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Natronospira bacteriovora sp. nov., and *Natronospira elongata* sp. nov., extremely salt-tolerant predatory proteolytic bacteria from soda lakes and proposal to classify the genus *Natronospira* into *Natronospiraceae* fam. nov., and *Natronospirales* ord. nov., within the class *Gammaproteobacteria*

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ABSTRACT

The genus *Natronospira* is represented by a single species of extremely salt-tolerant aerobic alkaliphilic proteolytic bacterium, isolated from hypersaline soda lakes. When cells of Gram-positive cocci were used as a substrate instead of proteins at extremely haloalkaline conditions, two new members of this genus were enriched and isolated in pure culture from the same sites. Strains AB-CW1 and AB-CW4 are obligate aerobic heterotrophic proteolytic bacteria able to feed on both live and dead cells of staphylococci and a range of proteins and peptides. Similar to the type species, *N. proteinivora*, the isolates are extremely salt-tolerant obligate alkaliphiles. However, *N. proteinivora* was unable to use bacterial cells as a substrate. Electron microscopy showed direct contact between the prey and predator cells. Functional analysis of the AB-CW1 and AB-CW4 genomes identified two sets of genes coding for extracellular enzymes potentially involved in the predation and proteolysis, respectively. The first set includes several copies of lysozyme-like GH23 peptidoglycan-lyase and murein-specific M23 [Zn]-dipeptidase enabling the cell wall degradation. The second set features multiple copies of secreted serine and metallopeptidases apparently allowing for the strong proteolytic phenotype. Phylogenomic analysis placed the isolates into the genus *Natronospira* as two novel species members, and furthermore indicated that this genus forms a deep-branching lineage of a new family (*Natronospiraceae*) and order (*Natronospirales*) within the class *Gammaproteobacteria.* On the basis of distinct phenotypic and genomic properties, strain AB-CW1^T (JCM 335396 = UQM 41579) is proposed to be classified as *Natronospira elongata* sp. nov., and AB-CW4^T (JCM 335397 = UQM 41580) as *Natronospira bacteriovora* sp. nov.

Introduction

Hypersaline soda lakes with salt concentration reaching saturation represent an unique type of inland salt lakes with molar concentrations of sodium carbonate/bicarbonate as a soluble alkalinity buffer, maintaining stable pH values at ca. 9.5–11. In contrast to chloride/sulfate hypersaline lakes and solar salterns, soda lakes are often characterized by a dense population of primary producers, including haloalkaliphilic cyanobacteria and unicellular algae, and by a highly productive and functionally diverse prokaryotic community (Krienitz and Schagerl, 2016; Oduor and Schagerl, 2007; Samylina et al., 2014). The functional microbial diversity of the soda lake communities in these unique habitats has been studied by both intensive culturing and phenotypic characterization of pure cultures and molecular biology studies over the past 20 years, fueled by both fundamental interest in life at double extreme conditions (reviewed by Sorokin, 2017; Sorokin et al., 2014; 2015; Grant and Jones, 2016) and the biotechnological potential of alkali-stable hydrolytic enzymes (Fujinami and Fujisawa, 2010; Sarethy et al.,

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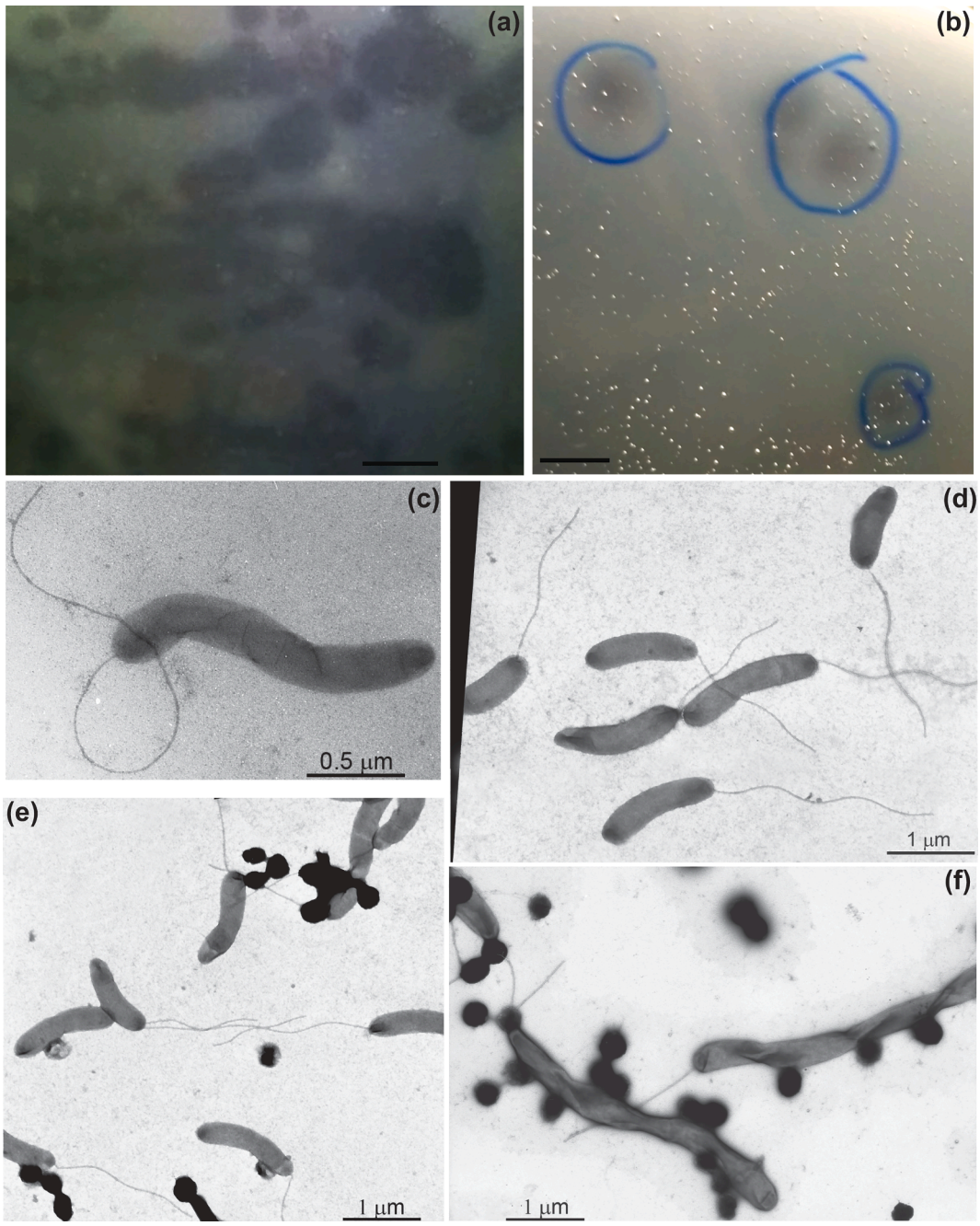

Fig. 1. Colony (**a-b**) and cell morphology (**c-f**) of predatory *Natronospira* strains AB-CW1 (right column) and AB-CW4 (left column) grown pH 9.5, 2 M total Na^+ and 37 °C. (**a** and **b**), surface colonies forming clearance lytic plaques on a solid medium with *Staphylococcus* cells, scale bar 5 mm; (**c** and **d**) electron microscopy microphotographs of cells grown on peptone; (**e** and **f**) electron microscopy microphotographs from cultures grown with *Staphylococcus* cells.

2011; Uma et al., 2020).

