxs |
| | | Ga0265412_1001626 | fig 66666666.276697.peg.632 | IspC/Dxr |
| | | Ga0265412_1001647 | fig 66666666.276697.peg.655 | IspD |
| | | Ga0265412_100157 | fig 66666666.276697.peg.71 | IspE |
| | | Ga0265412_1001648 | fig 66666666.276697.peg.656 | IspF |
| | Squalene | Ga0265412_1001803 | fig 66666666.276697.peg.802 | IspG |
| | synthesis | Ga0265412_10012613 | fig 66666666.276697.peg.2563 | IspH |
| | | | | Farnesyl diphosphate |
| | | Ga0265412_1001304 | fig 66666666.276697.peg.317 | synthase (GTT) |
| nent | | Ga0265412_10011010 | fig 66666666.276697.peg.1007 | HpnC |
| rcen | | Ga0265412_10011009 | fig 66666666.276697.peg.1006 | HpnD |
| info | | Ga0265412_10011008 | fig 66666666.276697.peg.1005 | HpnE |
| all re | | | | Phosphatidyl- |
| ll wa | | | | glycerolphosphate |
| ce | | Ga0265412_10011218 | fig 66666666.276697.peg.1200 | synthase |
| | | | | Phosphatidyl- |
| | | | | glycerolphosphate |
| | Cardiolipin | Ga0265412_10011563 | fig 66666666.276697.peg.1543 | synthase |
| | synthesis | | | Phosphatidyl- |
| | | | | glycerophosphatase |
| | | Ga0265412_10011050 | fig 66666666.276697.peg.1048 | А |
| | | Ga0265412_10011279 | fig 66666666.276697.peg.1261 | Cardiolipin synthase |
| | | Ga0265412_10011784 | fig 6666666.276697.peg.1748 | Cardiolipin synthase |
| | | Ga0265412_10011846 | fig 6666666.276697.peg.1810 | Cardiolipin synthase |
| | | Ga0265412_10011966 | fig 6666666.276697.peg.1927 | RnfA |
| | | Ga0265412_10011967 | fig 66666666.276697.peg.1928 | RnfB |
| | | Ga0265412_10011968 | fig 66666666.276697.peg.1929 | RnfC |
| | Na⁺-dependent | Ga0265412_10011969 | fig 66666666.276697.peg.1930 | RnfD |
| | NADH: quinone | Ga0265412_10011970 | fig 66666666.276697.peg.1931 | RnfG |
| sd | oxidoreductase (2 | Ga0265412_10011971 | fig 66666666.276697.peg.1932 | RnfE |
| und | complete | Ga0265412_10012911 | fig 66666666.276697.peg.2848 | RnfA |
| ary | operons) | Ga0265412_10012912 | fig 66666666.276697.peg.2849 | RnfB |
| prim | - | Ga0265412_10012913 | fig 66666666.276697.peg.2850 | RnfC |
| d | | Ga0265412_10012914 | fig 66666666.276697.peg.2851 | RnfD |
| | | Ga0265412_10012915 | fig 66666666.276697.peg.2852 | RnfG |
| | | Ga0265412_10012916 | fig 66666666.276697.peg.2853 | RnfE |
| | NADH | Ga0265412_1001451 | fig 66666666.276697.peg.460 | NuoA |
| | dehydrogenase | Ga0265412_1001452 | fig 66666666.276697.peg.461 | NuoB |
| | ucity arogenase | Ga0265412_1001453 | fig 66666666.276697.peg.462 | NuoC |
| | | | 2 | |

