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ABSTRACT

Two heterotrophic bacteroidetes strains were isolated as satellites from autotrophic enrichments inoculated with samples from hypersaline soda lakes in southwestern Siberia. Strain Z-1702^T is an obligate anaerobic fermentative saccharolytic bacterium from an iron-reducing enrichment culture, while *Ca.* Cyclonatronum proteinivorum Omega^T is an obligate aerobic proteolytic microorganism from a cyanobacterial enrichment. Cells of isolated bacteria are characterized by highly variable morphology. Both strains are chloride-independent moderate salt-tolerant obligate alkaliphiles and mesophiles. Strain Z-1702^T ferments glucose, maltose, fructose, mannose, sorbose, galactose, cellobiose, *N*-acetyl-glucosamine and alpha-glucans, including starch, glycogen, dextrin, and pullulan. Strain Omega^T is strictly proteolytic utilizing a range of proteins and peptones. The main polar lipid fatty acid in both strains is iso-$C_{15:0}$, while other major components are various C_{16} and C_{17} isomers. According to pairwise sequence alignments using BLAST *Gracilimonas* was the nearest cultured relative to both strains (< 90% of 16S rRNA gene sequence identity). Phylogenetic analysis placed strain Z-1702^T and strain Omega^T as two different genera in a deep-branching clade of the new family level within the order *Balneolales* with genus. Based on physiological characteristics and phylogenetic position of strain Z-1702^T it was proposed to represent a novel genus and species *Natronogracilivirga saccharolityca* gen. nov., sp. nov. (= DSMZ 109061^T = JCM 32930^T = VKM B 3262^T). Furthermore, phylogenetic and phenotypic parameters of *N. saccharolityca* and *C. proteinivorum* gen. nov., sp. nov., strain Omega^T (= JCM 31662^T, = UNIQEM U979^T), make it possible to include them into a new family with a proposed designation *Cyclonatronaceae* fam. nov..

Introduction

Soda lakes are a special type of inland salt lakes located in the semi-arid or arid climatic zones and characterized by high (≥9.0) pH caused by the presence of carbonate and bicarbonate ions and often by high mineralization resulting in extremely high alkaline buffering capacity. Intensive study of soda lakes performed during the last 30 years resulted in understanding that despite extreme parameters they characterized by high level of biomass production and active biogeochemical carbon, nitrogen, sulfur and iron cycling caused by the activity of functionally and phylogenetically diverse microorganisms, mainly represented by prokaryotes (last reviewed: (Antony et al., 2013; Sorokin, 2017; Sorokin et al., 2015; Sorokin et al., 2014), (Gracheva et al., 2017; Zavarzina et al., 2006; Zavarzina et al., 2020; Zhilina et al., 2015). Currently, a picture of trophic interactions in the haloalkaliphilic environments is fairly complete and the main actors and their trophic interactions are discovered. Particularly important role in mineralization of autochthonous organic matter is played by organoheterotrophic microorganisms, which include both aerobic and anaerobic bacteria and archaea.

The dominant primary producers of organic matter in Siberian saline soda lakes belong to haloalkaliphilic oxygenic benthic filamentous

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cyanobacteria of the genera *Nodosilinea* and *Geitlerinema* (Samylina et al., 2014). Their cell wall is rich in proteins and polysaccharides, and among all others this capacity is most characteristic of the filamentous diazotrophic species (Vargas et al., 1998). The aerobic haloalkaliphilic protein-utilizing strain Omega^T (*Balneolales*), that consistently developed as a heterotrophic satellite in several enrichment cultures of haloalkaliphilic filamentous cyanobacteria obtained from a soda lake in southwestern Siberia has previously been described as *Candidatus* Cyclonatronum proteinivorum with the focus on its metabolic capacities, revealed by cultivation and genomic analysis (Sorokin et al., 2018). Here we add some additional phenotypic and chemotaxonomy characteristics for this species to finalize its formal taxonomy description.

Carbohydrate-fermenting anaerobes are an essential primary step in the mineralization of organic matter by anaerobic microbial communities. In soda lakes, several alkaliphilic carbohydrate-utilizing anaerobic organotrophs were described (Garnova et al., 2004; Garnova et al., 2003; Hoover et al., 2003; Pikuta et al., 2009; Vishnuvardhan Reddy et al., 2013; Zavarzina et al., 2009; Zhilina et al., 2004; Zhilina et al., 2001; Zhilina et al., 2001; Zhilina et al., 1996). Some of them are capable of hydrolyzing partially soluble and insoluble polysaccharides (Sorokin et al., 2011; Sorokin et al., 2012; Zhilina et al., 2005; Zhilina et al., 2004). The others incapable of hydrolyzing polymeric substrates frequently act as satellites of polysaccharide-degrading co-habitants or participate in cell degradation of chemotrophic prokaryotes consuming mono- and oligomeric depolymerization products.

In this work we describe an alkaliphilic, anaerobic carbohydrate-fermenting bacterium, strain Z-1702^T, as a representative of the novel genus and species *Natronogracilivirga saccharolityca* gen. nov., sp. nov.. Together with the previously described proteolytic *Isachenkonia alkalipeptolitica* (Zavarzina et al., 2020) *N. saccharolityca* acted as an oligo-heterotrophic satellite in lithoautotrophic iron-reducing culture. Furthermore, a taxomic description of *Ca.* Cyclonatronum proteinivorum, strain Omega^T (Sorokin et al., 2018) is provided. *N. saccharolityca* and its closest relative *Ca.* Cyclonatronum proteinivorum are forming a deep-branching family-level lineage within the order *Balneolales* for which the name *Cyclonatronaceae* fam. nov. is proposed.

Materials and methods

Sampling and isolation

Strain Omega^T. A description of sampling and isolation of strain Omega^T was given previously (Sorokin et al., 2018). Here we briefly describe some details in isolation procedure, its main phenotypic properties as well as some important taxonomic features that were not included in the previous publication. Strain Omega^T was a dominant satellite in a mixed culture of filamentous cyanobacteria enriched from a biofilm on the surface of littoral sediments of the soda lake Bitter-3 (Altai region, Russia; N51°40′/E79°53′). It was cultivated on a solid medium using cell extract prepared from the same cyanobacterial biomass as the substrate, and isolated from a single, red-colored colony after several rounds of transfers onto solid aerobic sodium carbonate-based mineral medium (pH 10.0 and 1.2 M total Na^+). It was deposited in JCM (Japan Collection of Microorganisms) under the number JCM 31662^T, and in UNIQEM (Collection of Unique and Extremophilic Microorganisms) under the number U979^T.

Strain Z-1702^T. Soda lake Tanatar III (Altay Region, Russia, N51°37 29 /E 79 °48 28) sediment samples were collected by Dr. V. V. Kevbrin in June 2007, transported to the laboratory in sterile 100 ml vials and stored at 4 °C. At the time and location of sampling salinity and pH of the water–sediment slurry were 8.6 % (refractometer Atago); pH 10.3 (pH meter Hanna HI 991001), respectively. The inoculum, 10 % (v/v), was added to 15-ml Hungate tubes filled with 10 ml medium A used for cultivation of soda lake dissimilatory iron-reducing bacteria (g l^{-1}): NH_4Cl, 0.5; KH_2PO_4, 0.2; $MgCl_2{\cdot}6H_2O$, 0.1; $CaCl_2{\cdot}2H_2O$, 0.02; KCl 0.2; yeast extract (Difco), 0.05; Na_2CO_3, 68.0; $NaHCO_3$, 38.0; NaCl, 60.0; 1 ml of trace element solution (Kevbrin and Zavarzin, 1992); sodium formate, 2.0; ferrihydrite, synthesized as described previously (Zavarzina et al., 2006), 50 mmol Fe(III) l^{-1}; pH 9.5. The head space (5 ml) was filled with N_2 at atmospheric pressure. The tubes were autoclaved at 120 °C for 30 min. Inoculated tubes were incubated at 35 °C for 20 days.

After several transfers into the same medium, a single morphotype of short motile rods was observed. However, when this “pure culture” was transferred into organic-rich medium (sodium formate and ferrihydrite were replaced with 2 g l^{-1} of yeast extract or glucose) to reveal its purity, completely different uniform rods have grown. This peptolytic satellite developed with yeast extract was specified as recently described *Isachenkonia alkalipeptolitica* in *Clostridiaceae* (Firmicutes) (Zavarzina et al., 2020). In turn, carbohydrate-fermenting rods outnumbered the *I. alkalipeptolitica* when the culture cultivated on glucose. This enrichment was used for a pure culture isolation using dilution to extinction technique. The highest positive dilution (10^{-9}) was used for isolation of colonies in roll-tubes with the medium A contained glucose (2 g l^{-1}), and agar (Nobel, Difco) 30 g l^{-1}. For solid medium preparation a concentrated solution of carbonates was autoclaved separately and added to the medium before inoculation to a final concentration 2 M Na^+. The colonies that appeared in agar roll-tubes after 7 days of incubation at 35 °C were light yellow, fine-grained, isometric, 1.0–0.1 mm in diameter. Single colonies were picked with sterile glass capillaries and inoculated into the tubes with liquid basal medium, containing glucose (2 g l^{-1}). One of the isolates designated as strain Z-1702^T was selected for the detailed characterization. The purity of strain Z-1702^T was verified by genome sequencing and assembly.

The medium B optimized after pH and sodium carbonate growth tests (see below) and used for further cultivation and for substrate, biochemical, and physiological tests included (g l^{-1}): KH_2PO_4 0.2; $MgCl_2{\cdot}6H_2O$ 0.1; $CaCl_2{\cdot}2H_2O$ 0.02 g; Na_2CO_3 10; $NaHCO_3$ 60; NaCl 30; yeast extract (Difco) 0.05 or vitamin solution (Wolin et al., 1963); trace element solution, 1 ml l^{-1} $Na_2S{\cdot}9H_2O$ 0.5. After autoclaving, the pH was 8.7. For routine transfers glucose (2 g l^{-1}) was added as the substrate.

Strain Z-1702^T was deposited in DSMZ (German Collection of Microorganisms and Cell Cultures) under the number DSMZ 109061^T, JCM (Japan Collection of Microorganisms) under the number JCM 32930^T, and in VKM (All-Russian Collection of Microorganisms) under the number B-3262^T.

Phenotypic characterization

Cell morphology of strain Z-1702^T was examined under a 1000 × phase-contrast light microscope Zetopan (Reichert). Ultrathin sections cell sections were visualized using a JEM-100 transmission electron microscope (JEOL) as described previously (Zhilina et al., 1996). Bacterial growth in all physiological tests was monitored by direct cell counting under a phase-contrast microscope or by measuring OD_{600} in Hungate tubes put into a Unico 2100 spectrophotometer (USA). All experiments were done in two replicates.

The effect of temperature, pH and salinity on growth of strain Z-1702^T was analyzed in medium B with glucose. The temperature supporting growth was examined between 10 and 50 °C with intervals 3–5 °C. For the pH range, the basic minimal medium containing $NaHCO_3$ (10 g l^{-1}) and 140 g l^{-1} of NaCl was titrated to the necessary pH values with 6 M HCl or 12 M NaOH. The tubes with the medium were prepared in triplicates: two for cultivation and one for pH measuring performed after the autoclaving. Actual pH was monitored until the maximum OD values were reached. The pH level was examined between 7.0 and 10.7 with intervals 0.25–0.5 units (0.25 - close to extremes). Carbonates range was tested in the presence of 50 mM of

TABS buffer at pH 9.0 with carbonates replaced with equimolar amounts of Cl^- or SO_4^{2-} to maintain the same level of total anion concentrations. Salinity was examined between 0.1 and 3.75 M $Na^{\pm}$ with intervals 0.1–0.25 M total $Na^{\pm}$ (as carbonates) (0.1 - close to extremes). Oxygen sensitivity of strain Z-1702^T was tested in aerobic medium B, in the presence of 10 and 2.0 % (v/v) of O_2 in the gas phase or in anaerobic medium B, without addition of sodium sulfide. Catalase activity was determined by appearing of O_2 bubbles during treatment of the cells pellet with 3 % (v/v) H_2O_2 solution.