However, proteolytic prokaryotes from these soda lake environments are poorly represented in these studies. Only a single dedicated proteolytic anaerobe has ever been obtained in pure culture from soda lakes represented by a haloalkaliphilic *Bacillota* member, *Proteinivorax tanatarense* (Kevbrin et al., 2013). Aerobic proteolytic bacteria isolated from soda lakes include extremely salt-tolerant alkaliphilic *Bacteroidota* members, *Natronotalea proteinilytica* and *Longimonas alkaliphilus*, and a member of the *Gammaproteobacteria* – *Natronospira proteinivora* (Sorokin et al., 2017; Sorokin and Merkel, 2022). These isolates were not capable of microbial cell predation, although *Proteinivorax* originated from an enrichment with decaying cyanobacterial biomass. Recently, we used cells of Gram-positive cocci (possessing thick murein cell wall) to enrich potentially biomass-degrading aerobic proteolytic bacterial predators from hypersaline soda lakes at pH 10. The enrichments at low salt (0.6 M total Na^+) resulted in isolation of a moderately salt-tolerant proteolytic alkaliphile *Wenzhouxiangella* sp. AB-CW3, a gammaproteobacterium capable of killing its prey by producing peptide-based lantibiotic, which is unusual for Gram-negative bacteria, and lyzing the prey cells in direct contact using its abundant extracellular proteolytic complex (Sorokin et al., 2020). Similar type of enrichments but at higher salinity (2 and 4 M total Na^+) selected for two extremely salt-tolerant alkaliphilic proteolytic strains belonging to the genus *Natronospira* able to use bacterial cells as food, apparently using not only their proteases but also peptidoglycan hydrolases.

Here we describe the phenotypic and genomic properties of these

bacteria and propose to classify them as two new species of the genus *Natronospira*, which, in turn, is suggested to form a new family and order in the class *Gammaproteobacteria*.

Materials and methods

Inoculum and enrichment conditions

The upper 1 cm of oxic sediments and near-bottom brines from four hypersaline soda lakes were obtained from the south of Kulunda Steppe (Altai region, Russia) in July 2022. The salt concentration of the brines ranged from 250 − 430 g l^{-1}, the pH from 10.2 − 10.8 and the carbonate alkalinity from 3.5 − 4.0 M. The 1:1 sediment:brine slurries from individual lakes were mixed in equal proportions and were used as an inoculum (5 % v/v). The enrichment media containing either 2 or 4 M total Na^+ was based on sodium carbonate/bicarbonate buffer, each also containing 0.2 M of Na^+ as NaCl, with a final pH of 10. Cells of *Staphylococcus aureus* DSM 20231 were grown in the LB medium, separated by centrifugation and washed 2 times with sterile 0.1 M NaCl. The concentrated cell preparation was divided in two parts: one was kept alive at 4 °C and the second was autoclaved at 120 °C for 20 min and once again subjected to centrifugation and two washing steps to remove released soluble proteins. Both preparation were added to the final cultivation medium to an OD_{600} of 2.0. The enrichments were incubated at 37 °C on a rotary shaker at 150 rpm until visible decrease of turbidity and microscopic evidence of prey cell degradation and appearance of new morphotypes. These primary cultures were then serially diluted up to (10^{10}) in the same media and maximum positive dilutions (10^8-10^{10}) were surface-plated onto a solid medium prepared by 3:2 mixing of the liquid medium and melted 4.5 % washed agar at 50 °C (Daishin, Brunschwig Chemie BV, Amsterdam) resulting in plates in which *Staphylococcus* cells formed an uniform opaque background. The plates were incubated at 37 °C for 3–4 weeks until appearance of colonies forming clearance plaques (Fig. 1 **a, b**). Those were transferred into the liquid medium with 2 M total Na^+ (pH 9.5) with *Staphylococcus* cells, and eventually resulted in isolation of two pure bacterial cultures, strains AB-CW1 and AB-CW4, capable of predating on *Staphylococcus* cells.

Apart from *Staphylococcus, Micrococcus luteus* DSM 20030 with the same type of cell wall but with larger cells (also pregrown in the LB medium) was also used as a prey for the isolates. In addition, three pure cultures of haloalkaliphilic prokaryotes from soda lakes were tested: *Isoptericola* sp. (*Actinobaceteria*), a Gram-negative gammaproteobacterium *Halomonas alkaliphilus*, and a natronoarchaeon *Natronococcus amylolyticus* (all from a personal collection). The bacteria were grown on soluble starch at 1 M Na^+ and *Natronococcus* – at 4 M Na^+ (pH 9.5) and the cells were prepared in a similar way as for *Staphylococcus*.

Microscopy and chemotaxonomy

The progress of growth at predatory conditions was examined by phase contrast microscopy (Zeiss Axioplan Imaging 2 microscope, Göttingen, Germany) and electron microscopy was used to examine flagellation and cell–cell interaction. For the latter, the cells were centrifuged, resuspended in 2 M NaCl and fixed with *para*-formaldehyde (final concentration 3 %, v/v) at room temperature for 2 h, then washed again with the same NaCl solution, positively contrasted with 1 % (w/v) uranyl acetate and examined under a JEOL 100 electron microscope (Japan).

Membrane polar lipids and respiratory quinones were extracted from freeze-dried cells grown at 37 °C at optimal salt/pH conditions with peptone from casein until the late exponential growth phase. Intact polar lipids were extracted with a modified Bligh-Dyer procedure and analysed by Ultra High Pressure Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-$HRMS^n$), as described previously (Bale et al., 2021). For the polar lipid fatty acids profiling, the material was hydrolyzed in HCl/MeOH (1.5 N) followed by three successive extractions with dichloromethane. Fatty acids were derivatized with diazomethane (CH_2N_2) and alcohol groups were silylated with BSTFA. Identification and quantitation of (hydroxy) fatty acids was performed using an Agilent Technologies 7890B GC equipped with a silica column (CP Sil-5, 25 × 0.32 mm) coupled to an Agilent Technologies 5975C VL MSD mass spectrometer operated at 70 eV, with a mass range *m/z* 50–800 and a scan rate of 3 scans s^{-1} (Bale et al., 2019).

Growth physiology

A sodium carbonate/bicarbonate buffer containing 0.2–4.5 M total Na^+ with a pH of 9.5–10 was used for routine cultivation experiments and for testing the salinity range. To examine the pH, range the media contained 2 M total Na^+ as NaCl and 50 mM HEPES/50 mM K-P buffer (pH from 6 to 8), bicarbonate/NaCl (for pH 8–8.5) and bicarbonate/carbonate (for pH 8.5–11). The measured pH at the end of experiments was considered representative for the whole experiment. The temperature range for growth was measured at optimal Na^+-pH with peptone from casein as substrate within the range from 20 to 55 °C. All media were supplemented with 1 mM Mg sulfate and 1 ml/L of acidic trace metal solution (Pfennig and Lippert, 1966).

