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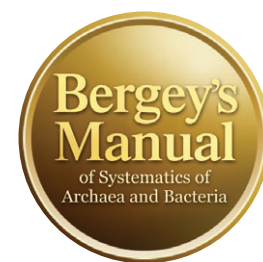
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Proteobacteria/Gammaproteobacteria/Natronospirales/Natronospiracea/

Natronospira

Sorokin et al. 2017^{VP}

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Na.tro.no.spi'ra. N.L. neut. n. *natron* (arbitrarily derived from the Arabic n. *natrun* or *natron*), soda; L. fem. n. *spira*, coil; N.L. fem. n. *Natronospira*, a soda-loving coil-shaped bacterium.

The genus *Natronospira* was originally classified as a member of the family *Ectothiorhodospiraceae*, order *Chromatiales*, and class *Gammaproteobacteria*, according to the 16S rRNA-based gene sequence comparison, while according to the phylogenomic analysis it forms a separate order-level branch within the *Gammaproteobacteria* unrelated to the *Chromatiales* members. It is an aerobic heterotroph that preferably utilizes proteins and peptides for growth. *Natronospira* is an extremely salt-tolerant, chloride-independent obligate alkaliphile. It inhabits oxic brines of hypersaline soda lakes, particularly in southwestern Siberia. The genus currently includes a single (type) species: *N. proteinivora*.

DNA G + C content (%): 59.8 (genome).

Type species: ***Natronospira proteinivora*** Sorokin et al. 2017^{VP}.

Natronospira cells are **spirilla of variable lengths**, depending on the growth conditions, motile with a **single lateral flagellum**, and with the Gram-negative type of cell wall. Produces membrane-bound yellow pigment. It is **strictly**

aerobic heterotroph utilizing various proteins and peptides for growth. The bacterium is **extremely salt-tolerant, chloride-independent obligate alkaliphile** growing optimally at 2–2.5 M total Na⁺ as carbonates and pH 9.5. **Found in hypersaline brines of soda lakes** in southwestern Siberia. The genus currently **includes a single species *N. proteinivora*** (Sorokin et al., 2017). The dominant respiratory quinone is Q8. The membrane polar lipids include several unidentified phospho- and aminolipids esterified mostly with two types of iso-C₁₇ fatty acids. *Natronospira* forms a **deep-branching separate phylogenetic lineage of the order level** within the *Gammaproteobacteria*.

DNA G + C content (%): 59.8 (genome).

Type species: ***Natronospira proteinivora*** Sorokin et al. 2017^{VP}.

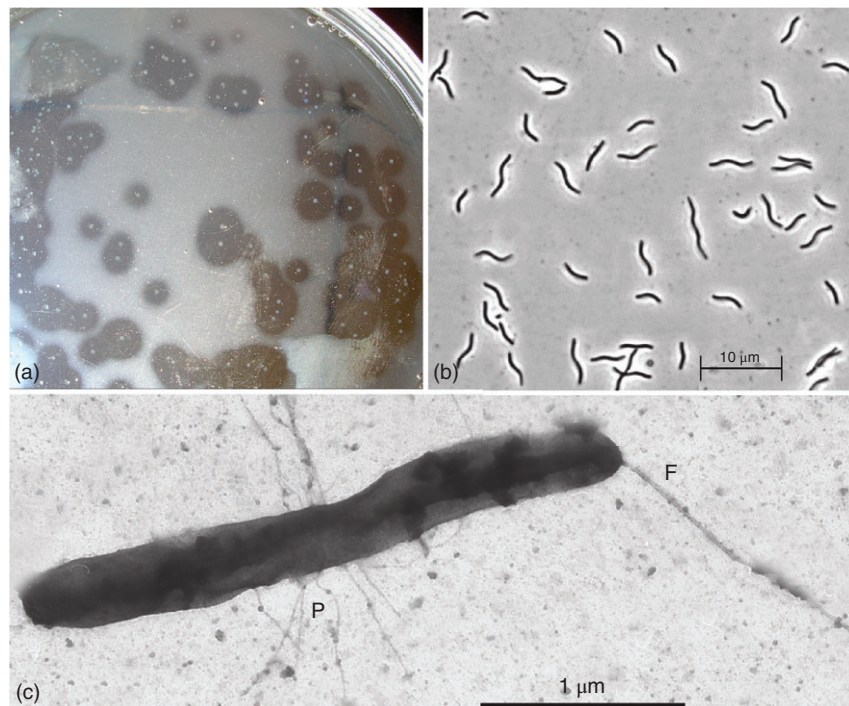
Number of species with validly published names: 1.

Family classification: The genus *Natronospira* is classified within the family "*Natronospiraceae*".