| | (NDH-1) primary | Ga0265412_1001454 | fig 66666666.276697.peg.463 | NuoD |
|-----------------|-----------------|--------------------|------------------------------|-----------|
| | proton pump | Ga0265412_1001455 | fig 66666666.276697.peg.464 | NuoE |
| | | Ga0265412_1001456 | fig 66666666.276697.peg.465 | NuoF |
| | | Ga0265412_1001457 | fig 6666666.276697.peg.466 | NuoG |
| | | Ga0265412_1001458 | fig 6666666.276697.peg.467 | NuoH |
| | | Ga0265412_1001459 | fig 6666666.276697.peg.468 | NuoI |
| | | Ga0265412_1001460 | fig 66666666.276697.peg.469 | NuoJ |
| | | Ga0265412_1001461 | fig 66666666.276697.peg.470 | NuoK |
| | | Ga0265412_1001462 | fig 66666666.276697.peg.471 | NuoL |
| | | Ga0265412_1001463 | fig 66666666.276697.peg.472 | NuoM |
| | | Ga0265412_1001464 | fig 66666666.276697.peg.473 | NuoN |
| | | Ga0265412_10011858 | fig 66666666.276697.peg.1826 | MrpG |
| secondary pumps | | Ga0265412_10011859 | fig 66666666.276697.peg.1825 | MrpF |
| | Mrp sodium | Ga0265412_10011860 | fig 66666666.276697.peg.1824 | MrpE |
| | /proton | Ga0265412_10011861 | fig 6666666.276697.peg.1823 | MrpD |
| | complex | Ga0265412_10011862 | fig 6666666.276697.peg.1822 | MrpC |
| | complex | Ga0265412_10011863 | fig 6666666.276697.peg.1821 | MrpB |
| | | Ga0265412_10011864 | fig 6666666.276697.peg.1820 | MrpA |
| | sodium/proton | Ga0265412_10011856 | fig 66666666.276697.peg.1818 | ArsB/NhaD |
| | antiporter NhaD | | | |
| | sulphate- | | | |
| | dependent | Ga0265412_10045 | fig 6666666 076607 mag 2001 | C11D |
| | bicarbonate | | 11g+0000000.270057.peg.3021 | Juli |
| | antiporter | | | |

Taxonomic characterisation of the Thioalkalivibrio sp. 10fs10

The full-length gene coding for the 16S rRNA of *Thioalkalivibrio* sp. 10fs10 isolate was extracted from draft genome. The sequences of the genes coding for the 16S rRNA of 77 different *Thioalkalivibrio* sp. strains were downloaded from Silva SSU database and from Joint Genome Institute (JGI) using IMG. All sequences were aligned using MUSCLE algorithm (gap open penalty -400.00, gap extension 0.0, UPGMA clustering method, 16 iterations), and trimmed to match the length of the shortest deposited sequence (1374 nucleotides). Maximum likelihood tree was computed using Tamura-Nei DNA evolutionary model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, +G = 0.4053 with evolutionary invariable 150 sites (+I =58.8102 %). Initial tree(s) for the heuristic search were obtained by applying NeighborIn review Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Highest log likelihood obtained was -5299.2315 and the accuracy of calculated tree was assessed by bootstrapping 1000 replicates.

Maximum likelihood tree based on Multi-Locus sequence (MLS) analysis

Maximum Likelihood MLS tree was computed using concatenated amino acid sequences of ClpA-DnaJ-GyrA-RpoH-RpoS-SecF proteins, codified by respective housekeeping genes obtained from genomic annotation of *Thioalkalivibrio* sp. 10fs10 and from available complete genomes of all *Thioalkalivibrio* spp. on IMG/M. DNA sequences of the aforementioned housekeeping genes were translated using genetic codes of table 11 (NCBI). The corresponding sequences of amino acids were aligned with MUSCLE algorithm (gap open penalty -400.00, gap extension 0.0, UPGMA clustering method, 16 iterations), equally trimmed to a final total length of 3158 amino acids and concatenated in the reported order. Maximum likelihood tree was computed using a method based on the Whelan and Goldman + Freq. model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.5336). The rate variation model allowed for some sites to be evolutionarily invariable sites (+I = 7.08 %). Initial tree(s) for the heuristic search were obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. Highest log likelihood obtained was -98651.57 and the accuracy of calculated tree was assessed by bootstrapping of 1000 replicates.

Classification of Thioalkalivibrio sp. 10fs10 by, Genome Taxonomy Database

Computation of Maximum likelihood tree, used for taxonomical classification as Genome Taxonomy Database protocol, was performed using a method based on the Whelan and Goldman + Freq. model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.7814). The rate variation model allowed for some sites to be evolutionarily invariable sites (+I = 9.02 %). Initial tree(s) for the heuristic search were obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, successively selecting the topology with superior log likelihood value. Highest log likelihood obtained was -68671.60 and the accuracy of calculated tree was assessed by bootstrapping of 500 replicates.

References of supplementary Materials and Methods

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