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DOI

[10.1002/9781118960608.gbm02088](https://doi.org/10.1002/9781118960608.gbm02088)

Publication date

2024

Document Version

Final published version

Published in

Bergey's Manual of Systematics of Archaea and Bacteria, Online

Citation (APA)

Sorokin, D. Y., & Merkel, A. Y. (2024). Cyclonatronum. In W. B. Whitman (Ed.), *Bergey's Manual of Systematics of Archaea and Bacteria, Online* (pp. 1-5). John Wiley and Sons. <https://doi.org/10.1002/9781118960608.gbm02088>

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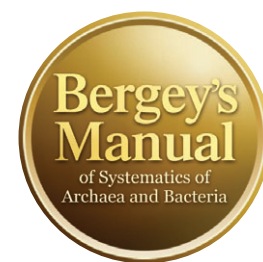
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Cyclonatronum

Zhilina et al. 2023, VL211

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Cy.clo.na.tro'num. Gr. masc. n. *kyklos*, a circle; N.L. neut. n. *natron*, soda; N.L. neut. n. *Cyclonatronum*, circle-shaped and soda-loving.

The genus *Cyclonatronum* is a member of the class *Rhodothermia* of the phylum *Bacteroidota*. It includes obligately aerobic organoheterotrophs that mostly utilize proteins and peptides, and possess an active sodium-pumping proteorhodopsin. The genus currently includes a single species, *Cyclonatronum proteinivorum*, which is represented by a mesophilic, moderately salt-tolerant, chloride-independent, and obligate alkaliphile found in saline soda lakes in Central Asia.

DNA G + C content (mol%): 51.5 (genome sequence).

Type species: *Cyclonatronum proteinivorum* Zhilina et al. 2023, VL211.

Description

Cells of *Cyclonatronum* are polymorphic, from semicircles to loose spirilla depending on growth conditions (Figure 1). Produce **red carotenoids** and **sodium-translocating proteorhodopsin**. The **dominant polar lipid fatty acids** include **iso-C₁₅** and **C_{16:1} ω7c**. The major respiratory lipoquinone is MK-7. The only described representative is **strictly aerobic**

heterotroph specialized in utilization of **peptides and proteins** as **growth substrates**. It is **moderately salt tolerant** and **obligately alkaliphilic** with maximum salt tolerance of **2.25 M total Na⁺** (in the form of sodium carbonate/bicarbonate) and **pH up to 10.2**. Found as a satellite of filamentous cyanobacteria enriched from moderately saline soda lakes in southwestern Siberia (Altai, Russia). The genus currently includes a **single species** *Cyclonatronum proteinivorum*, represented by a single strain Omega^T. *Cyclonatronum* forms deep-branching phylogenetic lineage of a **separate family-level** within the **order Balneolales** in the **class Rhodothermia**.

DNA G + C content (mol%): 51.5 (genome sequence).

Type species: *Cyclonatronum proteinivorum* Zhilina et al. 2023, VL211.

Number of species with validly published name: 1.