Strain Z-1702^T growth substrates were determined on the medium B at optimal growth conditions (37 °C, pH 8.7) with addition of 1 ml l^{-1} vitamins solution. All organic substrates (peptides, carbohydrates, alcohols and organic acids) were sterilized by filtration (0.2 μm, Millipore) and added to a final concentration of 0.3 % (w/v or v/v). Polymers (starch, pectin, xylan, pullulan, gelatin) were autoclaved as concentrated solutions in 2 M NaCl and added to the NaCl-free medium B before inoculation. The substrate was regarded as positive when the growth yield was significantly higher than in control medium (without substrate) after three consecutive transfers on the medium with the same substrate. The following substrates were tested as sole carbon and energy sources: simple carbohydrates (arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, ribose, sorbose, sucrose, trehalose, ramnose, ribose, xylose, *N*-acetyl-D-glucosamine,), polysaccharides (agarose, sodium alginate, amorphous cellulose, carboxymethilcellulose, cellulose (Avicel), α-cellulose, chitin, chitosan, dextrin (from potato and maize), dextran, galactan, glycogen, laminarin, mannan, starch, pectin, pullulan, xanthan gum, xylan), alcohols (ethanol, glycerol, inositol, mannitol, methanol), organic acids (fumarate, malate, pyruvate, succinate), peptides (heat-sterilized casein, bovine serum albumin, α- and β-keratin, gelatin, peptone, yeast extract, casamino acids) and biomass of *Spirulina* and *Methylococcus capsulatus* (gaprin).

The gaseous products of glucose fermentation were determined by gas chromatography Crystal 5000.2 (Chromatech, Russia) equipped with a TCD detector and a column (1 m) with a Molecular sieve of 5A and argon as a carrier gas. The soluble products in culture supernatant were acidified to pH 2 and analyzed in a Stayer HPLC (Aquilon, Russia) equipped with a refractometric detector (Knauer, Germany) and an Aminex HPX-87H column (Bio-Rad, USA), operated isocratically with 5 mM H_2SO_4 as eluent at 0.6 ml/min.

The capacity for anaerobic respiration of strain Z-1702^T with the following electron acceptors was studied on the medium B containing 2 g l^{-1} of starch: synthetized ferrihydrite (50 mM of Fe(III)), S^0 (1 g w/v), SO_3^{2-} (20 mM), $S_2O_3^{2-}$ (10 mM), SO_4^{2-} (20 mM), NO_3^- (20 mM), NO_2^- (10 mM), AsO_4^{3-} (5 mM), crotonate (10 mM), fumarate (10 mM). Reduction products were determined by measuring sulfide (Trüper and Schlegel, 1964) in case of growth with sulfur compounds as the acceptors; Fe(II) with ferrozine (Stookey, 1970) in case of synthetized ferrihydrite.

The ability of strain Z-1702^T to grow oligotrophically was tested by direct cell count under a phase-contrast microscope on medium B containing glucose in concentrations ranging from 0.01 to 0.1 (g l^{-1}). In the case of positive growth, the cultures were consequently transferred on the same medium at least three times to ensure the inoculum contents were not used as the growth substrate.

Antibiotic sensitivity was tested at optimal growth conditions with glucose as the substrate. The following (0.22 mμ filter-sterilized) antibiotics were tested at concentrations 25 and 50 mg l^{-1} (the latter in case of positive growth at 25 mg l^{-1}): ampicillin, bacitracin, chloramphenicol, cycloserine, erythromycin, gentamycin, kanamycin, neomycin, novobiocin, penicillin, polymyxin B, rifampicin, streptomycin and tetracycline.

For the membrane lipid fatty acid analysis, the cells of strain Omega^T were grown at 1 M total Na^+, pH 10 and 30 °C until the stationary growth phase was reached. The cells were harvested by centrifugation, washed once in 1 M NaCl and freeze dried. The cells of strain Z-1702^T, were grown at optimal conditions on medium B until the stationary growth phase was reached. The membrane lipid fatty acid composition of strains Omega^T and Z-1702^T was performed as described previously (Sorokin et al., 2012; Zavarzina et al., 2020). Respiratory lipoquinones of both strains were extracted by cold acetone and after raw separation by TLC a major UV-absorbing band was eluted and subjected to tandem mass spectrometry (LCG Advantage Max) with chemical ionization at atmospheric pressure. The quinones were identified by ionic mass after HPLC-MS.

Genome sequencing, assembly, and annotation

Genomic DNA was extracted with QIAamp DNA Mini Kit (Qiagen, Germany), according to manufacturer's instructions and fragmented on Bioruptor® sonication device (Diagenode, Belgium) to obtain fragments in the range of 400–600 bp. Illumina-compatible fragment genomic library of strain Z-1702^T was prepared from 100 ng of fragmented and size-selected genomic DNA with NEBNext® Ultra™ II DNA library preparation kit (New England Biolabs, USA) according to the manufacturer's instructions. Library was sequenced using an Illumina Miseq™ platform, using 2 × 150 paired end read chemistry. Quality filtering and trimming of sequencing adapters was performed with CLC Genomic Workbench 20.0.4 (Qiagen, Germany). Finally, 474,121 read pairs were used for *de novo* assembly with Spades assembler version 3.11.0 (Bankevich et al., 2012). Resulting assembly was of 3750541 bp in length and consisted of 50 scaffolds with N50 value of 263077 bp. Average assembly coverage was 39x. Analysis of assembly quality was performed with CheckM package (Parks et al., 2018).

Genome annotation

Genome annotation was performed during submission into NCBI by Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016). Analysis of mobile genetic elements was performed by ISSaga (Varani et al., 2011). Analysis of orthologous genes was performed with ProteinOrtho orthology prediction tool (Lechner et al., 2014) with default settings (max e-value 0.00001; minimal coverage 50 %). Pairwise AAI values were calculated using AAI calculator (Rodriguez-R and Konstantinidis, 2016). Clusters of orthologous genes (COGs) was assigned to proteins using eggNOG-mapper web server using default parameters (Cantalapiedra et al., 2021). CAZymes were searched with dbCAN2 (Zhang et al., 2018) using HMMER (Mistry et al., 2013) and DIAMOND (Buchfink et al., 2015). Signal peptides of secreted proteins and transmembrane domains were predicted using SignalP (Almagro Armenteros et al., 2019); and TMHMM (Le and Gascuel, 2008). Manual curation of protein function prediction was conducted using Blast (Altschul et al., 1997) and HHMMER (phmmer and hmmscan) packages (Potter et al., 2018) and UniprotKB (TrEMBL and Swiss-Prot) databases.

Data availability

Annotated draft genome sequence of strain Z-1702^T is available in DBJ/EMBL/GenBank under the PRJNA702628, JAFIDN01, GCF_017921895.1 for Bioproject, WGS and RefSeq assembly accession numbers.

Phylogenetic analysis

16S rRNA gene sequences of all type strains of validly published *Balneolales* representatives, two published but yet not passed the validation procedure *Balneolales* representatives, "*Ca*. Cyclonatronum proteinivorum", and the type strain of the *Rhodothermus marinus* DSM 4252^T (as an outgroup) were retrieved from the GenBank. Together

with the Z-1702^T 16S rRNA gene sequence, retrieved from the genome annotation, the total number of analyzed sequences was 23. The sequences were aligned using MAFFT v. 7 (L-INS-i algorithm, (Katoh et al., 2019). All positions with less than 95 % site coverage were eliminated. Phylogenetic tree was inferred in MEGA 10 (Kumar et al., 2018) using the General Time Reversible (GTR) model (G + I, 4 categories) with 1000 bootstrap replications.

For phylogenomic analysis the genomes of validly published *Balneolales* representatives as well as two currently non-valid representatives including "*Ca.* Cyclonatronum proteinivorum" were retrieved from the Genbank. Additionally, seven high quality (completeness > 90 %, contamination < 5 %) metagenome assembled genomes (MAGs) of the uncultured microorganisms, closely related to the "*Ca.* Cyclonatronum proteinivorum" and strain Z-1702^T were downloaded from the Genbank. Together with the Z-1702^T genome sequence the total number of analyzed sequences was 22. The bac120 set of conserved protein sequences, encoded by the genomes were identified and aligned with the GTDB-tk v.1.7.0 [32,]. Phylogeneitc analysis was performed in MEGA X (Kumar et al., 2018) using LG model (G + I, 5 categories, +F) (Le and Gascuel, 2008) and 100 bootstrap replications. All positions with less than 95 % site coverage were eliminated.

Results and discussion

Phenotypic properties of strain Omega^T

Strain Omega^T cells were nonmotile, with cell morphology from circular (diameter ~ 2.5–3 μm) to long flexible rods-spirilla up to 15 μm long, and 0.5 μm in width depending on salinity and substrates used (Fig. 1) (Sorokin et al., 2018). The cell wall was of the Gram-negative type. The biomass of the grown cells was pink-orange due to a presence of carotenoids. The absorption maxima of the methanol-acetone extract of the cells were at 450, 479 and 550 nm. The isolate also produces a functional sodium-pumping proteorhodopsin (Sorokin et al., 2018), which might serve as an additional membrane-energizing complex at natron-alkaline conditions.

Growth of strain Omega^T occurred at temperatures ranging from 25 to 45 °C (optimum at 33–35 °C), the pH (35 °C) from 8.5 to 10.5 (optimum at pH 9.5–10.0) and the total $Na^{\pm}$ concentration from 0.3 to 2.25 M (optimum at 0.6–1.0 M). The isolate is an obligate aerobe: anaerobic growth with casein peptone in the presence of NO_3^-, NO_2^-, S^0, $S_2O_3^{2-}$ and fumarate was not observed. It is an organoheterotroph specialized on utilization of various proteinaceous substrates growing best with peptone from casein, but also other peptones and yeast extract. Proteolytic growth was observed with heat-sterilized casein, bovine serum albumin and gelatin. Carbohydrates - galactose, glucose, cellobiose, fructose, lactose, maltose, mannose, sucrose, trehalose, xylose, and sugar polymers such as alginate, amorphous cellulose, chitin, starch, pectin did not support growth either aerobically or anaerobically (Table 1).

The polar lipid fatty acid (PLFA) profile of Omega^T was dominated (in order of abundance) by *iso*-$C_{15:0}$ (24.2) and anteiso-$C_{16:1\omega7c}$ (15.6), with *iso*-C_{16} (7.9), $C_{16:0}$ (7.8), anteiso-$C_{17:1\omega8}$ (6.6) and C_{17} (6.5) as subdominants (Suppl. Table 1). Surprisingly, strain Omega^T lacks 3-OH-*iso*-$C_{17:0}$ and anteiso-$C_{17:0}$ in the profile which are considered as markers of the *Bacteroidota sensu stricto* (Hahnke et al., 2003), possibly due to alkaliphilic nature of this bacterium or great phylogenetical distance between *Balneolales* and the other bacteroidetes. The major membrane respiratory lipoquinon identified in strain Omega^T was MK-7.

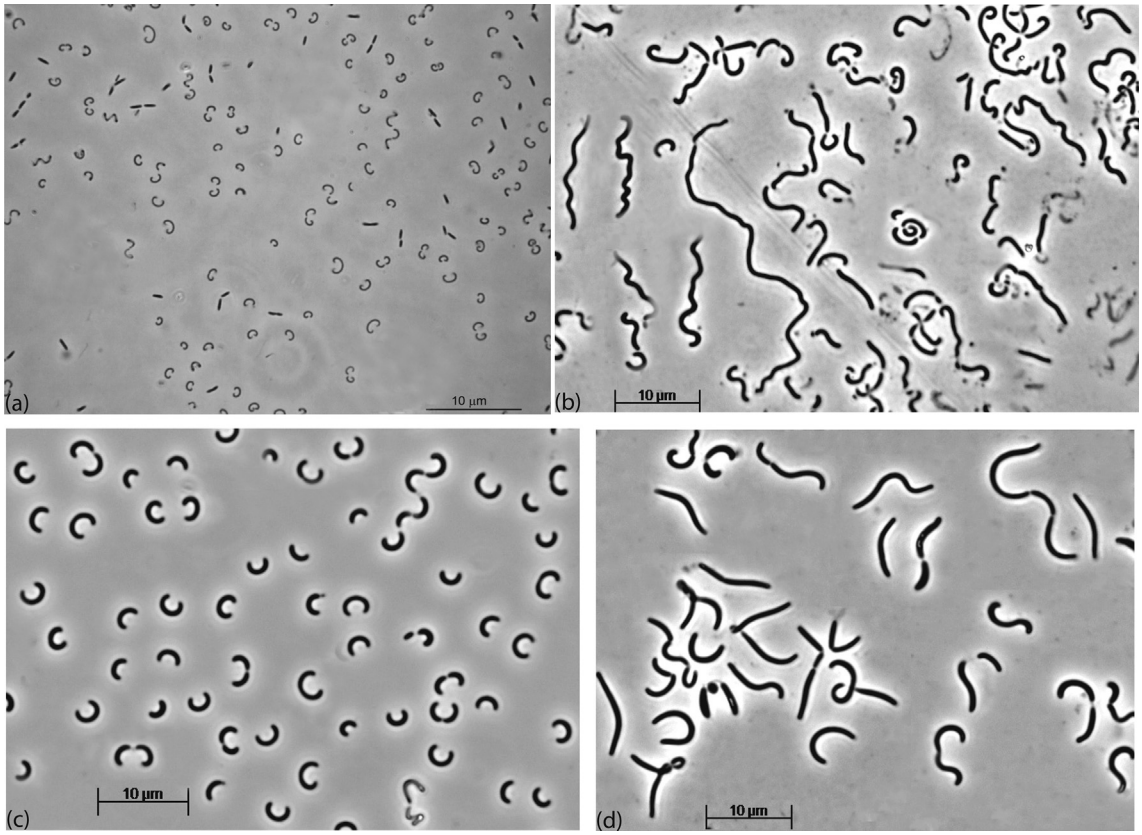

Fig. 1. Phase contrast microphotographs showing variable cell morphology of strain Omega^T grown at pH 10.0 depending on substrate and salinity. (a) in a mixed culture with oxygenic photorophic enrichment from a soda lake (1 M total Na^+); (b) cells from a colony developed on haloalkaline agar with cyanobacterial cell extract as substrate (1 M total Na^+); (c-d) in liquid culture with bovine serum albumin (2 M and 1 M total Na^+, respectively).