Further descriptive information

The cells are loose spiral-shaped motile rods of highly variable lengths. They have two types of appendages: a single thick flagellum and a network of multiple thin pili/fimbria-like filaments. Colonies on casein haloalkaline agar form characteristic clearance of insoluble casein around (Figure 1) and are slightly yellowish due to the presence of a membrane

FIGURE 1. Morphology of *Natronospira proteinivora* BSk^{er1}^T grown at 2 M total Na⁺ and pH 10 with casein as substrate. (a) Colonies forming casein clearance; (b) phase-contrast microphotograph of cells in liquid culture; (c) electron microphotograph of positively stained cell showing a single polar flagellum (F) and multiple thin pili-like (P) appendages.



pigment similar in its spectral characteristics to those found in other extremely salt-tolerant gammaproteobacteria, such as the genus *Thioalkalivibrio* (Takaichi et al., 2004). Such isoprenoid lipid-soluble pigments have been implicated in the stabilization of the cytoplasmic membrane at high salt-pH conditions by decreasing its water and proton permeability (Salvador-Castell et al., 2019).

The characteristic feature of the genus is its preference for proteins as growth substrate, including casein, bovin serum and lactalbumins, α -keratin, collagen, gelatin, soy protein, and hemoglobin. The protease activity is associated with the cells.

Habitat, enrichment, and isolation

N. proteinivora was enriched from a mixture of brines from several hypersaline soda lakes in the Kulunda Steppe (Altai, Russia) using α -keratin as a growth substrate. The enrichment medium was based on a 1:1 mixture of a sodium carbonate buffer at pH 10 and NaCl brine, both containing 4 M total Na⁺, with a final pH of 9.5. The pure culture isolation was achieved with the same medium (except that keratin was replaced by casein) by plating a maximal positive dilution

and isolating a single colony with a clearance zone (see Figure 1) of casein hydrolysis. Recently, we obtained two more putative members of this genus, representing different species (less than 97% of 16S rRNA gene identity to the type strain). These isolates were enriched from the same soda lakes but using cells of Gram-positive cocci as substrate. Similar to the type strain, they are strongly proteolytic, but the type strain lacks the ability to hydrolyze the bacterial cell walls (unpublished results).

Genome analysis

The draft genome of *N. proteinivora* BSk^{er1} was sequenced in Joint Genomic Institute (JGI Project ID: 1279557). It contains a single chromosome of 2.93 Mb and comprises 2,645 genes, from which 2,586 are protein coding. The main functional content of the genome is as follows:

1. The main physiological trend of the bacterium is its strong proteolytic nature. Correspondingly, the genome contains large repertoire of genes encoding proteases and especially peptidases of the metallo and serine families (Table 1).

TABLE 1. List of proteases/peptidases encoded in the genome of *Natronospira proteinivora*

Locus tag	Family	Location
<i>Metalloproteases</i>		
000301	M48 Zn-containing protease subfamily BepA	E/M
001800	M48 Zn-containing protease subfamily BepA	
001834	M48B Zn-containing protease HtpX	
000589	M50 family RIP Zn-protease RseP	M
000962	ATP-dependent zinc metalloprotease FtsH	M
001132	ATP-dependent zinc protease	C
001236	Zinc-dependent metalloprotease	E/M
001392	Zinc-dependent metalloprotease M10A/M12B	E
001298	CPBP family intramembrane metalloprotease	M
001552		
001795		
001654	Zn-dependent metalloprotease PmbA (M10)	E
001670	Zn-dependent metalloprotease TldD (M10)	E
<i>Serine proteases</i>		
000148	Rhomboid intramembrane serine protease family S54	M
001731		
001746		
002336		
002084	S41 family tail-specific serine protease	M
002289	Serine family S1C endoprotease DegQ	E
001464	S54 family rhomboid protease GlpG	M
000691	S13 family ATP-dependent Clp protease ATP-binding ClpA	C
001061	ATP-dependent Clp protease ATP-binding ClpX	C
001062	ATP-dependent Clp endopeptidase proteolytic subunit ClpP	C
001065	ATP-dependent protease chaperone ClpB	C
<i>Miscellaneous families proteases</i>		
002091	ATP-dependent protease ATPase subunit HslU	C
002092	T01.006 family ATP-dependent endopeptidase subunit HslV	C
<i>Serine family endopeptidases</i>		
000050	Trypsin-like family S1 endopeptidases	E
000637		
001026		
001432		
001894	S8 subtilisin-like family serine endopeptidases	E
001992		
001923		
002014		
002312		
000913	S9 prolyl oligopeptidases	E
001276		
001318		
001623		
001730		
001743		
001813		
002018		
002190		
002198		
002232		



TABLE 1. (continued)