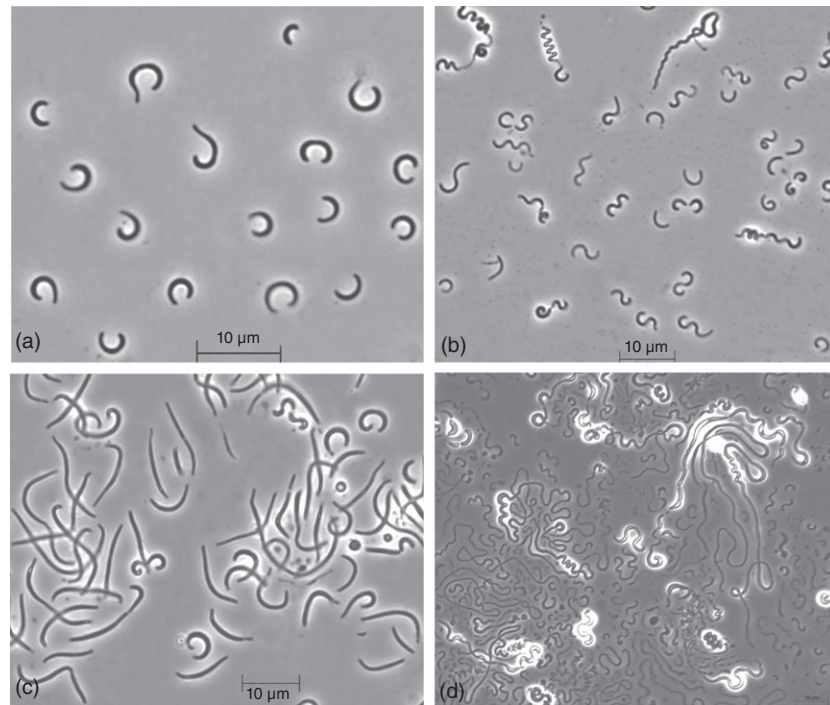
Family classification: The genus *Cyclonatronum* is classified within the family *Cyclonatronaceae*.

Further descriptive information

Cell morphology and chemotaxonomy

Cells of the type strain are highly polymorphic, ranging from “horse-shoe”-like semicircles to highly coiled spiral and long whip-like flexible rods (Figure 1). The colonies and the cell biomass grown in liquid cultures have intense

FIGURE 1. Cell morphology of *Cyclonatronum proteinivorum* Omega^T grown at pH 10 and 1 M total Na⁺ with different substrates: (a) casein, (b) alpha-keratin, (c) bovine serum albumin, and (d) peptone from casein.



pink color due to the presence of carotenoids (main peak 479 and shoulders at 450 and 505 nm in methanol:acetone extract, 7:3). Furthermore, *Cyclonatronum* is a first example of a soda lake *Bacteroidota* producing Na-pumping proteorhodopsin (Sorokin et al., 2018; Zhilina et al., 2023). The intact membrane polar lipids included six phospholipids and 1 glycolipid; however, they were not identified. The major membrane polar lipid fatty acids include (in order of abundance) iso-C₁₅, C_{16:1} ω7c, C₁₆, iso-C₁₆, iso-C_{17:1} ω8, and anteiso-C₁₇. The only detected respiratory lipoquinone was represented by the MK-7.

Growth physiology

The genus *Cyclonatronum* so far includes only a single strain, which is an obligately aerobic organoheterotroph. However, the presence of an active sodium-pumping proteorhodopsin might allow this aerobic bacterium to survive temporal exposure to anoxia. The main growth substrates utilized by strain Omega are various proteins including powdered alpha-keratin, casein, gelatin, bovine serum albumin, peptones from soy, casein, and meat, yeast extract, and unknown components excreted by cyanobacteria. As

is typical for the soda lake microbes, the strain Omega is a moderately salt-tolerant obligate alkaliphile growing optimally in sodium carbonate buffer with pH 9.5–10 and total Na⁺ concentrations around 0.5–1 M (Sorokin et al., 2018).

Habitat, enrichment, and isolation

Cyclonatronum proteinivorum was identified as one of the satellites in a laboratory phototrophic enrichment culture dominated by benthic filamentous cyanobacteria (from the genera *Sodalinea* and *Nodosilinea*) inoculated from surface sediment sample from a moderately saline soda lake Bitter-3 in Kulunda Steppe (Altai, Russia). The mineral medium contained 1.5 M total Na⁺ strongly buffered at pH 10 with sodium carbonates. The cyanobacterial culture was developing in two stages: first, only dense floating biomass of cyanobacteria was growing, and later on, heterotrophic bacteria started to proliferate in the lower part of the flask with intense pink color with several morphotypes, including semicircles. The latter formed pink colonies on a solid medium with the same basic composition containing filter-sterilized sonicate prepared from the cyanobacterial biomass from which a pure culture strain Omega^T was obtained (Sorokin et al., 2018).