Genome sequencing, phylogenomic analysis and functional genome analysis

Genomic DNA was obtained from the freshly grown cells of AB-CW1 and AB-CW4 using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, United States). A shotgun WGS library preparation and sequencing were performed using KAPA HyperPlus Library Preparation Kit (KAPA Biosystems, UK) and NovaSeq 6000 system (Illumina, San Diego, CA, USA). The genomes were assembled with Unicycler v.0.5.0 (Wick et al., 2017) and submitted for automatic annotation to the PGAP (Tatusova et al., 2016) in GenBank. The genome statistics are given in Supplementary Table S1**.** For phylogenomic reconstructions, 120 single copy conserved bacterial marker proteins were used according to the Genome Taxonomy Database (Rinke et al., 2021), aligned using GTDB-Tk v2.3.0 (Chaumeil et al., 2022) and trimmed by trimAl 2.rev0 build 2019–08-05 using "-automated1" (optimized for Maximum Likelihood phylogenetic tree reconstruction) and "-gt 0.98" modes (Capella-Gutiérrez et al., 2009) resulting in 17,657 aa length alignment. The trees were built with the IQ-TREE2 program v2.2.0.3 (Minh et al., 2020) with fast model selection via ModelFinder (Kalyaanamoorthy et al., 2017) and ultrafast bootstrap approximation (Minh et al., 2013) as well as approximate likelihood-ratio test for branches (Anisimova and Gascuel, 2006). Relative evolutionary divergence (RED) were calculated according to Parks et al. (2018) https://github.com/donovan-h-parks/PhyloRank) and bac120_r214 tree from GTDB repository.

The whole genome comparison included Average Nucleotide Identity (ANI), using Pyani 0.2.12 (Pritchard et al., 2016); Average Amino acid Identity (AAI) by the EzAAI v1.1 (Kim et al., 2021) and digital DNA-DNA hybridization (DDH) by the Genome-to-Genome Distance Calculator 3.0 online tool (https://ggdc.dsmz.de/ggdc.php). The genome assemblies of strains AB-CW1 and AB-CW4 are deposited in the GenBank under accession numbers GCA_034931365 and GCA_030848495, respectively.

Both genomes were blast-searched for lysozyme-like glycoside hydrolases in dbCAN3 (Zheng et al., 2023) and for extracellular peptidases-proteases in MEROPS 12.5 (Rawlings et al., 2018) databases and the selected protein sequences functionality were further manually checked in UniProt release 2023_05. Proteins potentially involved in haloalkaline adaptations were also identified.

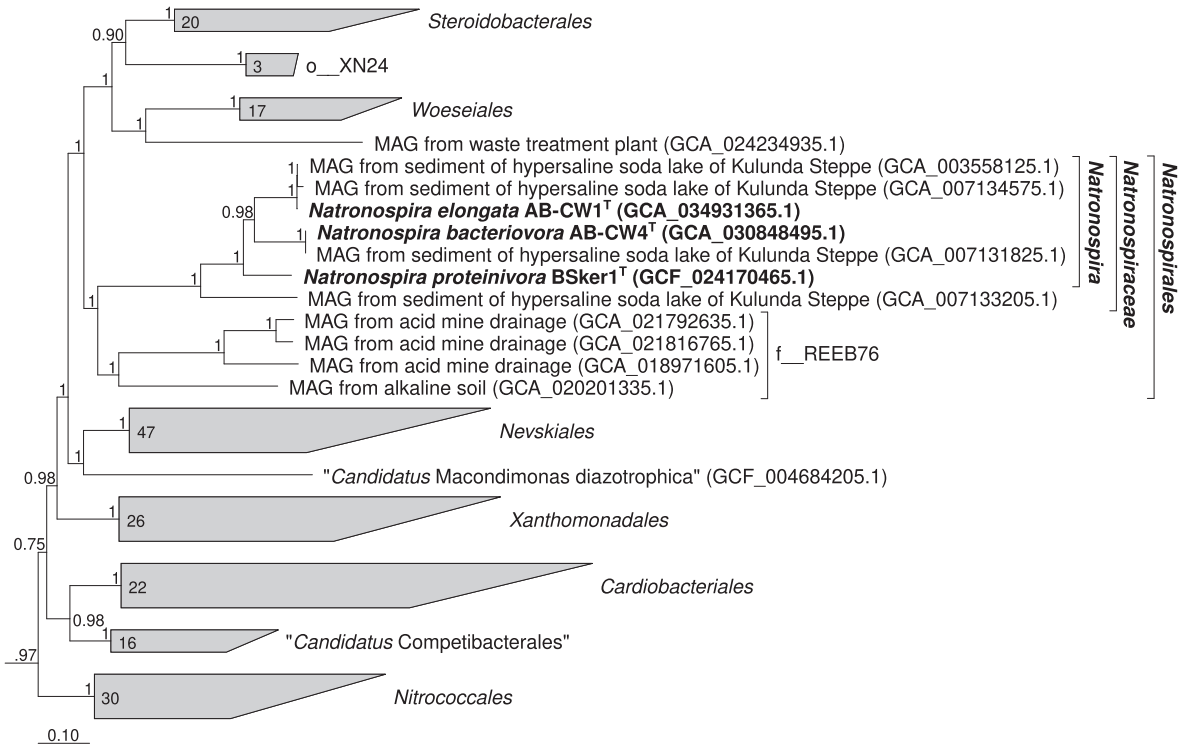

Fig. 2. Phylogenetic placement of strains AB-CW1 and AB-CW4 within the genus *Natronospira* and of the genus *Natronospira* as a separate family-order lineage within the class *Gammaproteobacteria* based on concatenated amino acid sequences of 120 bacterial single copy conserved marker proteins with taxonomic designations according to the Genome Taxonomy DataBase. The length of the alignment is 17,657 aa. Bootstrap consensus tree is shown with values placed at the nodes. Bar, 0.1 change per position.

Results and discussion

Phylogenomic analysis and classification

According to the 16S rRNA gene sequences analysis, strains AB-CW1 and AB-CW4 belonged to the genus *Natronospira* on the level of two new species with the sequence identity to *N. proteinivora* BSker1^T of 97.09 % and 96.76 %, respectively, and 98.58 % between each other (Supplementary Fig. S1). ANI, AAI, DDH and comparative phylogenomic analysis using 120 bacterial conserved protein markers confirmed this placement. ANI values of AB-CW1 and AB-CW4 compared with *N. proteinivora* BSker1^T were 84.1 % and 84.2 %, respectively, and 85.9 % when compared with each other. AAI values of AB-CW1 and AB-CW4 compared with *N. proteinivora* BSker1^T were 74.6 % and 74.1 %, respectively, and 76.1 % when compared with each other. The digital DDH values of AB-CW1 and AB-CW4 compared with *N. proteinivora* BSker1^T are 19.9 % and 20.3 %, respectively, and 22.3 % compared with each other. These values are in agreement with a new-species status of the predatory isolates within the genus *Natronospira*. Strains AB-CW1 and AB-CW4 have closely related MAGs assembled from the same soda lakes (Fig. 2) (Vavourakis et al., 2018; Vavourakis et al., 2019). All three MAGs were assembled from sediments where they accounted for 0.16 % to 0.36 % of the prokaryotic community.