Table 1
Comparison of the novel genera *Natronogracilivirga* and *Cyclonatronum* with the type species of other genera of *Balneolales*. 1 – strain Z-1702^T (this work); 2 – strain Omega^T (this work and (Sorokin et al., 2018); 3-*Balneola vulgaris* (Urios et al., 2006); 4 – *Gracilimonas tropica* (Cho et al., 2013; Choi et al., 2009); 5 - *Fodinibius salinus* (Wang et al., 2012); 6 - *Aliifodinibius roseus* (Wang et al., 2013); 7 - *Rhodohalobacter halophilus* (Xia et al., 2017; Han et al., 2018); 8 - *Soortia roseihalophila* (Amoozegar et al., 2017).

Characteristic	1	2	3	4	5	6	7	8
Isolation source	Sediments of soda lake Tanatar III, Altai region, Russia	Cyanobacterial biofilm from soda lake Bitter-3, Altai region, Russia	Water column in the bay of Bay of Banyuls, France	*Synechococcus* culture established from surface water of the tropical Pacific Ocean	Salt mine in Yunnan, south-western China	Salt mines in Yunnan, south-western China	Saltern near the city of Feicheng, China	Water sample from Badab-Soort travertine spring in North of Iran
Morphology	Cell morphology varied depending on substrate from thin short flexible rods 0.15–0.2 μm in width 0.5–4 μm in length to long optical dense flexible rods 0.3–0.5 μm in width 5.0–10.0 μm in length	Cell morphology varied depending on substrate and salinity, from semicircles to spiral or whip-like philaments	Rods 0.2 ± 0.06 μm in wide 2.5 ± 0.4 μm in length and	Long irregular rods of 0.2–0.3 μm in width and 6.5–17.7 μm in length	Rods 0.3 μm in width 1.0–3.0 μm in length and	Rods 0.3 μm in width 0.4–0.7 μm in length	Rods 0.3–0.6 μm in width 2.5–16.5 μm in length	Rods curved, spirillum, coccoid and crescentus forms 0.3–0.4 μm in width 1.5–2.5 μm in length
Motility	+	−	+	−	−	−	−	+
Spore formation	−	−	−	+	−	−	−	−
Colony color	cream	reddish	orange	orange	pink	rose red	reddish	orange
Biomass color	reddish	reddish	orange	orange	pink	rose red	reddish	nd
Gram-negative cell wall structure	+	+	+	+	+	+	+	+
Temperature range/ optimum, °C	20/37–40/43	25/33–35/45	10/30/40	20/35/40	25/37/45	10/28/42	20/40/50	28/30/34
pH range/ optimum	7.5/8.7–9.0/10.2	8.5/9.5–10.0/10.5	5.0/8.0/10.0	6.0/7.0–8.0/10.0	6.0/7.5–8.5/ 9.0	6.5/7.0/8.0	7.0/7.5–8.5/9.0	6.0/7.0/8.0
Na^+ range/ optimum, M	0.6/1.8/1.2	0.3/0.6–1/2.25	nd	nd	nd	nd	nd	nd
NaCl	requirement	−	+	−	−	+	+	+
+								
Anaerobic	(facultatively anaerobic +/-)	+	−	−	+/-	−	+/-	+/-
−								
Catalase	+	+	+	+	+	+	+	−
Growth	**substrates**							
Cellobiose	+	−	nd	nd	nd	nd	nd	+
Galactose	+	−	−	−	+	+	+	+
Glucose	+	−	+	+	+	+	+	+
Maltose	+	−	+	nd	+	+	nd	+
Mannose	+	−	nd	nd	+	+	+	+
Sorbose	+	−	nd	nd	nd	nd	nd	nd
Dextrin	+	nd	nd	nd	nd	+	+	nd
Glycogen	+	nd	nd	nd	nd	−	nd	nd
N-acetyl-D-	glucosamine	+	nd	+	+	nd	nd	+
nd								
Starch	+	−	nd	+	−	−	+	+
Pullulan	+	nd	nd	nd	nd	nd	nd	nd
Peptone	−	+	nd	nd	nd	nd	nd	nd
Yeast extract	−	+	nd	nd	nd	nd	nd	nd
Casein	−	+	nd	nd	−	−	nd	−

(*continued on next page*)

Table 1 (*continued*)

Characteristic	1	2	3	4	5	6	7	8
Bovine serum albumin	–	+	nd	nd	nd	nd	nd	nd
Gelatin	–	+	nd	+	+	+	+	+
Genomic G + C, %	49.7	51.4	39.8	42.9	42.5	48.3	44.4	40.5
Major fatty acids	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$, anteiso-$C_{17:0}$, anteiso-$C_{17:1\omega8}$, *iso*-$C_{17:1\omega8}$, *iso*-$C_{17:0}$, 3-OH-*iso*-$C_{17:0}$	*iso*-C_{15}, anteiso-$C_{16:1\omega7c}$, *iso*-C_{16}, $C_{16:0}$, anteiso-$C_{17:1\omega8}$, C_{17}	*iso*-$C_{15:0}$, 2-OH-*iso*-$C_{15:0}$, $C_{17:1}\omega$ 8c, $C_{15:0}$, $C_{15:1}\omega$ 6c, *iso*-$C_{13:0}$, *iso*-$C_{17:1}$ ω9c	*iso*-$C_{15:0}$, *iso*-$C_{17:1}\omega$9c	*iso*-$C_{17:1}\omega$9c/ 10-methyl-$C_{16:0}$, *iso*-$C_{15:0}$, $C_{16:1}\omega$7c/$C_{16:1}$ ω6c	*iso*-$C_{15:0}$, anteiso-$C_{15:0}$, summed feature 3 ($C1_{6:1\ \omega6c}$ and/or $C_{16:1\omega7c}$), summed feature (*iso*-$C_{17:1\omega9c}$ and/or $C_{16:0\ 10\text{-methyl}}$)	$C_{16:1}\omega$7c and/or *iso*-$C_{15:0}$2-OH;, *iso*-$C_{15:0}$, *iso*-$C_{17:1}$ ω9c, anteiso-$C_{15:0}$, $C_{16:0}$, $C_{14:0}$, $C16:1$ ω5c	*iso*-$C_{15:0}$, *iso*-$C_{13:0}$, anteiso-$C_{17:0}$ and *iso*-C_{14}
Genome size, Mbp	3.75	4.29	2.86	3.83	2.86	5.08	3.12	nd

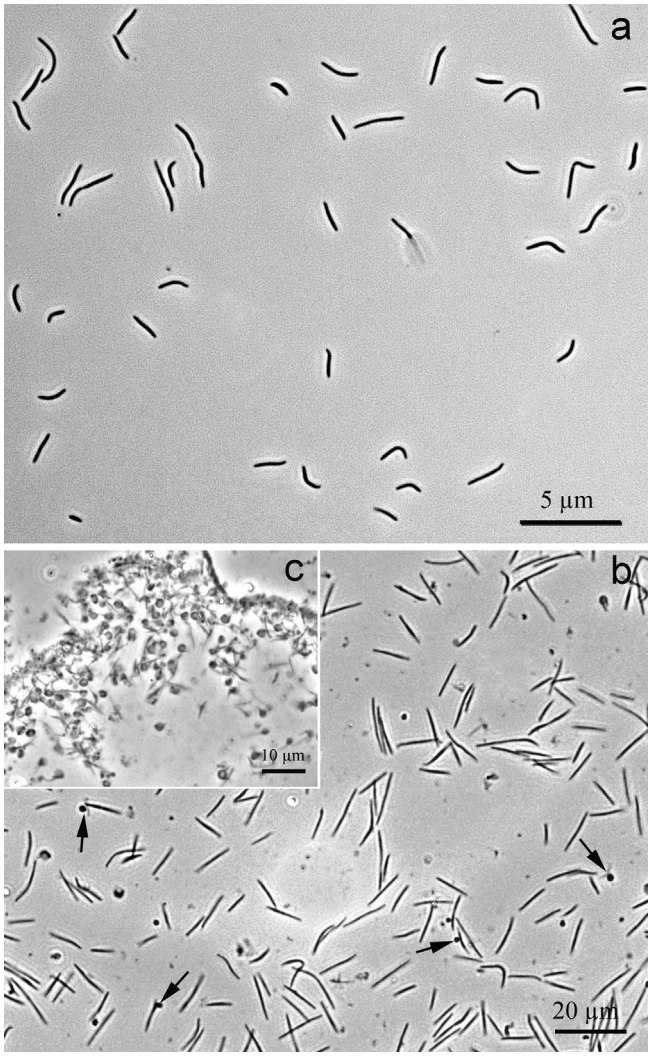

Fig. 2. Micrographs of strain Z-1702^T cells demonstrating (a) morphology on early exponential phase; (b) morphology on late exponential phase formation of dense spherical cells located at the end or side of the cell indicated by arrows; (c) spheroplasts forming on the stationary phase.

Phenotypic properties of strain Z-1702^T

Strain Z-1702^T also has a highly variable cell morphology. At optimal condition at early exponential growth phase cells were thin flexible rods with slightly tapering ends 0.15–0.2 µm in diameter with length varying from 0.5 to 4.0 µm (Fig. 2a). In the late exponential and stationary phases of growth on liquid medium, at suboptimal conditions, or on solid media the cells of strain Z-1702^T became thicker (0.3–0.5 µm) and longer (up to 10.0 µm) (Fig. 2b, Suppl. Fig. 1). Formation of dense spherical mini-cells located at the end or side of the normal cells is characteristic to this strain (Fig. 2b arrows). Formation of sphaeroplasts followed by cell lyzis were common at stationary growth phase (Fig. 2c). Cells were motile by means of peritrichous flagella (Fig. 3a). Spore-forming cells were not observed. Ultrathin sections revealed cell wall of Gram-negative type (Fig. 3b).