Genome analysis

The finished genome of the strain Omega is 4.29 Mb in size and comprises 3,196 genes encoding 3,123 proteins. The genome has already been functionally analyzed in depth (Sorokin et al., 2018), and here only the essential conclusions are provided on the main functional content encoded.

The proteolytic nature of *Cyclonatronum* is corroborated by the presence in genome of a large number of genes coding for extracellular peptidases (51 out of 134) with a domination of the serine families S8, S9, and S12 and Zn-peptidase M28. Two encoded S51 family exopeptidases might be involved in cyanophycin (an organic N-storage polymer in cyanobacteria) degradation. It is also predicted to be auxotrophic on a number of amino acids (10), hence depending on an external source.

The genome contains a bicistronic operon *betAB* encoding biosynthesis pathway of compatible solute glycine betaine from choline. Furthermore, glycine betaine could be imported by the OpuD BCC transporter. The encoded alkaliphilic bioenergetic elements include a multisubunit Na⁺/H⁺ antiporter MrpABCDEFG, single subunit Na⁺/H⁺ antiporters NhaC and CPA2, and TrkAH K⁺:H⁺ symporter. Two primary membrane sodium pumps are present. One is a sodium-pumping proteorhodopsin in which sodium-pumping activity has been confirmed *in vivo* (Sorokin et al., 2018). The second enzyme is electrogenic Na⁺/K⁺-translocating pyrophosphatase HppA (Luoto et al., 2011). The NADH-menaquinone oxidoreductase and the ATP synthase seem to be of the proton-translocating type.

What was overlooked in the previous genome analysis is the presence of an operon encoding dissimilatory ammonifying nitrite reductase NrfAH (WP_240644826-27). Homologous operons are present in many *Bacteroidota* species, but to our knowledge, the actual potential for anaerobic growth by nitrite respiration to ammonia has never been proven in cultured members of this phylum. In the case of *Cyclonatronum*, we did check its ability to grow anaerobically with nitrate (5 mM) and nitrite (2 mM) in the presence of peptone from casein as substrate, and the results were negative. We also tested several sugars and glycerol as potential donors in the presence of 0.2 g/l of peptone as potential electron donors with the same negative outcome.

Maintenance and preservation

Active liquid cultures of *Cyclonatronum proteinivorum* remained viable at 4°C for up to 1 months. Long-term

preservation by deep freezing is possible with 15% glycerol as a cryoprotectant.

Taxonomy

Based on the phylogenetic analysis of 120 single-copy bacterial conserved protein markers, *Cyclonatronum* forms a separate deep-branching, family-level lineage within the *Bacteroidota* class *Rhodothermia* and order *Balneolales*. Apart from the Omega^T isolate, there are several environmental metagenome-assembled genomes (MAGs) in the GenBank from soda lakes in the same area and in similar lakes in British Columbia (Canada), which either belong to the same genus or the sister genera in the same family (Figure 2).

List of species of the genus *Cyclonatronum*

Cyclonatronum proteinivorum
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pro.te.i.ni.vo'rum N.L. neut. n. *proteinum*, protein; L. v. *voror*, to devour; N.L. neut. adj. *proteinivorum*, protein devouring.

Cells are Gram-stain-negative, nonmotile, with a variable cell morphology from circular to long flexible rods spirilla with the average cell width of 0.5 μm. Cells contain carotenoids and produce a sodium-pumping proteorhodopsin. Strictly aerobic and organoheterotrophic. Utilizes various soluble and insoluble proteins and peptones as energy, carbon, and nitrogen sources. Sugars and sugar polymers, organic acids, and alcohols do not support growth. Moderately salt-tolerant obligate alkaliphile, growing at pH from 8.5 to 10.5 and in total Na⁺ from 0.3 to 2.25 M. Has a genetic potential to produce osmolyte glycine betaine from choline. The optimum growth temperature is 33–35°C. The dominant polar lipid fatty acids are iso-C₁₅ and C_{16:1} ω7c. The major respiratory lipoquinone is MK-7. The type strain was isolated from an enrichment culture of benthic filamentous cyanobacteria obtained from the sediments of a hypersaline soda lake in southwestern Siberia (Russia).