Locus tag	Family	Location
000006	S16 family LON/La endopeptidase (caseinase)	E
001060	S16 family LON/La endopeptidase (caseinase)	E
001146	C40 family dipeptidyl-carboxypeptidase^a	E
001324	S15 dipeptidase family Xaa-Pro (casein hydrolysis)	E
001694	S58 prolyl-aminopeptidase DmpA	E
001730	S9 family prolyl oligopeptidase	E
002321	S10 family carboxypeptidase C	M
<i>Metallopeptidases</i>		
002539	M1 family alanine aminopeptidase	E
002145	M3 family oligopeptidase	E
002000	M13 family neprilysin-like Zn-endopeptidase	E/M
000441	M14A family Zn-carboxypeptidase	E
000155	M17 family Mn-containing leucyl aminopeptidase	C
000207		
000865		
000734	M20A family glutamate carboxypeptidase	E
000079	M23 family beta-lytic endopeptidase^a	E
000988	M28A family Zn-amino/carboxypeptidase	E
002430		
000442	M48A family Ste24-p Zn-peptidase	M
000741		
002331		
002415		
001108	M48B subfamily HtpX Zn-peptidase	M
000490	Zn peptidase YugP family M7/M10	M
000736	Zn peptidase MtfA family M90	C
001655	Zn-peptidase with the zinc-ribbon_6 domain	C
002318		
000457	D-alanyl-D-alanine Zn-carboxypeptidase family M15	C
001364		
000534	Type I M24 family Co-containing methionyl-exopeptidase	E
000581		
001238	M78 ImmA/IrrE family Zn-peptidase	C
001317	M24 prolyl-aminopeptidase P	E
001509		
001658	M38 Zn-containing isoaspartyl peptidase/asparaginase	C
001688	M19 family Zn-dipeptidase	M
<i>Unassigned families</i>		
001161	U32 family endopeptidase/collagenase	E
001162		

^aUsed by predatory bacteria, such as *Lysobacter* and *Bacillus subtilis*, for lytic attack on the cell wall of their preys. C, cytoplasmic; E, extracellular; M, membrane.

Functionally significant enzymes emphasizing the phenotype are represented in bold.

2. Haloalkaliphilic adaptation includes the following:
- Two multisubunit sodium:proton antiporters MnhABC D1D2EFG and MrpAB1B2CD1D2EF and a singleton sodium:proton antiporter NhaC; potassium:proton antiporter CPA1; Na⁺:Ca²⁺ antiporter of the CaCA superfamily.
 - Potassium import by potassium:proton symporter TrkAH.
 - Compatible solute biosynthesis is represented by the ectoine pathway (EctABC) and ectoine hydroxylase EctD (in a separate locus) forming hydroxyectoine. In addition, the genome also encodes three copies of the BCC transporter allowing the import of glycine betaine and choline.
3. The respiratory complexes include H⁺-translocating NADH dehydrogenase NuoABCDEFGHJK, H⁺-translocating F1F0-ATP synthetase, cytochrome *bc*₁ complex, quinol oxidase *bd*, and two types of the heme-copper family cytochrome *c* oxidases – *cbb*₃ and *aa*₃.
4. An interesting operon is present in the genome of *N. proteinvora*, apparently encoding the biosynthesis of a *nisin*-like lantibiotic, consisting of structural subunits lanthionine synthase LanB and lantibiotic dehydratase LanC; two ABC-type toxin-exporting subunits HlyD (hemolysin) and lantibiotic-exporter LanT; and an S9-family peptidase potentially involved in the cleaving of a lantibiotic leader peptide (Siegiers et al., 1996). The fact is unusual, since such peptide antibiotics are mostly produced by the Gram-positive bacteria (McAuliffe et al., 2001). However, recently we found a similar trend in a haloalkaliphilic predatory proteolytic gammaproteobacterium *Wenzhouxiangella* sp. AB-CW3 (isolated from the same lakes as *Natronospira* but at lower salinity) feeding on Gram-positive cocci (Sorokin et al., 2020).

Maintenance and preservation

Active liquid cultures of *N. proteinvora* can be kept viable at 4°C for up to 3 months. Long-term preservation by deep freezing is possible with 15% glycerol as a cryoprotectant.

Taxonomy

According to the 16S rRNA gene phylogenetic analysis, the genus *Natronospira* was originally classified as a member of the family *Ectothiorhodospiraceae* and order *Chromatiales* (Sorokin et al., 2017). The nearest related genus seems to be *Aquisalimonas*, which includes extremely salt- and alkalitolerant aero-

bic heterotrophic members also found in hypersaline alkaline lakes (Márquez et al., 2007). However, a more recent phylogenomic reconstruction based on the analysis of 120 single-copy bacterial conserved protein markers showed that *Natronospira* and four MAGs recovered from the Cock Soda Lake in the same area from which *N. proteinvora* was isolated form a separate deep-branching lineage of a novel order level within the *Gammaproteobacteria* (Figure 2, only a high-quality MAG GCA_003558125.1 with completeness >90% is shown).