Growth of strain Z-1702^T occurred at temperatures ranging from 20 to 43 °C, with an optimum at 37–40 °C. Cells of strain Z-1702^T were not thermostable and did not withstand 80 °C heating for 20 min. The pH (35 °C) range for growth was 7.5–10.2 with an optimum at pH 8.7–9.0. Strain Z-1702^T was obligate natronophile required $Na^{\pm}$ ions in the carbonate form. It did not grow when carbonates were replaced by equimolar amounts of NaCl or Na_2SO_4 in the presence

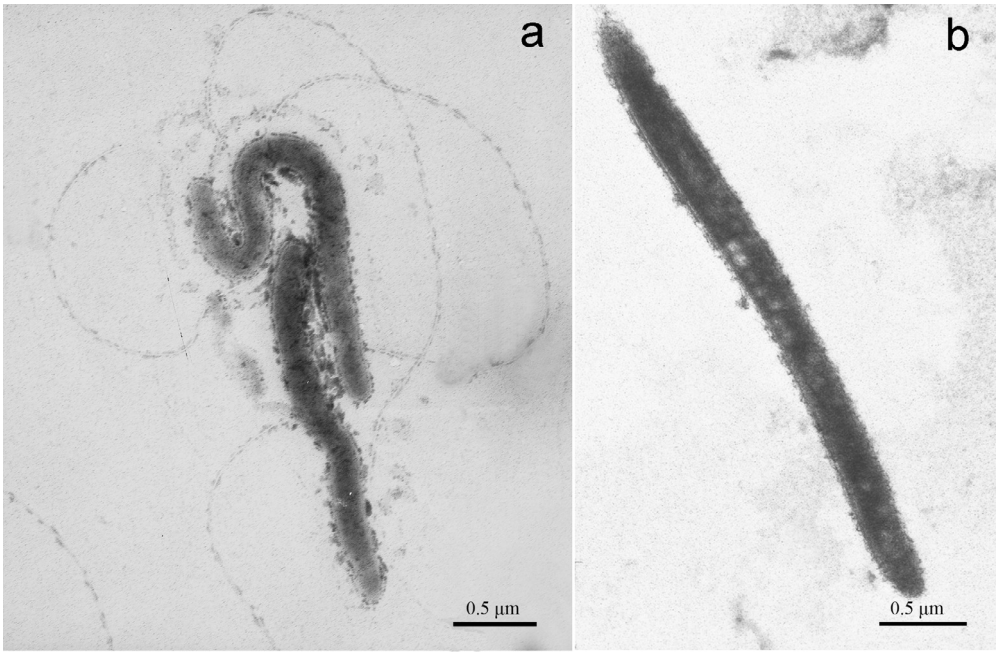

Fig. 3. Electron micrographs of strain Z-1702^T cells demonstrating (a) flagellation; (b) transmission electron micrograph of a thin section of the cells, showing Gram-negative structure of cell wall.

of TABS buffer at pH 9.0. The strain grew at total $Na^{\pm}$ concentrations ranging from 1.2 to 3.24 M with an optimum at 2.0 M and total CO_3^{2-}/ HCO^{3-} concentrations ranging from 0.6 to 1.8 M with an optimum at 1.2 M. Strain Z-1702^T was halotolerant and could grow without Cl^- in the medium. Strain Z-1702^T was obligate anaerobe, growing on anaerobically prepared medium with reducing agent (sodium sulfide). No growth was observed on aerobically prepared medium, or with 2.0–10 % O_2 in the gas phase or on anaerobically prepared medium without sodium sulfide. Catalase activity test was positive.

Strain Z-1702^T had strict fermentative type of metabolism and was not capable for anaerobic respiration. It was a saccharolytic organoheterotroph fermenting cellobiose, galactose, glucose, fructose, maltose, mannose, sorbose and *N*-acetyl-D-glucosamine. Furthermore, it also can utilize starch and several starch-like alpha-glucans, including dextrin (from potato and maize), glycogen, pullulan. Arabinose, lactose, ribose, sucrose, trehalose, ramnose, ribose, xylose, agarose, sodium alginate, carboxymethylcellulose, cellulose, α-cellulose, amorphous cellulose, chitin, chitosan, dextran, galactan, pectin, xanthan gum,

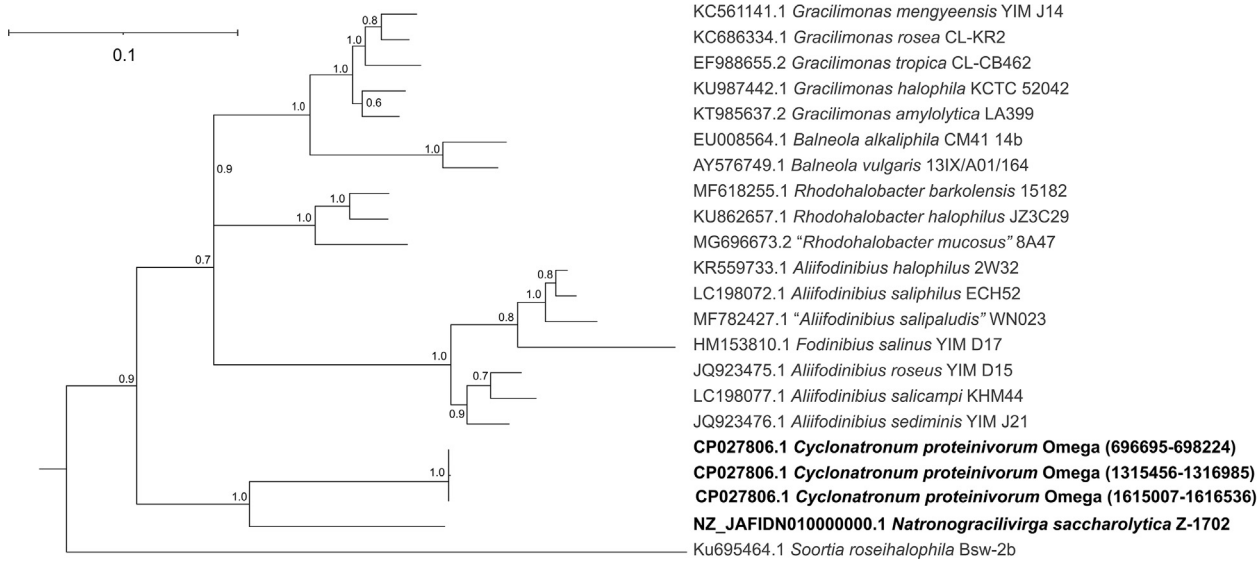

Fig. 4. Maximum-likelihood phylogenetic trees, showing position of *Natronogracilivirga saccharolityca* Z-1702^T and *Cyclonatronum proteinivorum* Omega^T (in bold) within the order *Balneolales*. The branch lengths correspond to the number of substitutions per site according to the corrections associated with used models. All positions with less than 95 % site coverage were eliminated. A proportion of trees in which the associated taxa clustered together (bootstrap test) are shown at the nodes. *Rhodothermus marinus* DSM 4252^T (not shown) was used as an outgroup in both trees. (**a**) 16S rRNA gene sequence-based tree consisted of 23 nucleotide sequences and 1387 positions analyzed. The sequences of three Omega^T 16S rRNA genes are specified by their coordinates in the CP027806.1 genome sequence (in parentheses); (**b**) the bac120 set of conserved proteins sequence tree consisted of 22 aminoacid sequences and 31,510 positions. Isolation source of Z-1702^T, Omega^T and seven closely related MAGs are indicated to the right of the sequence designations.

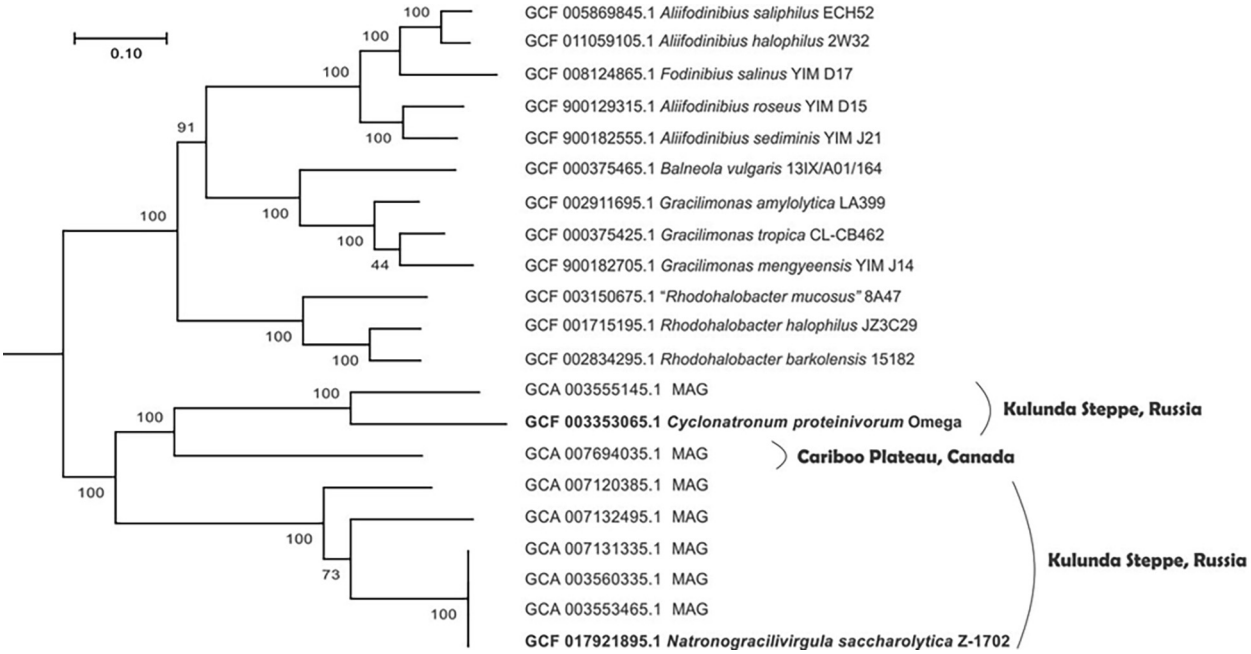

Fig. 4 (*continued*)

xylan, gaprin, dry *Spirulina's* biomass, casamino acids, alcohols, organic acids and peptides did not support growth of strain Z-1702^T(Table 1).

Strain Z-1702^T could grow oligotrophically with glucose at a minimal concentration of 0.05 g l^{-1} reaching the cell density of 9.1 × 10^7 cells ml^{-1}. Optimal growth occurred with 2.5–3.0 g l^{-1} of glucose with the growth yields 2.1 × 10^8 cells ml^{-1}. From 7 mM of oxidized glucose strain Z-1702^T produced 20.8 mM of acetate, 3.5 mM of succinate and < 3.0 mM of an unidentified product. The novel isolate did not require yeast extract for growth but did not grow without vitamin solution.

Chloramphenicol, erythromycin, and rifampicin completely inhibited growth of the strain Z-1702^T at already 25 mg l^{-1} while cycloserine and tetracycline showed inhibition at 50 mg l^{-1}. Other tested antibiotics (ampicillin, bacitracin, gentamycin, kanamycin, neomycin, novobiocin, penicillin, polymyxin B and streptomycin) had no inhibitory effect at up to 50 mg l^{-1}.

Among the cellular fatty acids of strain Z-1702^T were straight-chain and branched chain (iso and anteiso) saturated (67.8 %) and unsaturated (22.7 %) fatty acids and hydroxy acids (9.5 %) (Table S1). The main fatty acids (79.1 % of the total) in the lipids of Z-1702^T were: *iso*-$C_{15:0}$ (20.8), $C_{16:0}$ (12.1), *iso*-$C_{16:0}$ (11.6), anteiso-$C_{17:0}$ (8.2), anteiso-$C_{17:1\omega8}$ (6.9), *iso*-$C_{17:1\omega8}$ (6.9), *iso*-$C_{17:0}$ (6.5), 3-OH-*iso*-$C_{17:0}$ (6.1) (Suppl. Table 2**)**. The major respiratory lipoquinon identified in strain Z-1702^T was MK-7.

General genome properties

Draft genome assembly of strain Z-1702^T consisted of 50 genomic scaffolds of 3750541 bp total length. GC content was 49.67 %, which is similar to its closest cultivated relative, strain Omega^T (51.39 %). Analysis of Bacteria-specific marker genes with lineage-specific workflow of CheckM package showed that predicted assembly completeness and contamination values were 97.54 and 2.46 %, respectively. CheckM analysis of complete circular genome of strain Omega^T, showed exactly the same number of missing and duplicated genes (Suppl. Table 2). That fact indicates that non-ideal assembly quality metrics stem from the lineage-specific genomic characteristics, rather than the potential misassembles or contamination. 3060 genes were predicted by Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016), including 2983 protein-coding genes, 22 pseudogenes and 55 RNA genes. One full-length ribosomal operon was detected; however, coverage of this region indicates that at least three identical rRNA operons are presented in the genome. Due to fragmented nature of the assembly, detailed analysis of laterally transferred genes was impossible in case of strain Z-1702^T genome. Nevertheless, diversity of mobile genetic elements, predicted by ISSaga (Varani et al., 2011) may reflect low level of Z-1702^T genome mobility: only three complete IS element-related ORFs were detected, in contrast to genome of strain Omega^T, possessing 26 complete mobile element-associated ORFs (Sorokin et al., 2018).