Phylogenetic reconstruction based on 120 bacterial conserved protein markers (Parks et al., 2018) placed all three strains as a single genus-level cluster with 100 % statistically branch support (Fig. 2). This is in agreement with our earlier suggestion (Sorokin and Merkel, 2022) that this genus, which is formerly classified as a member of the family *Ectothiorhodospiraceae* (REF), is a part of a deep independent branch within the class *Gammaproteobacteria* at the level of a separate order. Genus *Natronospira,* together with a MAG GCA_007133205.1 from a surface sediment of a hypersaline soda lake of Kulunda Steppe (Vavourakis et al., 2019), form a family-level lineage that is called f_SLND01 in GTDB 08-RS214 and has 100 % branch support in our and GTDB reconstructions. Consequently, we propose the *Natronospiraceae* family for this cluster (Fig. 2). The *Natronospiraceae* and a phylogenetic cluster called f_REEB76 in GTDB 08-RS214, which includes three MAGs from acid mine drainage and one MAG from an alkaline soil, together form an order-level lineage that has 73% branch support in the GTDB reconstruction, but 100% in our reconstruction (Fig. 2). Consequently, we propose *Natronospirales* for this new order-level cluster. It has 0.655 relative evolutionary divergence (RED) value, which is much closer to the median value of 0.610 for bacterial orders in GTDB 08-RS214, than in case of separate orders o_SLND01 (RED value 0.827) and o_REEB76 (RED value 0.862) proposed in GTDB 08-RS214. Thus, f_REEB76 is a group of yet uncultured bacteria belonging to the order *Natronospirales*. It consists of a group of MAGs assembled from acid mine drainage (Gao et al., 2022) and one deep-branching MAG from alkaline desert soil (Mandakovic et al., 2020), while the entire family *Natronospiraceae* consists only of isolates and MAGs from the hypersaline soda lakes of Kulunda Steppe. This would suggests a limited environmental occurrence of members of the order *Natronospirales*. However, 32 16S rRNA gene sequences that were >95 % similar to those of the *Natronospira* species were identified. All of them were from alkaline coastal soils from the Gulf of Cambay, Gujarat, India (Keshri et al., 2015) or from alkaline saline soils of the former lake Texcoco (Valenzuela-Encinas et al., 2009). This indicates a wider environmental occurrence of the members of the *Natronospirales* although they seem to be restricted to alkaline environments.

Morphology and predatory behaviour

Cells of both isolates grown on peptones were vibrio to short spirilla with a single polar flagellum (Fig. 1 **c, d**). Cells grown on peptones, both colonial and in liquid culture, contained a yellow membrane-bound pigment with an absorption maximum at 480 nm in methanol:

acetone (7:3, v:v) extract, similar to the type species of *Natronospira*.

When grown with *Staphylococcus* cells as substrate, cells of AB-CW4 remained short, while AB-CW1 formed elongated, loosely coiled spirilla. Electron microscopy revealed that the predatory activity of both strains was accomplished by direct contact with the prey cells (Fig. 1 **e, f**). This has been similarly observed in a moderately salt-tolerant predatory *Wenzhouxiangella* sp. AB-CW3 (Sorokin et al., 2020). While cells of the latter produced multiple fimbria-like filaments, most probably responsible for the adhesion to prey and also encoded a large fimbrial protein of 3,068 aa (WP_190974951), neither the fimbria and the gene were present in the cells and genomes of the AB-CW1 and AB-CW4 isolates. Apparently, AB-CW strains are using another mechanism for the prey cell attack. A possible option are the pili (part of the secretion systems type II and IV), which are also microtubular surface structures but shorter than fimbria and serving multiple purposes, such as secretion of toxins and extracellular hydrolases (type II) and cell adhesion and uptake of extracellular DNA (type IV) (de Masi et al., 2013). Both genomes contain several loci encoding such systems (Supplementary Table S2).

The respiratory quinone and membrane lipid composition of both strains is similar. The only quinone species present is ubiquinone UQ-8. The dominant identified membrane phospholipids in both isolates include phosphatidylcholines (PC) and less abundant phosphatidylethanolamines (PE) and phosphatidylglycerols (PG). The dominant polar lipid fatty acids included *i*-C17:0 and *i*-C17:1ω9c, similar to the type *Natronospira* species. In addition, novel isolates also have a significant fraction of the *i*-C19:1ω9c and a much higher proportion of the C16:0 (Supplementary Table S3).

Growth physiology

Similar to *N. proteinivorans*, AB-CW1 and AB-CW4 are obligately aerobic organoheterotrophs utilizing various proteins and peptides as the growth substrate, including alpha-keratin (fine powdered fraction), gelatin, casein, filter-sterilized bovine serum albumin and lactalbumin, soy protein, bovine collagen, and various peptones and yeast extract. Furthermore, a weak growth was observed with soluble starch for AB-CW1 and, for both strains, with maltose. None of the other tested single carbon compounds including C2-C6 organic acids, alcohols (glycerol, methanol, ethanol) and C5-C6 sugars supported growth. Tests for anaerobic growth, either fermentative with maltose or respiratory with maltose as substrate and nitrate or sulfur as electron acceptors were negative. Ammonium and amino acid nitrogen can be used as the N-source by both isolates, while urea and nitrate did not support growth with maltose as the carbon and energy substrate.

A major phenotypic property of the new isolates is their ability to predate on bacterial cells. The most active growth was observed on heat-sterilized cells of *Staphylococcus*, followed by live cells of the latter with nearly full prey digestion within 1 and 2 weeks, respectively. They were also able to grow on cells of two haloalkaliphilic bacteria isolated from the same soda lakes belonging to *Actinobacteria* and *Gammaproteobacteria*, although much less actively and with incomplete degradation of prey cells. No proliferation and cell degradation occurred when the prey cells were represented by a natronarchaeon *Natronococcus amylolyticus*. A possible reason for this may be that the sarcina-like tetracocci formed by *Natronococcus* are covered with a thick polysaccharide matrix and do not lyse even in distilled water, in contrast to most known haloarchaeal species (Albers and Meyer, 2011), making them inaccessible for the proteolytic complex produced by the predatory AB-CW isolates. It would be interesting to test other natronoarchaea with different type of cell wall as a prey, but it probably would need a separate enrichment to select for a bacterial predator capable of degrading the haloarchaeal *S*-layer glycoproteins. In contrast to the AB-CW strains, none of the predatory activity was observed in the type species *N. proteinivorans*.

Salt (as sodium carbonates) and pH (at 2 M total Na^+) profiles of AB-CW1 and AB-CW4 grown on peptone were, in general, similar to what has been reported for the type strain of *Natronospira*. This characterizes the new isolates as extremely salt-tolerant obligate alkaliphiles: the salinity ranged from 0.75 to 1.0 to 3.5–4.0 M total Na^+ (optimum at 2–2.5 M) and the pH from 8.1 to 10.5 with an optimum at 9.5. The temperature range (optimum) for both strains determined at pH 9.5 and 2 M total Na^+ were 20–48 and 35 °C, respectively. A phenotypic comparison of AB-CW1, AB-CW4 and *N. proteinivora* BSker1^T is given in Table 1.

Table 1
Comparative properties of predatory strains AB-CW1 and AB-CW4 and the type strain of the genus *Natronospira*.

Property	**AB-CW1**	**AB-CW4**	*Natronospira proteinivora* BSker1^T
Cell morphology	Motile curved rod	Motile short spirillum	Motile spirillum
Yellow pigment	+	+	+
Relation to oxygen	Obligate aerobe	Obligate aerobe	Obligate aerobe
Growth substrates			
bacterial cells	G + -cocci *Isoptericola* sp. (partial) *Halomonas alkaliphilus*	G + -cocci *Isoptericola* sp. (partial) *Halomonas alkaliphilus* (partial)	−* −* −*
proteins	gelatine, casein, albumins,collagen, alpha-keratin	gelatine, casein, albumin,collagen, alpha-keratin	gelatine, casein, albumin, alpha-keratin
peptones (casein, meat)	(w)	(w)	+
starch			−
maltose	++ (w)+ (w)	+ (w)+ (w)	−
Salinity range (opt.), M Na^+ (at pH 9.5)	0.75–3.5 (1.5–2.0)	1.0–4.0 (2.0)	1.0–4.5 (2.0–2.5)
pH range (opt.) at 2 M Na^+	8.2–10.55 (9.5)	8.1–10.42 (9.5)	8.5–10.25 (9.5)
Max. temperature (°C) (at 2.0 M Na^+ and pH 9.5)	48	45	45
Predominant polar lipid fatty acids	*i*-C17:0, *i*-C17:1ω9c, *i*-C19:1ω9c, C18:1ω9, C16:0	*i*-C17:0, *i*-C17:1ω9c, *i*-C19:1ω9c, C16:0	iC17:0, iC17:1ω9c
Respiratory lipoquinone	UQ-8	UQ-8	UQ-8
Genome size (Mbp)	3.1	3.0	2.9
G + C, % (genomic)	61.5	62.5	60.0
Habitat	Hypersaline soda lakes		

Features common for all 3 strains: inability to utilize urea and nitrate as the N-source; ability to utilize sulfate as the sulfur source; positive tests for cytochrome oxidase and catalase and negative for lipase (with Tween80); absence of tryptophanase gene *trnA,* indicative of the inability to produce indole from tryptophane.
* this work; w, weak growth; partial, incomplete prey cells lysis.