List of species of the genus *Natronospira*

Natronospira proteinvora Sorokin et al. 2017^{VP} (emended)

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pro.te.i.ni.vo'ra N.L. neut. n. *proteinum*, protein; N.L. fem. adj. suff. *-vora*, devouring; N.L. fem. adj. *proteinvora*, devouring proteins.

Cells are curved rods, 0.4 × 2–30 μm, motile with a single flagellum, and forming multiple pili-like appendages, probably used to attach to insoluble protein-containing particles. Colonies are up to 3 mm, yellowish, compact, flat, and circular, forming zones of casein hydrolysis. The polar lipids include several unidentified phospho- and aminolipids. The polar lipid fatty acids are dominated by iso-C_{17:0} and iso-C_{17:1} ω9c. Have a genetic capability to produce ectoine and hydroxyectoine as compatible solutes. Strictly aerobic, proteolytic, utilizing various proteins (casein, bovin serum and lactalbumins, α-keratin, collagen, gelatin, soy protein, and hemoglobin) and peptides (peptones from casein, meat and soy, casein hydrolysate, and yeast extract) for growth. Obligately alkaliphilic with a pH range for growth from 8.5 to 10.25 and an optimum at pH 9.5. Chloride-independent extremely salt-tolerant natronophile, growing in sodium carbonate brines containing 1.0–4.5 M total Na⁺ with optimum growth at 2–2.5 M. The upper temperature limit for growth (at optimal pH and salinity) is 45°C.

DNA G + C content (mol%): 59.8 (genome).

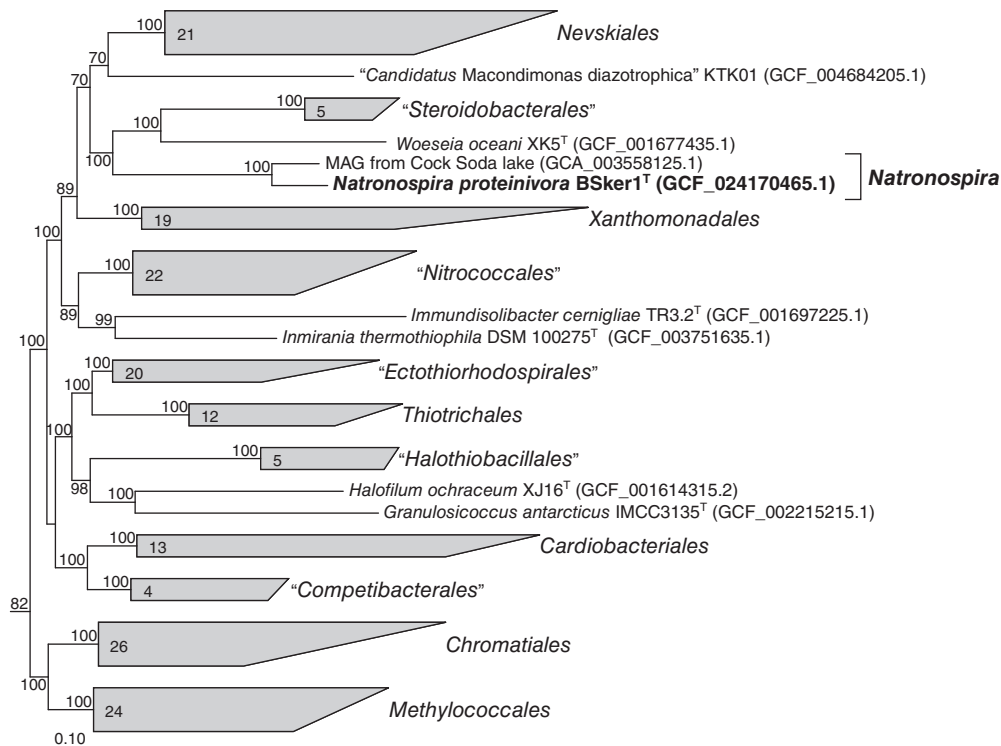
Type strain: BSk1 (=JCM31312=UNIQEM U1008^T).

EMBL/GenBank accession number (16S rRNA gene): KU720568.

Acknowledgment

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FIGURE 2. Phylogenetic position of *Natronospira* (in bold) within the *Gamma*proteobacteria based on sequence analyses of concatenated alignment of 120 single-copy conserved bacterial protein markers (Parks et al., 2020; taxonomic designations correspond to the Genome Taxonomy DataBase 207). The trees were built using the IQ-TREE 2 program (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). Bootstrap consensus tree is shown, with values above 70% placed at the nodes. Bar, 0.10 changes per position.



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