Phylogenetic analysis

16S rRNA gene sequence BLAST search indicated that Z-1702^T belongs to the order *Balneolales* with strain Omega^T as the nearest relative and *Gracilimonas halophila* (Lu et al., 2017) as the nearest relative with a validly published name. The 16S rRNA gene sequence identities between strain Z-1702^T and strain Omega^T or *Gracilimonas halophila* were 89.86 or 88.45 %, respectively, which is below the genus-level border, and close but still above the family-level border (94.5 % and 86.5 %, respectively), according to (Yarza et al., 2014). Both, 16S rRNA gene sequence phylogenetic and 120 conserved single-copy protein marker proteins phylogenomic trees (Fig. 4a, b) placed strain Z-1702^T and strain Omega^T as a deep-branching lineage within the *Balneolales* (Hahnke et al., 2003; Mistry et al., 2013).

16S rRNA gene sequence-based phylogenetic analysis revealed that the *Balneolales* comprised of three clusters: i) located most closely to the root *Soortia roseihalophila* (Amoozegar et al., 2017), ii) the cluster consisting of strain Z-1702^T and stain Omega^T (Sorokin et al., 2018) and iii) the cluster containing the rest of the *Balneolales* members. Considering that *Soortia roseihalophila* and all other *Balneolales* represented 2 different families: *Soortiaceae* and *Balneolaceae*, respectively, and taking into account the evolutionary distance of the bifurcation points of the "Z-1702^T – Omega^T" cluster, it would be a solid assumption to consider the latter as a novel-family lineage. The bac120 tree structure resembled the 16S rRNA gene sequence tree except it lacked *Soortia roseihalophila* which genomic sequence is publically unavailable.

Table 2
Descriptions of *Cyclonatronum* gen. nov. and *Cyclonatronum proteinivorum* sp. nov.

Genus name	*Cyclonatronum*	
Species name		*Cyclonatronum proteinivorum*
Genus status	gen. nov.	
Genus etymology	Cy.clo.na.tro'num. Gr. masc. n. *kyklos*, a circle; N.L. n. *natron* (arbitrarily derived from the Arabic n. *natrun* or *natron*), soda; N.L. neut. n. *Cyclonatronum*,, circle-shaped soda-loving	
Type species of the genus	*Cyclonatronum proteinivorum*	
Specific epithet	–	*proteinivorum*
Species status	–	sp. nov.
Species etymology	–	pro.te.i.ni.vo'rum. N.L. neut. n. *proteinum*, protein; L. v. *voro*, to devour; N.L. neut. adj. *proteinivorum*, protein devouring
Description of the new taxon and diagnostic traits	Strictly aerobic, mezophilic, alkaliphilic, natronophilic bacterium. Cells are nonmotile circular rods with variable morphology. Gram-negative. Proteolytic organoheterotroph. The main fatty acid is *iso*-$C_{15:0}$. The type species is *Cyclonatronum proteinivorum*.	Cell morphology is variable from circular (diameter around 2.5–3 μm) to long flexible rods-spirilla up to 15 μm, and the average cell width of 0.5 μm. Cells contain carotenoids with absorption maxima in MeOH-aceton extract at 450, 479 (main) and 505 nm and a sodium-pumping proteorhodopsin. Gram-negative and nonmotile. The major fatty acids are iso$C_{15:0}$ and $C_{16:1\omega7c}$, anteiso-$C_{16:1\omega7c}$, *iso*-$C_{16:0}$, $C_{16:0}$, anteiso-$C_{17:1\omega8}$ and $C_{17:0}$. Mezophile with growth temperature range of 25–45 °C and an optimum at 33–35 °C. Obligate alkaliphile with a pH range for growth 8.5–10.5 (optimum at 9.5–10.0). Obligate natronophile and halophile requiring Na^+ concentration from 0.3 to 2.25 M (optimum at 0.6–1.0 M). Strictly aerobic chemoorganoheterotroph proteolytic. Utilizes casein, albumin, gelatin, peptones from meat, soy and casein and yeast extract as energy, carbon and nitrogen source. Sugars and sugar polymers, organic acids and alcohols are not utilized. The type strain is sensitive to chloramphenicol, erythromycin and rifampicin and cycloserine and tetracycline only at concentrations of 50 mg l^{-1}. Habitat - soda lakes.
Country of origin	–	Russian Federation
Region of origin	–	Altai
Date of isolation (dd/mm/yyyy)	–	09/2016
Source of isolation	–	Biofilm on the surface of littoral sediments of the soda lake Bitter-3
Sampling date (dd/mm/yyyy)	–	10. 07/2015
Latitude (xx°xx′xx″ N/S)	–	N51°40′′
Longitude (xx°xx′xx″ E/W)	–	E79°53′
16S rRNA gene accession nr.	–	KF830693
Genome accession number	–	CP027806
Genome status	–	complete
Genome size	–	4.29
GC % (genomic)	–	51.4
Number of strains in study	–	1
Information related to the Nagoya Protocol	–	Not applicable
Designation of the Type Strain	–	Omega^T
Strain Collection Numbers	–	JCM 31662^T, UNIQEM U979^T

AAI value between proteomes of strain Z-1702^T and strain Omega^T was of 52 %; between "Z-1702^T – Omega^T" pair and *Balneolaceae* representatives – 49–52 %; within *Balneolaceae* family – 55–84 %, supporting a distant position of "Z-1702^T – Omega^T" pair.

Thus, based on the two phylogenetic and phylogenomic analyses, 16S rRNA gene sequence identity values, RED values and pairwise AAI calculations of *Balneolales* representatives we propose that strain Z-1702^T and strain Omega^T are being novel species of two novel genera *Natronogracilivirga saccharolityca* gen. nov., sp. nov. and *Cyclonatronum proteinivorum* gen. nov., sp. nov., respectively, and, together, are forming a novel family *Cyclonatronaceae* fam. nov. (see Tables 2 and 3).

It should be noted that all the members of the *Cyclonatronaceae* including uncultivated microorganisms inhabit halo-alkalophilic environments. Five MAGs related to the strain Z-1702^T (three of them are almost identical according to bac120 tree) were from the DNA, also isolated from Kulunda Steppe saline lakes as was one of the MAGs, related to the strain Omega^T. Another, deeply branched sequence belonged to the MAG, obtained from Cariboo Plateau, Canada (Fig. 4b), pointing at widespread distribution of the *Cyclonatronaceae* representatives in halo-alkaline lakes. It is also worth mentioning that as all *Balneolaceae* members and more deeply rooted *Soortia roseihalophila* (Fig. 4a) are neutrophilic microorganisms, indicating a common ancestor of *Cyclonatronaceae* has switched its physiology from neutrophilic to alkaliphilic.

Genome analysis

Analysis of COG distribution of *in silico* proteome of the strain Z-1702^T, compared to other members of *Balneolales* and *Rhodothermaeota* Munoz et al., 2016 showed high proportion of genes involved in carbohydrate utilization (Suppl. Table 2). Among the chosen gen-

Table 3
Descriptions of *Natronogracilivirga* gen. nov. and *Natronogracilivirga saccharolytica* sp. nov.

Genus name	*Natronogracilivirga*	
Species name		*Natronogracilivirga saccharolytica*
Genus status	gen. nov.	
Genus etymology	Na.tro.no.gra.ci.li.vir'ga. N.L. n. *natron* (arbitrarily derived from the Arabic n. *natrun* or *natron*) soda, sodium carbonate; L. masc./fem. adj. *gracilis,* thin; L. fem. n. *virga*, rod; N.L. fem. n. *Natronogracilivirga*, a soda-requiring thin rod	
Type species of the genus	*Natronogracilivirga saccharolytica*	
Specific epithet	–	*saccharolytica*
Species status	–	sp. nov.
Species etymology	–	sac.cha.ro.ly'ti.ca. Gr. neut. n. *saccharon* sugar; N.L. fem. adj. *lytica* from Gr. fem. adj. *lutikê* able to loosen; N.L. fem. adj. *saccharolytica* saccharolytic, breaking down multiple sugars
Description of the new taxon and diagnostic traits	A strictly anaerobic, fermentative, mezophilic, alkaliphilic, natronophilic bacterium. Cells are motile thin flexible rods with variable morphology. Gram-negative. Fermentative metabolism. The main polar lipid fatty acid is *iso*-$C_{15:0}$. The type species is *Natronogracilivirga saccharolytica*.	At early exponential growth phase and optimal conditions cells are thin optical light flexible rods with slightly tapering ends 0.15–0.2 μm in diameter 0.5–4.0 μm in length. At late exponential and stationary phases, at not optimal conditions, or on solid media cells are optical dense 0.3–0.5 μm in diameter 5–10 μm in length. Form dense spherical cells located at the end or at side of the cell. Cell lised, formed spheroplasts. Gram-negative and motile. Mezophile with growth temperature range of 20–43 °C and an optimum at 37–40 °C. Alkaliphile with pH range for growth is 7.5–10.2 with optimum at 8.7–9.0. Obligate halotolerant natronophile required Na^+ ions in the carbonate form with Na^+ concentrations growth range 1.2–3.2 M with an optimum at 2.0 M and total CO_3^{2-}/HCO^{3-} concentrations range 0.6–1.8 M with an optimum at 1.2 M. Sodium chloride and yeast extract are not required for growth. Strictly fermentative type of metabolism not capable for anaerobic respiration. Growth occurs with cellobiose, galactose, glucose, fructose, maltose, mannose, sorbose, dextrin (from potato and maize), glycogen, *N*-acetyl-D-glucosamine, starch, pullulan. Capable to grow oligotrophically with glucose at concentrations of 0.05 g l^{-1}. The type strain is sensitive to chloramphenicol, erythromycin and rifampicin and cycloserine and tetracycline only at concentrations of 50 mg l^{-1}. Strain Z-1702^T was not sensitive to ampicillin, bacitracin, gentamycin, kanamycin, neomycin, novobiocin, penicillin, polymyxin B and streptomycin added at both concentrations. The major fatty acids are *iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$, anteiso-$C_{17:0}$, anteiso-$C_{17:1\omega8}$, *iso*-$C_{17:1\omega8}$, *iso*-$C_{17:0}$, 3-OH-*iso*-$C_{17:0}$.
Country of origin	–	Russian Federation
Region of origin	–	Altai
Date of isolation (dd/mm/yyyy)	–	17/9/2017
Source of isolation	–	Sediments of soda lake Tanatar III
Sampling date (dd/mm/yyyy)	–	22/06/2007
Latitude (xx°xx′xx″ N/S)	–	N51°37'29"
Longitude (xx°xx′xx″ E/W)	–	E79°48'28"
16S rRNA gene accession nr.	–	NATSA_RS15180
Genome assembly number	–	GCF_017921895.1
Genome status	–	complete
Genome size	–	3.75
GC mol%	–	49.67
Number of strains in study	–	1
Information related to the Nagoya Protocol	–	Not applicable
Designation of the Type Strain	–	Z-1702^T
Strain Collection Numbers	–	=DSMZ 109061^T, =JCM 32930^T, =VKM B 3262^T

omes the proportion of COG functional category "Carbohydrate transport and metabolism" in the genome of strain Z-1702^T was second highest after the genome of *Rhodotermus marinus* DSM 4252^T, which is well known for its active carbohydrate-consuming lifestyle (Alfredsson et al., 1988). It should be also noted that in strain Z-1702^T the proportion of genes not assigned to COGs was second highest after strain Omega^T, reflecting the phylogenetic remoteness of the strain. Thus, COG analysis confirmed both phenotypic observations and novelty of the strain Z-1702^T.

The presence of 104 genes, encoding CAZymes (Drula et al., 2022) among which the glycosidases (GHs) represented almost half of the total number (Fig. 5a) is in accordance with strain Z-1702^T capability to grow on polysaccharides. On the contrary the proteolytic strain Omega^T, for which the growth on carbohydrates was not shown, has

a lesser of both, CAZymes in total and GHs. Besides that, strain Z-1702^T genome encoded the only polysaccharide lyase NATSA_14165 of PL12 family, which, together with glycosyltransferase NATSA_14170 of GT4 family, is most probably involved in complex heteropolysaccharide metabolism. No PL genes were found in the genome of strain Omega^T, while the mentioned by Sorokin et al., (Sorokin et al., 2018) PL22 seems to be wrongly annotated. The saccharolytic strain Z-1702^T and the proteolytic strain Omega^T have similar number of GTs and carbohydrate esterases (CEs) implying their involvement in structural or storing polysaccharides biosynthesis. The discrepancy between the two genomes was not only in the total GHs number but also in their distribution among the families (Fig. 5b). Both genomes encode high number of GH13 representatives, which are involved in metabolism of starch-like alpha-glucans (https://www.cazy.org/). Relatively high number of GH3 members indicate a potential ability to hydrolize various oligosaccharides or glycosidic bonds located close to the termini of the polysaccharide molecules. Presumably, these enzymes are involved in cell wall' polysaccharides hydrolysis. In comparison with strain Z-1702^T strain Omega^T has higher number of the lysozyme-encoding (GH23) genes (2 and 4, respectively), which is in accordance with its capacity to grow on cyanobacterial cells. Moreover, strain Omega^T has a higher number of GH109 and GH171 probably involved in peptidoglycan degradation. Finally, a number of GHs were exclusively present in strain Omega^T (Fig. 5b), all of which seems to be also involved in cyanobacterial cell wall degradation. In turn, the GHs exclusively present in strain Z-1702^T were predicted to hydrolyze oligo- and polysaccharides with various types of beta-glycosidic bonds including not only glucosides but also mannosides, galactosides etc. (Fig. 5b) reflecting its saccharolytic lifestyle. Thus, both strains have similar repertoire of CAZymes involved in oligo- and polysaccharides synthesis as well as degradation of storage polysaccharides but differed in the enzymatic machinery for degradation of complex microbial cell wall polysaccharides and the presence (in case of strain Z-1702^T) or absence (in the case of strain Omega^T) of enzymes for hydrolysis of polysaccharides other than glucans (mannans, galactans).