Functional genome analysis

In the functional genomic analysis, we focused on the genetic repertoire potentially responsible for the predatory phenotype of the new isolates and haloalkaliphilic adaptation. According to what is known from other specialized predatory bacteria (Bratanis et al., 2020), two major sets of extracellular, and, to a lesser extent, membrane-bound proteins, which might be involved in predation, were encoded by both genomes. The first set includes murein-specific glycosyl hydrolases and di-peptidases which, acting together, can hydrolyze the cell wall.

Table 2
Extracellular peptidoglycan hydrolases, peptidases and cell-invasion factors encoded in the genomes of *Natronospira* strains AB-CW1 and AB-CW4.

AB-CW1			AB-CW4		
Locus MEA544+	**Enzyme**	**Signal**	**Locus** WP_30672+	**Enzyme**	**Signal**
Peptidoglycan degradation/invasion systems					
4211	peptidoglycan *N*-acetylglucosamine deacetylase CE4	Sec/SPII	6803	M23 peptidoglycan DD- Zn-endopeptidase + LysM*	Sec/SPII
4212	peptidoglycan glucosaminidase GH73	membrane	6804	DedA (toxin:H+ efflux pump)	membrane
4221-4226	Tol-Pal system: TolABQR-Pal-YgbC-YbgF (self-protection from colicins)	membrane Sec/SPI/SPII	7004	peptidoglycan glucosaminidase GH73	membrane
			7005	peptidoglycan *N*-acetylglucosamine deacetylase CE4	Sec/SPII
4573	lyzozyme/peptidoglycan lyase GH23 + LysM	Sec/SPII	7173–7174	TonB/TolB lipoprotein colicin translocation system (killing)	Sec/SPII
4776	lyzozyme/peptidoglycan lyase GH23	Sec/SPI	7175	lyzozyme/peptidoglycan lyase GH23	Sec/SPII
4778	LysM peptidoglycan-binding protein	Sec/SPII	7177	exodeoxyribonuclease VII large subunit	–
4831	M23 peptidoglycan DD- Zn-endopeptidase	membrane	7178	M23 peptidoglycan DD- Zn-endopeptidase	Sec/SPII
4967	M23 peptidoglycan DD- Zn-endopeptidase	Sec/SPI	7180	M48 intramembrane glutamic Zn-endopeptidase	membrane
4968	S41 oligo-endopeptidase	Sec/SPI	7206	lyzozyme/peptidoglycan lyase GH23 + LysM	Sec/SPI
5115	ArnA lipoprotein (antibiotic resistance)	globular	7239	S11 D-alanyl-D-alanine carboxypeptidase (murein recycling)	Sec/SPI
5116	lyzozyme/peptidoglycan lyase GH23	Sec/SPI	7447	lyzozyme/peptidoglycan lyase GH23 + LysM	Sec/SPII
5118	porin: IgA1 protease OMP protein autotransporter	Sec/SPI	7941	lyzozyme/peptidoglycan lyase GH23	Sec/SPI
5119	S9 prolyl endopeptidase	Sec/SPI	7943	LysM peptidoglycan-binding protein	Sec/SPII
5142	lyzozyme/peptidoglycan lyase GH23	Sec/SPII	7999	M23 peptidoglycan DD- Zn-endopeptidase	Sec/SPII
5223	M23 peptidoglycan DD- Zn-endopeptidase	Sec/SPI	8357	S41 oligo-endopeptidase	Sec/SPI
5224	anhydro-N-acetylmuramic acid kinase AnmK (murein recycling)	–	8358	M23 peptidoglycan Zn DD- –endopeptidase	Sec/SPI
5381	S9C prolyl oligopeptidase	Sec/SPI	8602	muropeptide:H^+ symporter AmpG	membrane
5382	antibiotic biosynthesis monooxygenase	–	8603	CotH/Fibronectin III (putative invasion factor)	Sec/SPII
5383	lytic murein transglycosylase/lysozyme GH103	Sec/SPI	8605	S8 endopeptidse (excreted)	globular
5384	proline iminopeptidase (exo) S33 (C-term. membrane)	Sec/SPI	8607	lytic murein transglycosylase/lysozyme GH103	Sec/SPI
5385	OMP lipoprotein	Sec/SPI/SPII	8608–8609	Type II toxin/antitoxin system BrnTA	–
5389–5390	2x TonB (interact with colicin transporter CirAB)	Sec/SPI	8767	OmpA outer membrane protein/porin (colicin export)	Sec/SPI Sec/SPI
5391	M50 Zn-peptidase	Sec/SPI	8768	PilO (type IV secretion: promote cell adhesion)	
5413	M23 peptidoglycan Zn DD-endopeptidase	Sec/SPI	8769	lyzozyme/peptidoglycan lyase GH23 + LysM	Sec/SPI
5435	S9 serine endopeptidase	Sec/SPI	9151	peptidoglycan DD- Zn-endopeptidase M23	Sec/SPI
5440	lyzozyme/peptidoglycan lyase GH23 + LysM	Sec/SPI	9318	CBM9/glycoprotein-N-acetylglucosamine-aminidase	Sec/SPI
5441	M24 Xaa-Pro Mn-dipeptidase	–	9393	LysM peptidoglycan-binding domain	Sec/SPII
5922	S1-C chymotrypsin-like serine protease	Sec/SPII			
5926	LysM peptidoglycan-binding protein	Sec/SPII			
6346	peptidoglycan *N*-acetylglucosamine deacetylase CE4	Sec/SPI			
6349	lyzozyme/peptidoglycan lyase GH23	Sec/SPII			
6518–6519	Lpp20 lipoproteins (putative invasive colonization)	Sec/SPII			
6520	lyzozyme/peptidoglycan lyase GH23 + LysM	Sec/SPII			
6521	M12B Zn-endopeptidase: invasin homologue; promote invasive behavior of the enteropathogens	Sec/SPI			
Extracellular peptidases/proteases					
4299	M14 Zn-carboxypeptidase	Sec/SPI	6884	S41 carboxypeptidase	Sec/SPI
4300	M48B Zn-endoprotease	Sec/SPI	6909	M15B/C/D Zn-carboxy/di-peptidase (endolysin)	Sec/SPI
4312	M15B/C Zn-carboxypeptidase (endolysin)	Sec/SPI	6922	M48 Zn-protease	Sec/SPI
4336	S41 carboxypeptidase	Sec/SPI	6923	M14 Zn-carboxypeptidase	Sec/SPI
4427	M12B Zn-protease (putative caseinase/gelatinase)	Sec/SPII	7052	C40 di-peptidase	Sec/SPI/SPII
4440	M12B Zn-endopeptidase + Fibronectin III binding domain	Sec/SPI	7055	M56 Zn-endopeptidase (antibiotic resistance)	membrane
4478	C40 di-peptidase	Sec/SPI/SPII	7099	Zn-protease M12B(putative caseainase/gelatinase)	Sec/SPI/SPII
4491	M14C Zn-carboxypeptidase (putative murein cycling)	membrane	7121	haemolysin III:killing toxin (pore-forming) (1)	membrane
4669–4670	Fic-family invasion factor	–	7148	S8 endopeptidase	Sec/SPI
4671	S9 dipeptidyl peptidase	Sec/SPI	7201	S9 endopeptidase	Sec/SPI
4745	S8 endopeptidase	Sec/SPI	7362	M48 Zn-protease	Sec/SPII
4879–4881	3 x S8 endopeptidases	Sec/SPI	7449	S49 endopeptidase	membrane
4935	M3 Zn-oligopeptidase	Sec/SPII	7511	S1C chymotrypsin carboxypeptidase	Sec/SPII
5043	S9A prolyl oligopeptidase	Sec/SPI	7597	M28A Zn-aminopeptidase	Sec/SPI
5074	M14C Zn-carboxypeptidase (putative murein cycling)	Sec/SPI	7658	S9A prolyl oligopeptidase	Sec/SPI
5119	prolyl oligopeptidase S9C	Sec/SPI	7677	S8 endopeptidase (extracellular)	globular
5128	S9 endopeptidase	Sec/SPI	7818	S8 endopeptidase (extracellular)	globular
5133–5134	S8 endopeptidases	Sec/SPI	7849	haemolysin III:killing toxin (pore-forming) (2)	membrane
5155	M13 Zn-endopeptidase	Sec/SPII	7874–7876	3 x S8 endopeptidases	Sec/SPI
5163	M3A Zn-oligopeptidase	Sec/SPII	7977	S9 endopeptidase	Sec/SPI
5381	prolyl oligopeptidase S9C	Sec/SPI	8027	S1C chymotrypsin carboxypeptidase (extracellular)	membrane
5399	M12B Zn-endoprotease	Sec/SPI/SPII	8103	S1C chymotrypsin carboxypeptidase (with membrane anchor)	Sec/SPI
5474	S11/13 D-alanyl-D-alanine carboxypeptidase	Sec/SPI	8186	M14C Zn-carboxypeptidase (putative murein cycling)	Sec/SPI
5570	M28A Zn-aminopeptidase	Sec/SPI	8237	S9C prolyl oligopeptidase	Sec/SPII