The genomes of both strains contain all the genes, encoding Embden-Meyerhof-Parnas (EMP) pathway crucial for sugars fermentation. An important difference was observed at the final step of glycolysis – oxidative decarboxylation of pyruvate. Anaerobic saccharolytic strain Z-1702^T has two homologs of pyruvate: ferredoxin oxidoreductase (Por), a bacterial-type single-subunit protein NATSA_01295 and an archaeal-type multisubunit complex NATSA_1825-1835. The latter, however, has rather distant homologs with evidence at protein level that makes predicting its activity less valid. In turn, aerobic proteolytic strain Omega^T does not have any of these ferredoxin-dependent oxygen-sensitive enzymes. Meanwhile, both strains have a NAD-dependent pyruvate dehydrogenase (PdhABC, NATSA_11365-11375 & CYPRO_1777-1779) playing analogous role in aerobic microorganisms (Bothe, 1975).

Despite strictly anaerobic fermentative metabolism of strain Z-1702^T all genes, coding for oxidative TCA (oTCA) proteins were identified in its genome: citrate synthase (NATSA_07745), aconitase (NATSA_07865), isocitrate dehydrogenase (NATSA_06900), 2-oxoglutarate dehydrogenase (Kdh, NATSA_06145) and 2-oxoglutarate synthase (Kor, NATSA_13225-13230), Succinyl-CoA synthetase (NATSA_01000, NATSA_12960), succinate dehydrogenase (Complex II, NATSA-00035–00045), fumarate hydratase (NATSA_00310) and malate dehydrogenase (NATSA_12280). Of two possible enzymes, catalyzing oxidative decarboxylation of 2-oxoglutarate, 2-oxoglutarate dehydrogenase (Kdh, EC 1.2.4.2) and ferredoxin-dependent 2-oxoglutarate synthase (Kor, EC 1.2.7.3), the second one is more likely to be active (if oTCA is indeed active in this microorganism) taking into account its high sensitivity to oxygen (Baughn et al., 2009) and obligatory anaerobic lifestyle of strain Z-1702^T.

Genome of strain Z-1702^T encodes a NADH dehydrogenase (Complex I, NATSA_06055- NATSA_06080, NATSA_07165-NATSA_07200) and two cytochrome oxidases (Complex III, NATSA_00210-00225, NATSA_01910-01925). Both cytochrome oxidases are homologous to *Melioribacter roseus* enzymes which were shown to act at high (*c(b/o)o_3*-type, Mros_0035-0038 – NATSA_00210-00225) and low (*cbb$_3$*-type Mros_1513-1515 – NATSA_01910-01925) oxygen concentrations (Gavrilov et al., 2017). However, it should be noted that another homolog of "high-oxygen" (59 % identity to catalytic subunits NATSA_00215 and Mros_0038) *c(b/o)o_3*-type cytochrome oxidase was previously shown to act in oxygen detoxification in *Desulfovibrio vulgaris* (Ramel et al., 2013). Similar role could be proposed for this enzyme in strain Z-1702^T considering its strict anaerobic lifestyle, while the role of the *cbb$_3$* cytochrome *c* oxidase remains unclear.

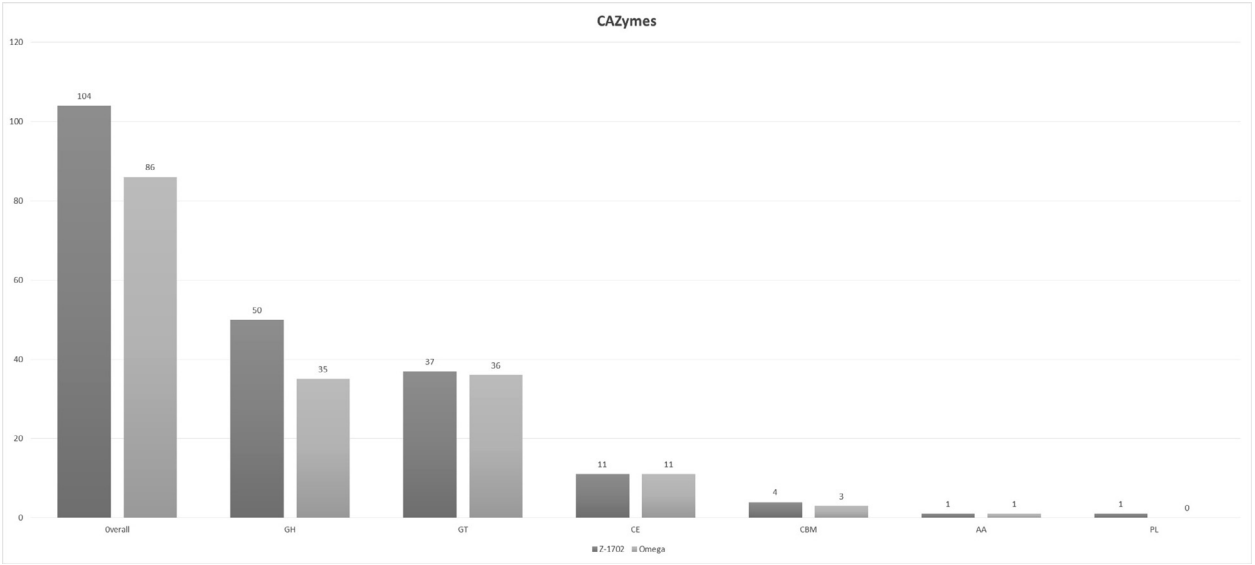

Fig. 5. Distribution of CAZymes-encoding genes within the genomes of *Natronogracilivirga saccharolityca* Z-1702^T and *Cyclonatronum proteinivorum* Omega^T. (**a**) CAZymes classes. GH, glycosidases; GT, glycosyltransferases; CE, carbohydrate esterases; CBM, carbohydrate-binding modules; PL, polysaccharide lyases; AA, auxiliary activities. (**b**) Glycosidases (GHs) families. Strain Z-1702^T is in dark grey, strain Omega^T is in light grey. Vertical axis, number of genes.

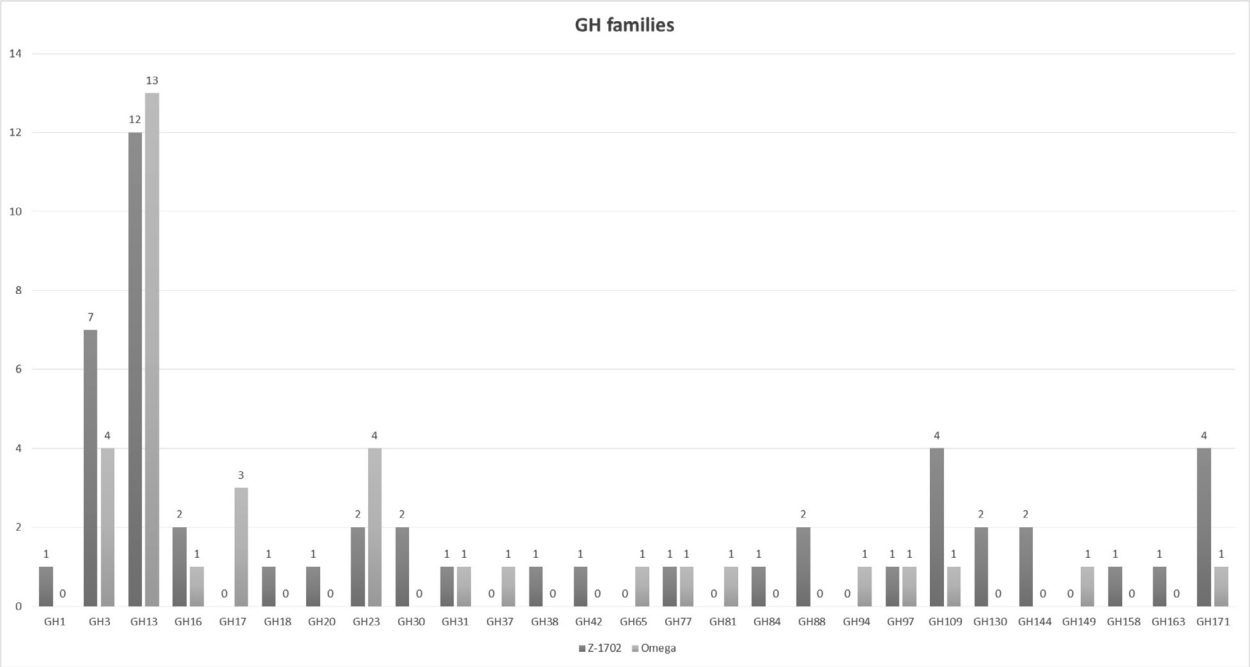

Fig. 5 (*continued*)

All (with a few exceptions) of the TCA and aerobic respiration proteins of strain Z-1702^T have close homologs in strain Omega^T (having identity values close or above the mean AAI value for the proteomes of these two microorganisms, which is 52 %) indicating a vertical inheritance of these enzymes from the common ancestor. The exceptions are isocitrate dehydrogenase (NATSA_06900 and CYPRO_1551, 39 % identity), and succinate dehydrogenase cytochrome b (SdhC) subunit (NATSA_00045 and CYPRO_0900, 46 % identity).

A gene, encoding proteorhodopsin highly similar (71 % amino acid identity) to its homolog in strain Omega^T is present in the genome of strain Z-1702^T. In strain Omega^T it was shown to be an active light-dependent and O_2-independent primary sodium pump, which might be profitable in an alkaliphilic bacterium associated with benthic cyanobacteria living at oxygen-limiting conditions on the surface of sulfidic sediments. Similar role might be proposed for bacteriorhodopsin of anaerobic strain Z-1702^T, although this suggestion needs biochemical confirmation.

Genomic analysis revealed two complex iron-sulfur molybdoenzymes (CISM) clusters in strain Z-1702^T with the catalytic subunits belonging to ArrA arsenate reductase family. The role of these enzymatic complexes in strain Z-1702^T is enigmatic since the strain does not grow on arsenate. It was shown (Gavrilov et al., 2017) that molybdopterin oxidoreductases families' specificity is rather flexible, however, other possible CISM activities including thiosulfate reductase and polysulfide reductase were not confirmed in growth experiments. The genome of strain Omega^T does not contain any molybdopterin family oxidoreductases which is reflecting its strictly aerobic lifestyle.

In contrast to its proteolytic relative, strain Z-1702^T genome encoded only one cyanophycinase, a member of S51 family serine peptidases. The NATSA_15045 has a similar to CYPRO_2145 single-domain organization (Sorokin et al., 2018). Cianophycin is a non-ribosomally synthesised multi-L-arginyl-poly-L-aspartic acid peptide, acting as a storage (primarily, nitrogen) compound in cyanobacteria and many other bacteria including *Bacteroidetes* and *Balneolales*. It is synthesized by cyanophycin synthetase, which is a homooligomeric enzyme, acting at the expense of ATP molecules. In strain Z-1702^T genome the cyanophycin synthetase gene is located next to the cyanophycinase gene - *NATSA_15050*. Another closely located gene - *NATSA_15040* - encodes an isoaspartyl dipeptidase which might play a role in either a correction of altered cyanophycin peptides during its synthesis or in a degradation of cyanophycins. This gene context implies cyanophycin synthesis and degradation are regulated in strain Z-1702^T. In contrast, strain Omega^T lacks the cyanophycin synthetase gene in its genome, obviously due to its peptolytic lifestyle imparting no need for nitrogen storing which could be important to saccharolytic strain Z-1702^T. In turn, a homolog of isoaspartyl dipeptidase is present - CYPRO_0331.

Strain Z-1702^T encoded four S8 family peptidases (subtilases) - *NATSA_01070, NATSA_03980, NATSA_12345, NATSA_14965*, twofold lower than in peptolytic strain Omega^T (Sorokin et al., 2018) genome encoding nine S8 genes.

According to the genomic prediction, both strains Omega^T and Z-1702^T have a potential for biosynthesis of glycine betaine - a most common osmoprotectant (osmolyte) in halophilic bacteria (Roberts, 2005). However, they have two different pathways for this. Strain Omega^T apparently uses a two-step oxidation of choline (which may originate from aminolipids) via betaine aldehyde encoded by betAB, common for aerobic halophiles (Roberts, 2005), while the genome of strain Z-1702^T contains a bicistronic operon encoding two-step sequential methylation of glycine to glycine betaine by SAM-dependent methyl-transferases (NATSA_08095-08100) first discovered in anoxygenic purple sulfur bacterium *Halorhodospira halochloris* (Nyyssölä et al., 2000). Furthermore, strain Z-1702^T might potentially be able to import external glycine betaine and its precursors from the environment. The genome encodes two types of glycine-betaine transporters in a single genomic locus (NATSA_02055-02070): a monosubunit BCC family transporter and an ABC glycine betaine transporting cassette OpuAA/AB/AC (Hoffmann and Bremer, 2011). No homologs of these genes were detected in the genome of strain Omega^T.

Conclusions

Two bacterial isolates originated from soda lakes in the Kulunda Steppe (Altai Region, Russia) are both mesophilic, obligate alkaliphilic, chloride-independent natronophiles (Table 1). Thus, the strains are very similar in terms of physico-chemical growth parame-

ters. Despite that both act as satellites of autotrophic bacteria, their physiology is quite different reflecting specific positions in the trophic structure of haloalkaliphilic communities. Both bacteria are organotrophs, but strain Omega^T specializes in aerobic peptide degradation, whereas strain Z-1702^T is a fermenting saccharolytic utilizing carbohydrates and alpha-glucans. These phenotypic characteristics were generally confirmed by the analysis of the genomes of both strains. In contrast to proteolytic strain Omega^T, strain Z-1702^T genome exhibits overrepresentation of CAZymes and the opposite trend was observed for peptidases. Anaerobic strain Z-1702^T also has all the genes encoding sugars fermentation enzymes including a terminal step of ferredoxin-dependent oxidative decarboxylation of pyruvate into acetyl-CoA, lacking in aerobic strain Omega^T. At the same time in many other functional aspects the two genomes showed similar patterns with the most striking similarity is in the presence of all genes encoding the TCA cycle and oxygen respiration enzymes which was not expected considering strictly anaerobic growth of strain Z-1702^T. It is possible that in strain Z-1702^T the TCA cycle is used only for anabolic purposes while cytochrome oxidases are involved in oxygen detoxification or that the genes have some disruptions, thus being unexpressed or making their products inactive. These should be clarified by in-depth genomic as well as transcriptomic/proteomic analyses.

Description of Cyclonatronaceae fam. nov.

Cyclonatronaceae (Cy.clo.na.tro.na.ce'ae. N.L. neut. n. *Cyclonatronum*, type genus of the family; *-aceae*, ending to denote a family; N.L. fem. pl. n. *Cyclonatronaceae*, the Cyclonatronum family).

Includes members with highly variable cell morphology and the Gram-negative type of cell wall. They are mezophilic, obligately alkaliphilic and moderately salt-tolerant aerobic or anaerobic organoheterotrophs utilizing either peptides or carbohydrates. DNA genomic G + C content is in the range 49–52 %. The family consists of type genus *Cyclonatronum* and the genus *Natronogracilivirga*. The family is a member of the order *Balneolales*.

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Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.syapm.2023.126403.

References

Alfredsson, G.A., Kristjansson, J.K., Hjorleifsdottir, S., Stetter, K.O., 1988. Rhodothermus marinus, gen., a thermophilic, halophilic bacterium from submarine hot springs in Iceland. J. Gen. Microbiol. 134, 299–306. https://doi.org/10.1099/00221287-134-2-299.

Almagro Armenteros, J.J., Tsirigos, K.D., Sønderby, C.K., Petersen, T.N., Winther, O., Brunak, S., von Heijne, G., Nielsen, H., 2019. SignalP 5.0 improves signal peptide predictions using deep neural networks. Nat. Biotechnol. 37 (4), 420–423. https://doi.org/10.1038/s41587-019-0036-z.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic. Acids. Res. 25 (17), 3389–3402. https://doi.org/10.1093/nar/25.17.3389.

Amoozegar, M.A., Khansha, J., Mehrshad, M., Shahzadeh Fazeli, S.A., Ramezani, M., de la Haba, R.R., Sánchez-Porro, C., Ventosa, A., 2017. *Soortia roseihalophila* gen. nov., sp. nov., a new taxon in the order *Balneolales* isolated from a travertine spring, and description of *Soortiaceae* fam. nov. Int. J. Syst. Evol. Microbiol. 67 (1), 113–120. https://doi.org/10.1099/ijsem.0.001583.

Antony, C.P., Kumaresan, D., Hunger, S., Drake, H.L., Murrell, J.C., Shouche, Y.S., 2013. Microbiology of Lonar lake and other soda lakes. ISME J. 7, 468–476. https://doi.org/10.1038/ismej.2012.137.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A., Dvorkin, M., Kulikov, A.S., Lesin, V., Nikolenko, S., Pham, S., Prjibelski, A., Pyshkin, A., Sirotkin, A., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (5), 455–477. https://doi.org/10.1089/cmb.2012.0021.

Baughn, A.D., Garforth, S.J., Vilcheze, C., Jacobs Jr., W.R., 2009. An anaerobic-type alpha-ketoglutarate ferredoxin oxidoreductase completes the oxidative tricarboxylic acid cycle of *Mycobacterium tuberculosis*. PLoS Pathog. 5, e1000662.

Bothe, H., 1975. The occurrence of the pyruvate dehydrogenase complex and the pyruvate-ferredoxin oxidoreductase in blue-green bacteria. Biochem. Soc. Trans. 3 (3), 376–377. https://doi.org/10.1042/bst0030376.

Buchfink, B., Xie, C., Huson, D.H., 2015. Fast and sensitive protein alignment using DIAMOND. Nat. Methods. 12 (1), 59–60. https://doi.org/10.1038/nmeth.3176.

Cantalapiedra, C.P., Hernández-Plaza, A., Letunic, I., Bork, P., Huerta-Cepas, J., 2021. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. Mol. Biol. Evol. 38 (12), 5825–5829. https://doi.org/10.1093/molbev/msab293.

Cho, Y., Chung, H., Jang, G.I., Choi, D.H., Noh, J.H., Cho, B.C., 2013. *Gracilimonas rosea* sp. nov., isolated from tropical seawater, and emended description of the genus *Gracilimonas*. Int. J. Syst. Evol. Microbiol. 63 (11), 4006–4011. https://doi.org/10.1099/ijs.0.052340-0.

Choi, D.H., Zhang, G.I., Noh, J.H., Kim, W.S., Cho, B.C., 2009. *Gracilimonas tropica* gen. nov., sp. nov., isolated from a Synechococcus culture. Int. J. Syst. Evol. Microbiol. 59 (5), 1167–1172. https://doi.org/10.1099/ijs.0.005512-0.

Drula, E., Garron, M.-L., Dogan, S., Lombard, V., Henrissat, B., Terrapon, N., 2022. The carbohydrate-active enzyme database: functions and literature. Nucleic Acids Res. 50 (D1), D571–D577. https://doi.org/10.1093/nar/gkab1045.

Garnova, E.S., Zhilina, T.N., Tourova, T.P., Lysenko, A.M., 2003. *Anoxynatronum sibiricum* gen. nov., sp. nov. alkaliphilic saccharolytic anaerobe from cellulolytic community of Nizhnee Beloe (Transbaikal region). Extremophiles 7 (3), 213–220. https://doi.org/10.1007/s00792-002-0312-5.

Garnova, E.S., Zhilina, T.N., Tourova, T.P., Kostrikina, N.A., Zavarzin, G.A., 2004. Anaerobic, alkaliphilic, saccharolytic bacterium *Alkalibacter saccharofermentans* gen. nov., sp nov from a soda lake in the Transbaikal region of Russia. Extremophiles 8 (4), 309–316. https://doi.org/10.1007/s00792-004-0390-7.

Gavrilov, S.N., Podosokorskaya, O.A., Alexeev, D.G., Merkel, A.Y., Khomyakova, M.A., Muntyan, M.S., Altukhov, I.A., Butenko, I.O., Bonch-Osmolovskaya, E.A., Govorun, V.M., Kublanov, I.V., 2017. Respiratory pathways reconstructed by multi-Omics analysis in *Melioribacter roseus*, residing in a deep thermal aquifer of the West-Siberian Megabasin. Front. Microbiol. 8 (1228), 1–13. https://doi.org/10.3389/fmicb.2017.01228.

Gracheva, M.A., Chistyakova, N.I., Antonova, A.V., Rusakov, V.S., Zhilina, T.N., Zavarzina, D.G., 2017. Mössbauer study of iron minerals transformations by *Fuchsiella ferrireducens*. Hyperfine Interact. 238, 84. https://doi.org/10.1007/s10751-017-1460-4.

Hahnke, R.L., Meier-Kolthoff, J.P., García-López, M., Mukherjee, S., Huntemann, M., Ivanova, N.N., Woyke, T., Kyrpides, N.C., Klenk, H.P., Göker, M., 2003. (2016) Genome-based taxonomic classification of *Bacteroidetes*. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.02003.

Han, S.B., Yu, Y.H., Ju, Z., Li, Y., Zhang, R., Hou, X.J., Ma, X.Y., Yu, X.Y., Sun, C., Wu, M., 2018. *Rhodohalobacter barkolensis* sp. nov., isolated from a saline lake and emended description of the genus *Rhodohalobacter*. Int. J. Syst. Evol. Microbiol. 68 (6), 1949–1954. https://doi.org/10.1099/ijsem.0.002775.

Hoffmann, T., Bremer, E., 2011. Protection of *Bacillus subtilis* against cold stress via compatible-solute acquisition. J Bacteriol. 193 (7), 1552–1562. https://doi.org/10.1128/JB.01319-10.

Hoover, R.B., Pikuta, E.V., Bej, A.K., Marsic, D., Whitman, W.B., Tang, J., Krader, P., 2003. *Spirochaeta americana* sp. nov., a new haloalkaliphilic, obligately anaerobic spirochaete isolated from soda Mono Lake in California. Int. J. Syst. Evol. Microbiol. 53, 815–821. https://doi.org/10.1099/ijs.0.02535-0.

Katoh, K., Rozewicki, J., Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform. 20 (4), 1160–1166. https://doi.org/10.1093/bib/bbx108.