(*continued on next page*)

Table 2 (*continued*)

AB-CW1			AB-CW4		
Locus MEA544+	**Enzyme**	**Signal**	**Locus** WP_30672+	**Enzyme**	**Signal**
5639	S9C acyl-aminopeptidase (cleave off *N*-acetylglucosamine residue from glycoprotein)	Sec/SPI	8282	M3A Zn-oligopeptidase	Sec/SPII
			8442	beta-aspartyl dipeptidase T2 (works on glycoproteins)	Sec/SPII
5642	P1/S58 L-aminopeptidase (putative peptide antibiotic synthesis)	Sec/SPI	8444	S8A subtilase endopeptidase	Sec/SPI
5649	M19 Zn-dipeptidase	Sec/SPI	8473	M19 Zn-dipeptidase	Sec/SPI
5678	beta-aspartyl dipeptidase T2 (works on glycoproteins)	Sec/SPII	8479	P1/S58 aminopeptidase (putative peptide antibiotic synthesis)	Sec/SPI
5778	S9A prolyl oligopeptidase	Sec/SPI	8509	S9A prolyl oligopeptidase	Sec/SPI
5853	S9A prolyl oligopeptidase	Sec/SPI	8520	S9A prolyl oligopeptidase	Sec/SPII
5856	M28 Zn-aminopeptidase	Sec/SPII	8550	M48 Zn-endoprotease	Sec/SPI
5866	S41 endopeptidase	Sec/SPII	8564	S9A prolyl oligopeptidase	Sec/SPI
5921	integrin (cell–cell adhesion)	Sec/SPI	8584	M12B Zn-endoprotease (membrane anchored)	Sec/SPI
5922	S1-C chymotrypsin-like serine protease	Sec/SPII	8659	M14B Zn-carboxypeptidase	Sec/SPI
6067	S8 endopeptidase	Sec/SPI	8660	S8A fibrinolytic endopeptidase + fibronectin III domain	Sec/SPI
6095	M1 Zn-aminopeptidase	Sec/SPII	8734	M13 Zn-endopeptidase	Sec/SPII
6124	S8 endopeptidase (membrane-anchored at C-terminal)	Sec/SPI	8750–8751	S8A subtilase-like endopeptidases	Sec/SPI
6133–6134	S8 endopeptidases (membrane-anchored at C-terminal)	Sec/SPI	8756	S9C acyl-aminopeptidase (cleave off *N*-acetylglucosamine residue from glycoprotein)	Sec/SPII
6213	S1C chymotrypsin-like endoprotease	Sec/SPI	8837	S8A subtilase endopeptidase	Sec/SPI
6362	S9 endopeptidase	Sec/SPI	8870	Xaa-Pro Mn-aminopeptidase M24B	**TAT/SPI**
6382	S1-C chymotrypsin protease (globular, external)	–	8954	S1C chymotrypsin-like carboxypeptidase	Sec/SPI
6571	Xaa-Pro Mn-aminopeptidase M24B	**TAT/SPI**	8996	M1 Zn-aminopeptidase	Sec/SPII
6664	S1-C chymotrypsin protease	Sec/SPI	9065	S8A subtilase endopeptidase	Sec/SPI
6705	S41A carboxypeptidase	Sec/SPI	9107–9108	S8A subtilase endopeptidases	Sec/SPI
6911–6912	M10 Zn-endopeptidases (virulence invasive factor)	Sec/SPI	9201	S9C di-peptidase	Sec/SPII
			9312	M56 Zn-peptidase (antibiotic resistance)	membrane
			9323	M48 Zn-endoprotease	Sec/SPII
			9328	S10 carboxypeptidase	Sec/SPI
			9343	S1C chymotrypsin-like carboxypeptidase	Sec/SPI
			9349	S41A carboxypeptidase	Sec/SPI
			9358	M28 Zn-aminopeptidase	Sec/SPII
			9361	S9B di-peptidase 4	Sec/SPI
			9385	S9B di-peptidase 4	Sec/SPII

*LysM – peptidoglycan-binding domain similar to CBM50.

Particularly those are lysozyme-like peptidoglycan lyase from the GH23 family (with or without LysM/CBM50 carbohydrate-binding domain), glucosaminidase from the GH73 family and peptidoglycan-active [Zn]-dipeptidases from the M23 family (Table 2). Furthermore, those often also included cell invasion factors, such as M10 and M12 family endpoeptidases, Fic family, haemolysin III, which might be part of the predatory system. Interestingly, however, the genome of *N. proteinivora* also features a similar genetic potential, albeit less abundantly represented, even though this species does not possess the predatory potential. Apparently, in case of *N. proteinivora* the encoded hydrolases are likely involved in the internal murein recycle.

A second set of encoded proteins potentially important for a predatory life style includes multiple copies of extracellular peptidases/proteases among which the most abundant are the serine families S8, S9 and S41 and metallopeptidases (mostly Zn-dependent) from the families M12, M14 and M48. Practically all those excreted hydrolases have Sec/SPI/SPII signal peptide (except only for a single case with the TAT signal), indicating that they do not stop in the periplasm but are crossing the outer membrane and, thus, are capable of direct interaction with the extracellular polymers and the whole (prey) cells (Table 2).