Kevbrin, V.V., Zavarzin, G.A., 1992. Effect of sulfur compounds on the growth of the halophilic homoacetogenic bacterium *Acetohalobium arabaticum*. Microbiology 61, 563–567.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.
Le, S.Q., Gascuel, O., 2008. An improved general amino acid replacement matrix. Mol. Biol. Evol. 25 (7), 1307–1320. https://doi.org/10.1093/molbev/msn067.
Lechner, M., Hernandez-Rosales, M., Doerr, D., Wieseke, N., Thévenin, A., Stoye, J., Hartmann, R.K., Prohaska, S.J., Stadler, P.F., 2014. Orthology detection combining clustering and synteny for very large datasets. PLoS One 9 (8), e105015.
Lu, D.C., Xia, J., Dunlap, C.A., Rooney, A.P., Du, Z.J., 2017. *Gracilimonas halophila* sp. nov., isolated from a marine solar saltern. Int. J. Syst. Evol. Microbiol. 67 (9), 3251–3255. https://doi.org/10.1099/ijsem.0.002093.
Mistry, J., Finn, R.D., Eddy, S.R., Bateman, A., Punta, M., 2013. Challenges in homology search: HMMER3 and convergent evolution of coiled-coil regions. Nucleic. Acids Res. 41 (12), e121.
Munoz, R., Rossello-Mora, R., Amann, R., 2016. Revised phylogeny of *Bacteroidetes* and proposal of sixteen new taxa and two new combination including *Rhodothermaeota* phyl. nov. Syst. Appl. Microbiol. 39, 281–296. https://doi.org/10.1016/j.syapm.2016.04.004.
Nyyssölä, A., Kerovuo, J., Kaukinen, P., Weymarn, N., Reinikainen, T., 2000. Extreme halophiles synthesize betaine from glycine by methylation. J Biol Chem 29, 22196–22201.
Parks, D.H., Chuvochina, M., Waite, D.W., Rinke, C., Skarshewski, A., Chaumeil, P.-A., Hugenholtz, P., 2018. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat. Biotechnol. 36 (10), 996–1004. https://doi.org/10.1038/nbt.4229.
Pikuta, E.V., Hoover, R.B., Bej, A.K., Marsic, D., Whitman, W.B., Krader, P., 2009. *Spirochaeta dissipatitropha* sp. nov., an alkaliphilic, obligately anaerobic bacterium, and emended description of the genus *Spirochaeta* Ehrenberg 1835. Int. J. Syst. Evol. Microbiol. 59, 1798–1804. https://doi.org/10.1099/ijs.0.65862-0.
Potter, S.C., Luciani, A., Eddy, S.R., Park, Y., Lopez, R., Finn, R.D., 2018. HMMER web server: 2018 update. Nucleic Acids Res. 46, W200–W204. https://doi.org/10.1093/nar/gky448.
Ramel, F., Amrani, A., Pieulle, L., Lamrabet, O., Voordouw, G., Seddiki, N., Brèthes, D., Company, M., Dolla, A., Brasseur, G., 2013. Membrane-bound oxygen reductases of the anaerobic sulfate-reducing *Desulfovibrio vulgaris* Hildenborough: roles in oxygen defense and electron link with periplasmic hydrogen oxidation. Microbiology (Reading). 159 (12), 2663–2673. https://doi.org/10.1099/mic.0.071282-0.
Roberts, M.F., 2005. Organic compatible solutes of halotolerant and halophilic icroorganisms. Saline Syst 1, 5. https://doi.org/10.1186/1746-1448-1-5.
Rodriguez-R, L., Konstantinidis, K. (2016) The enveomics collection: a toolbox for 411 specialized analyses of microbial genomes and metagenomes. PeerJ Prepr. 4, e1900v1, doi: 412 10.7287/peerj.preprints.1900.
Samylina, O.S., Sapozhnikov, F.V., Gainova, O.Y., Ryabova, A.V., Nikitin, M.A., Sorokin, D.Y., 2014. Algo-bacterial phototrophic communities of soda lakes in Kulunda Steppe (Altai, Russia). Microbiology 83, 849–860. https://doi.org/10.1134/S0026261714060162.
Sorokin, D.Y., Panteleeva, A.N., Tourova, T.P., Kaparullina, E.N., Muyzer, G., 2011. *Natronoflexus pectinivorans* gen. nov. sp. nov., an obligately anaerobic and alkaliphilic fermentative member of *Bacteroidetes* from soda lakes. Extremophiles 15 (6), 691–696. https://doi.org/10.1007/s00792-011-0399-7.
Sorokin, D.Y., Tourova, T.P., Panteleeva, A.N., Kaparullina, E.N., Muyzer, G., 2012. Anaerobic utilization of pectinous substrates at extremely haloalkaline conditions by *Natranaerovirga pectinivora* gen. nov., sp. nov., and *Natranaerovirga hydrolytica* sp. nov., isolated from hypersaline soda lakes. Extremophiles 16 (2), 307–315. https://doi.org/10.1007/s00792-012-0431-6.
Sorokin, D.Y., Berben, T., Melton, E.D., Overmars, L., Vavourakis, C., Muyzer, G., 2014. Microbial diversity and biogeochemical cycling in soda lakes. Extremophiles 18, 791–809. https://doi.org/10.1007/s00792-014-0670-9.
Sorokin, D.Y., Banciu, H.A., Muyzer, G., 2015. Functional microbiology of soda lakes. Curr. Opin. Microbiol. 25, 88–96. https://doi.org/10.1016/j.mib.2015.05.004.
Sorokin, D., Muntyan, M., Toshchakov, S., Korzhenkov, A., Kublanov, I., 2018. Phenotypic and genomic properties of a novel deep-lineage haloalkaliphilic member of the phylum *Balneolaeota* from soda lakes possessing Na+-translocating proteorhodopsin. Front. Microbiol. 9, 2672. https://doi.org/10.3389/fmicb.2018.02672.
Sorokin, D.Y., 2017. Anaerobic haloalkaliphiles. In: Encyclopedia of Life Science, JohnWiley & Sons, Ltd., Chichester, https://doi.org/10.1002/9780470015902.a0027654.
Stookey, L.L., 1970. Ferrozine – a new spectrophotometric reagent for iron. Anal. Chem. 42, 779–781. https://doi.org/10.1021/ac60289a016.
Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E.P., Zaslavsky, L., Lomsadze, A., Pruitt, K.D., Borodovsky, M., Ostell, J., 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 44 (14), 6614–6624. https://doi.org/10.1093/nar/gkw569.
Trüper, H.G., Schlegel, H.G., 1964. Sulphur metabolism in Thiorhodaceae I. Quantitative measurements on growing cells of *Chromatium okenii*. Antonie Van Leeuwenhoek 30, 225–238. https://doi.org/10.1007/BF02046728.
Urios, L., Agogué, H., Lesongeur, F., Stackebrandt, E., Lebaron, P., 2006. *Balneola vulgaris* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* from the north-western Mediterranean Sea. Int. J. Syst. Evol. Microbiol. 56, 1883–1887. https://doi.org/10.1099/ijs.0.64285-0.
Varani, A.M., Siguier, P., Gourbeyre, E., Charneau, V., Chandler, M., 2011. ISsaga is an ensemble of web-based methods for high throughput identification and semi-automatic annotation of insertion sequences in prokaryotic genomes. Genome Biol. 12 (3), R30. https://doi.org/10.1186/gb-2011-12-3-r30.
Vargas, M.A., Rodríguez, H., Moreno, J., Olivares, H., Del Campo, J.A., Rivas, J., 1998. Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. J. Phycol. 34, 812–817. https://doi.org/10.1046/j.1529-8817.1998.340812.x.
Vishnuvardhan Reddy, S., Aspana, S., Tushar, D.L., Sasikala, C., Ramana, C.V., 2013. *Spirochaeta sphaeroplastigenens* sp. nov., a halo-alkaliphilic, obligately anaerobic spirochaete isolated from soda lake Lonar. Int. J. Syst. Evol. Microbiol. 63, 2223–2228. https://doi.org/10.1099/ijs.0.046292-0.
Wang, Y.X., Liu, J.H., Xiao, W., Zhang, X.X., Li, Y.Q., Lai, Y.H., Ji, K.Y., Wen, M.L., Cui, X.L., 2012. *Fodinibius salinus* gen. nov., sp. nov., a moderately halophilic bacterium isolated from a salt mine. Int. J. Syst. Evol. Microbiol. 62 (2), 390–396. https://doi.org/10.1099/ijs.0.025502-0.
Wang, Y.X., Liu, J.H., Xiao, W., Ma, X.L., Lai, Y.H., Li, Z.Y., Ji, K.Y., Wen, M.L., Cui, X.L., 2013. *Aliifodinibius roseus* gen. nov., sp. nov., and *Aliifodinibius sediminis* sp. nov., two moderately halophilic bacteria isolated from salt mine samples. Int. J. Syst. Evol. Microbiol. 63 (8), 2907–2913. https://doi.org/10.1099/ijs.0.043869-0.
Wolin, E.A., Wolin, M.J., Wolfe, R.S., 1963. Formation of methane by bacterial extracts. J. Biol. Chem. 238 (8), 2882–2886. https://doi.org/10.1016/S0021-9258(18)67912-8.
Xia, J., Xie, Z.H., Dunlap, C.A., Rooney, A.P., Du, Z.J., 2017. *Rhodohalobacter halophilus* gen. nov., sp. nov., a moderately halophilic member of the family *Balneolaceae*. Int. J. Syst. Evol. Microbiol. 67 (5), 1281–1287. https://doi.org/10.1099/ijsem.0.001806.
Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.-H., Whitman, W.B., Euzéby, J., Amann, R., Rosselló-Móra, R., 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat. Rev. Microbiol. 12, 635–645. https://doi.org/10.1038/nrmicro3330.
Zavarzina, D.G., Kolganova, T.V., Boulygina, E.S., Kostrikina, N.A., Tourova, T.P., Zavarzin, G.A., 2006. *Geoalkalibacter ferrihydriticus* gen. nov., sp. nov., the first alkaliphilic representative of the family *Geobacteraceae*, isolated from a soda lake. Microbiology 78, 673–682. https://doi.org/10.1134/S0026261706060099.
Zavarzina, D.G., Tourova, T.P., Kolganova, T.V., Bouligina, E.S., Zhilina, T.N., 2009. Description of *Anaerobacillus alkalilacustre* gen. nov., sp. nov. - strictly anaerobic diazotrophic bacillus isolated from soda lake and transfer of *Bacillus arseniciselenatis, Bacillus macyae*, and *Bacillus alkalidiazotrophicus* to *Anaerobacillus* as the new combinations *A. arseniciselenatis* comb. nov., *A. macyae* comb. nov., and *A. alkalidiazotrophicus* comb. nov. Microbiology 78, 723–731. https://doi.org/10.1134/S0026261709060095.
Zavarzina, D.G., Zhilina, T.N., Kostrikina, N.A., Toshchakov, S.V., Kublanov, I.V., 2020. *Isachenkonia alkalipeptolytica* gen. nov. sp. nov., a new anaerobic, alkaliphilic proteolytic bacterium capable of reducing Fe-(III) and sulfur. Int. J. Syst. Evol. Microbiol. 70, 4730–4738. https://doi.org/10.1099/ijsem.0.004341.
Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., Yang, Z., Busk, P.K., Xu, Y., Yin, Y., 2018. dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. 46 (W1), W95–W. https://doi.org/10.1093/nar/gky418.
Zhilina, T.N., Zavarzin, G.A., Rainey, F., Kevbrin, V.V., Kostrikina, N.A., Lysenko, A.M., 1996. *Spirochaeta alkalica* sp. nov., *Spirochaeta africana* sp. nov., and *Spirochaeta asiatica* sp. nov., alkaliphilic anaerobes from the continental soda lakes in Central Asia and the East African Rift. Int. J. Syst. Bacteriol. 46, 305–312. https://doi.org/10.1099/00207713-46-1-305.
Zhilina, T.N., Garnova, E.S., Tourova, T.P., Kostrikina, N.A., Zavarzin, G.A., 2001. *Halonatronum saccharophilum* gen. nov. sp. nov.: a new haloalkaliphilic bacterium of the order *Haloanaerobiale*s from Lake Magadi. Microbiology 70, 64–72. https://doi.org/10.1023/A:1004896905579.
Zhilina, T.N., Garnova, E.S., Tourova, T.P., Kostrikina, N.A., Zavarzin, G.A., 2001. *Amphibacillus fermentum* sp. nov. and *Amphibacillus tropicus* sp. nov., new alkaliphilic, facultatively anaerobic, saccharolytic bacilli from Lake Magadi. Microbiology 70, 711–722. https://doi.org/10.1023/A:1013196017556.
Zhilina, T.N., Appel, R., Probian, C., Brossa, E.L., Harder, J., Widdel, F., Zavarzin, G.A., 2004. *Alkaliflexus imshenetskii* gen. nov. sp. nov., a new alkaliphilic gliding carbohydrate-fermenting bacterium with propionate formation from a soda lake. Arch. Microbiol. 182, 244–253. https://doi.org/10.1007/s00203-004-0722-0.
Zhilina, T.N., Kevbrin, V.V., Turova, T.P., Lysenko, A.M., Kostrikina, N.A., Zavarzin, G. A., 2005. *Clostridium alkalicellum* sp. nov., an obligately alkaliphilic cellulolytic bacterium from a soda lake in the Baikal region. Microbiology 74, 557–566. https://doi.org/10.1007/s11021-005-0103-y.
Zhilina, T.N., Zavarzina, D.G., Detkova, E.N., Patutina, E.O., Kuznetsov, B.B., 2015. *Fuchsiella ferrireducens* sp. nov., a novel haloalkaliphilic, lithoautitrophic homoacetogen capable pf iron reduction, and emendation of the description of the genus *Fuchsiella*. Int. J. Syst. Evol. Microbiol. 65, 2432–2440. https://doi.org/10.1099/ijs.0.000278.