With respect to the haloalkaliphilic adaptation, the genomes of both strains encode the biosynthesis pathway for compatible solutes ectoine and hydroxy-ectoine in a single operon *ectABCD*; two multisubunit Na^+:H^+ antiporters (*mnhEFGABCD1D2D3/mrpEFGBCD1D2D3*); two monosubunit Na^+:H^+ antiporters (*nhaC* and *CPA1*); a K^+:H^+ antiporter *CPA2* and a K^+:H^+ symporter *trkAH*, as has been observed for *N. proteinivora* (Sorokin and Merkel, 2022).

Overall, strains AB-CW1 and AB-CW4 represent the first example of extremely salt-tolerant natronophilic bacteria from a soda lake habitat with a potent predatory potential. On the basis of distant phylogenomics and unique phenotypic properties, the novel isolates are proposed to form two novel species in the genus *Natronospira*, whose genus diagnosis also needs to be amended. Moreover, phylogenomic analysis also suggested that this genus is to be reclassified into a separate family *Natronospiraceae* fam. nov., and order *Natronospirales* ord. nov. The new species and the higher taxa protologues are presented in Table 3.

Amended description of the genus Natronospira Sorokin et al. 2017

In addition to the properties reported earlier (Sorokin et al., 2017; Sorokin and Merkel, 2022), the major polar phospholipids in the members of the genus were identified as phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Some of the *Natronospira* members have the ability to predate on bacterial cells.

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CRediT authorship contribution statement

Dimitry Y. Sorokin: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Alexander Y. Merkel:** Writing – original draft, Methodology, Investigation. **Tatyana V. Kalganova:** Writing – original draft, Methodology. **Nicole J. Bale:**

Table 3
Description of *Natronospirales* ord. nov., *Natronospiraceae* fam. nov., *Natronospira elongata* sp. nov., and *Natronospira bacteriovora* sp. nov.

Parameter	Order: ***Natronospirales***	Family: ***Natronospiraceae***	Species: ***Natronospira elongata***	Species: ***Natronospira bacteriovora***
Order name	*Natronospirales*			
Family name		*Natronospiraceae*		
Species name			*Natronospira elongata*	*Natronospira bacteriovora*
Status	ord. nov.	fam. nov.	sp.nov.	sp.nov.
Description of a new taxon	Na.tro.no.spi.ra'les (N.L. fem. n. *Natronospira*, a bacterial genus; −*ales*, ending to denote an order; N. L. fem. pl. n. *Natronospirales*, the *Natronospira* order). The order encompasses extremely salt-tolerant and obligately alkaliphilic aerobic heterotrophic bacteria utilizing mostly various proteins for growth. Currently include a single family *Natronospiraceae*. A member of the class *Gammaproteobacteria*. The type genus is *Natronospira*.	Na.tro.no.spi.ra.ce'ae (N.L. fem. n. *Natronospira*, a bacterial genus; −*aceae*, ending to denote a family; N.L. fem. pl. n. *Natronospiraceae*, the *Natronospira* family). The family includes extremely salt-tolerant and obligately alkaliphilic aerobic heterotrophic bacteria utilizing mostly various proteins for growth. Currently it includes a single (type) genus *Natronospira*. A member of the order *Natronospirales*, class G*ammaproteobacteria*. The type genus is *Natronospira*.	*Natronospira elongata* (e.lon.ga'ta. L. fem. part. adj. *elongata*, elongated). Cells are Gram-negative, from vibrio to long loose spirilla, 0.25–0.3 x 1–20 μm, motile by a single polar flagellum. The colonies are yellowish, up to 4 mm, flat and round. The polar phospholipids are dominated by phosphatidylcholine and phosphatidylethanolamine with phosphatidylglycerol as a minor component. The polar lipid fatty acids are dominated by *i*17:0, *i*17:1ω9c, 18:1ω9, *i*19:1ω9c and 16:0. The only respiratory lipoquinone is UQ-8. Strictly aerobic organoheterotrophs using mostly proteins and peptides for growth. Also have the capacity to predate on bacterial cells. Obligately alkaliphilic with a pH range for growth from 8.2 to 10.55 and an optimum at pH 9.5. Extremely salt tolerant with the salt range (in the form of sodium carbonates) from 0.75 to 3.5 M of total Na^+ (optimum at 2–2.5 M). The upper temperature limit for growth (at optimal pH and salinity) is 48 °C. The G + C content of the genomic DNA is 61.5 % (genome). The type strain, AB-CW1 (JCM 335396 = UQM 41579), was isolated from a mix sample of aerobic surface sediments and brines of hypersaline soda lakes in Kulunda Steppe (Altai, Russia).	*Natronospira bacteriovora* (bac.te.rio.vo'ra. Gr. neut. n. *bakterion*, a small rod; L. press. part. *vorans*, devouring; N.L fam. adj. *bactriovorans*, devouring bacteria). Cells are Gram-negative, from vibrio to small spirilla, 0.25 x 1–3 μm, motile by a single polar flagellum. The colonies are yellowish, up to 3 mm, flat and round. The polar phospholipids are dominated by phosphatidylcholine and phosphatidyl-ethanolamine with phosphatidylglycerol as a minor component. The polar lipid fatty acids are dominated by *i*17:0, *i*17:1ω9c and *i*19:1ω9c with a less abundant 16:0. The only respiratory lipoquinone is UQ-8. Strictly aerobic organoheterotrophs using mostly proteins and peptides for growth. Also have the capacity to predate on bacterial cells. Obligately alkaliphilic with a pH range for growth from 8.1 to 10.40 and an optimum at pH 9.5. Extremely salt tolerant with the salt range (in the form of sodium carbonates) from 1.0 to 4.0 M of total Na^+ (optimum at 2.0 M). The upper temperature limit for growth (at optimal pH and salinity) is 45 °C. The G + C content of the genomic DNA is 62.5 % (genome). The type strain, AB-CW4 (JCM 335397 = UQM 41580), was isolated from a mix sample of aerobic surface sediments and brines of hypersaline soda lakes in Kulunda Steppe (Altai, Russia).
Type strain			AB-CW1^T	AB-CW4^T
Culture collection numbers			JCM 335396; UQM 41579	JCM 335397; UQM 41580
Genome status			Draft	Draft
GenBank genome assembly			GCA_034931365	GCF_030848495
Genome size (Mbp)			3.1	3.0
16S-rRNA gene locus in the genome			50901–52441	100637–102177
Country of origine			Russia	
Region			Altai region,	
Source of isolation			Aerobic sediments and brines from hypersaline soda lakes	
Latitude			51°39′ N; 49°10′ N; 48°14′ N	
Longitude			79°48′ E; 46°39′ E; 46°35′ E	
Sampling date			July 2022	
pH of the sample			10.2–10.8	
Salinity of the sample			25–43 %	
Number of strains in study			1	
Information regarding to Nagoya protocol			Not applicable	

Writing – review & editing, Writing – original draft, Methodology, Investigation. **Jaap Sininghe Damsté:** Writing – review & editing.

Data availability

Data will be made available on request.

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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.2024.126519.

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Natronospira bacteriovora sp. nov., and *Natronospira elongata* sp. nov., extremely salt-tolerant soda lake predatory proteolytics and proposal to classify the genus *Natronospira* into *Natronospiraceae* fam. nov., and *Natronospirales* ord. nov., within the class *Gammaproteobacteria*.

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Supplementary Information

Table S1. General features of *Natronospira* genomes.

Table S2. Secretion systems type II and the pili type IV in genomes of strains AB-CW1 and AB-CW4.

Table S3. Comparative composition of PLFA in members of the genus *Natronospira* grown at pH 9.5, 37°C until late exponential growth phase. The AB-CW strains very grown on peptone at 3 M total Na^+ and *Natronospira proteinivora* BSker1^T – at 4 M total Na^+ with casein. The major compounds are in bold.

Figure S1. Phylogenetic placement of strains AB-CW1 and AB-CW4 within the genus *Natronospira* based on 16S rRNA gene sequences. Bootstrap consensus tree is shown with values placed at the nodes. Bar, 0.1 change per position.

Table S1.

	N. elongata AB-CW1^T	*N. bacteriovora* AB-CW4^T	*N. proteinivora* BSker1^T
Total length, Mb	3.1	3	2.9
number of contigs	102	15	4
GC, %	61.5	62.5	60
N50	83.5 kb	372 kb	1.6 Mb
Genome coverage	1000.0x	630.0x	512.0x
Completeness, %	95.43	97.18	97.99
Contamination, %	0.42	0.04	0.02
Genes (total)	2792	2,725	2,650
Genes (protein coding)	2729	2,660	2,578
Genes (RNA)	51	52	51
Pseudo genes (total)	12	13	21
Complete rRNAs	1, 1, 1 (5S, 16S, 23S)	1, 1, 1 (5S, 16S, 23S)	1, 1, 1 (5S, 16S, 23S)
tRNAs	44	45	44
GenBank	GCA_034931365.1	GCA_030848495.1	GCF_024170465.1

Table S2.

AB-CW1			AB-CW4		
Locus: MEA544+	Gene	Protein	Locus: WP_30672+	Gene	Protein
Type IV secretion system (pili for cell adhesion, extracellular DNA uptake)					
4695	pilE	type IV pilin	7304	fimT	pseudopilin
4696	pilC	type IV pilin (surface virulence activator)	7305	pilV	pilus assembly protein
4697	pilX	pilus assembly protein	7306	pilW	pilus assembly protein
4698	pilW	pilus assembly protein	7307	pilX	pilus assembly protein
4699	pilV	pilus assembly protein	7308	pilC	type IV pilin (surface virulence activator)
4670; 4746	fimT	pseudopilin	7309	pilE	type IV pilin
4822	fimA	fimbrial pilin with lectin domain	7989	pilA	type IV pilin assembly protein
4823	pilB	type IV-A pilus assembly ATPase	7990	fimA	fimbrial pilin with lectin domain
4824	pilA	type IV pilin assembly protein	7991	pilB	type IV-A pilus assembly ATPase
5262-5263	pilU	type IV-A pilus ATPase (twitching motility)	9113-9114	pilU	type IV-A pilus ATPase (twitching motility)
5906	pilT	type IV pilus twitching motility protein	9114	pilT	type IV pilus twitching motility protein
Type II secretion (pili for toxin and extracellular proteins translocation)					
4924	gspG	type II secretion system proteins	7730	gspM	type II secretion system proteins
4925	gspF		7731	gspL	
4926	gspE		7732	gspN	
4927	gspF		7733	gspK	
5264	gspL		7734	gspL	
5265	gspK		7735	gspJ	
5266	gspJ		7736	gspH	
5267	gspI		7737	gspG	
5268	gspH		8375	gspF	
5269	gspG		8376	gspG	
6182	gspI		8378	gspI	
6183	gspO		8379	gspH	
6184	gspP		8380	gspO	
6185	lamG	lectin associated with metalloprotease	8381	gspP	
			8382	lamG	lectin associated with metalloprotease

Supplementary Table S3.

PLFA (>0.5%)	**AB-CW1**	**AB-CW4**	**Bsker1^{T}**
3-OH C11:0	0.8		
i3-OH C11:0			1.1
C14:0	2.4		
iC15:0	3.3	2.8	0.7
C16:0	**12.4**	**5.1**	**5.6**
iC16:0		1.9	0.6
C16:1 ω9	0.7	0.8	
iC17:0	**23.6**	**28.7**	**48.1**
ai C17:0	1.8	0.9	0.8
iC17:1 ω9	**22.1**	**34.3**	**36.4**
C18:0	**5.5**	2.6	1.8
iC18:0		1.4	1.8
C18:1 ω7			1.8
C18:1 ω9	**7.0**		1.6
C18:1 ω11	1.5	1.9	
iC19:0		0.7	
iC19:1 ω9	**14.0**	**17.3**	
i19:0 + C20:1	0.9		

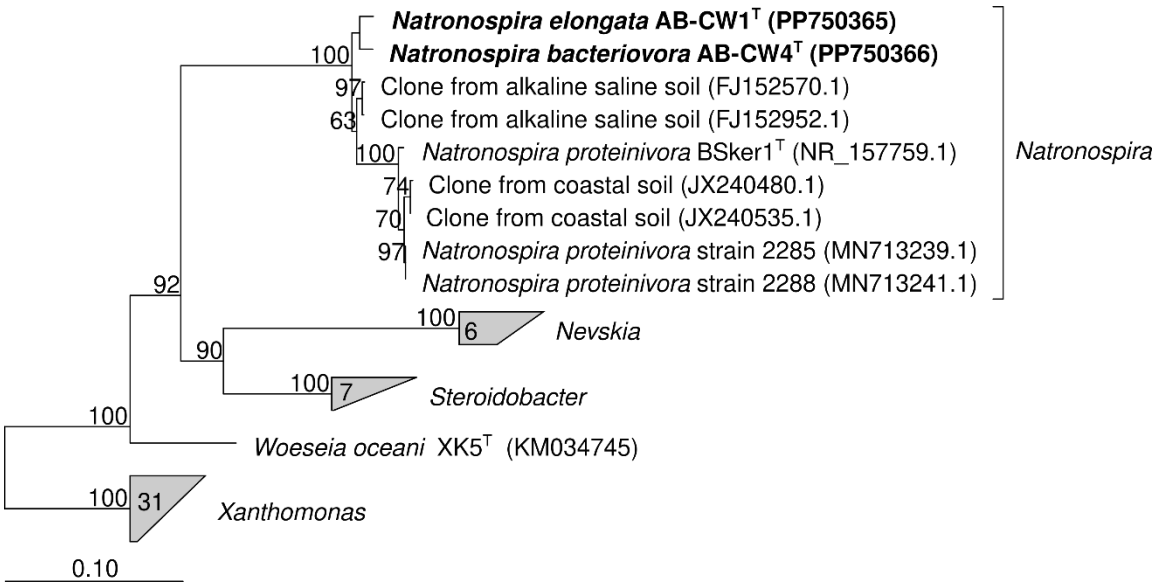
Natronospira elongata AB-CW1T (PP750365)
Natronospira bacteriovora AB-CW4T (PP750366)
Clone from alkaline saline soil (FJ152570.1)
Clone from alkaline saline soil (FJ152952.1)
Natronospira proteinivora BSker1T (NR_157759.1)
Clone from coastal soil (JX240480.1)
Clone from coastal soil (JX240535.1)
Natronospira proteinivora strain 2285 (MN713239.1)
Natronospira proteinivora strain 2288 (MN713241.1)
Natronospira
100
97
63
100
74
70
97
92
100
6
Nevskia
90
100
7
Steroidobacter
100
Woeseia oceani XK5T (KM034745)
100
31
Xanthomonas
0.10

Fig. S1.