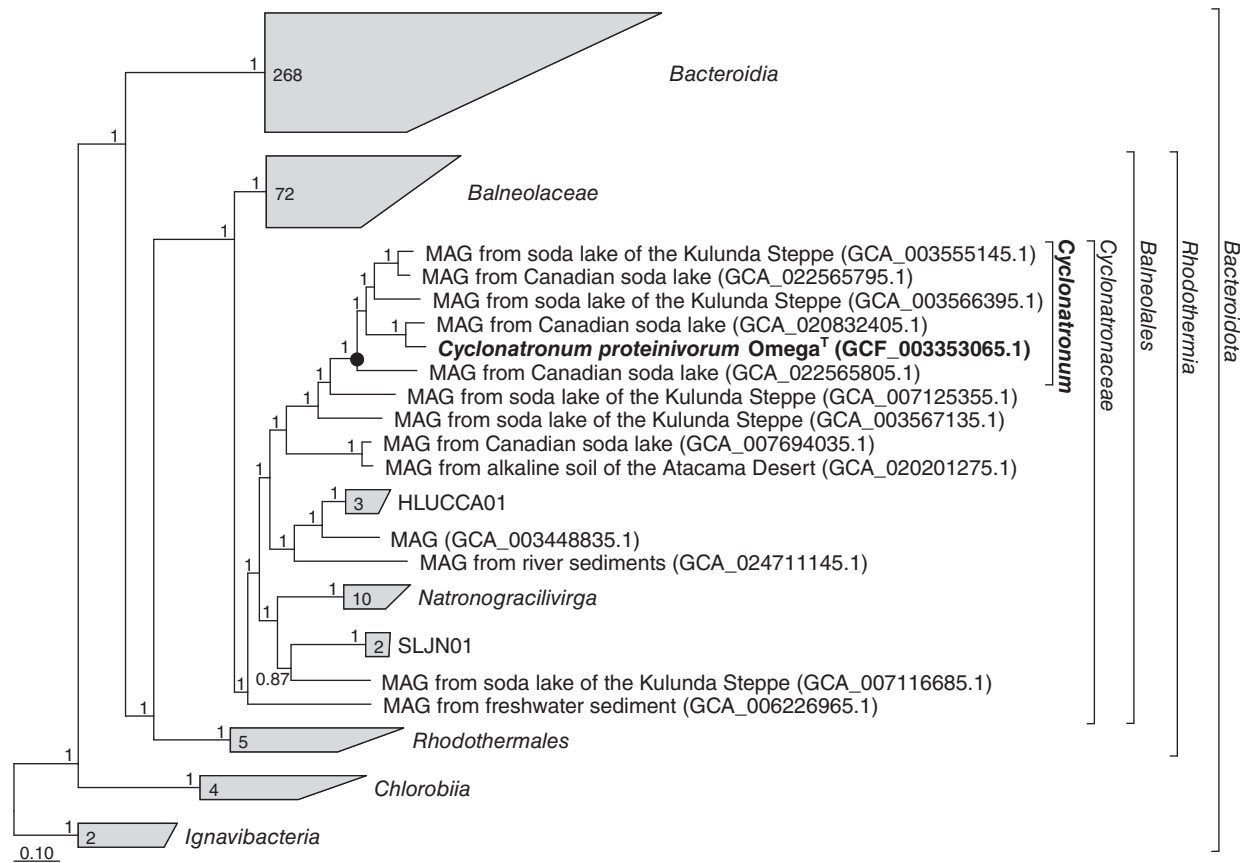
DNA G + C content (mol%): 51.5 (genome sequence).

Type strain: Omega = JCM 31662 = UNIQEM U979.

EMBL/GenBank accession number (16S rRNA gene): KF 830693.

GenBank accession number (genome): CP027806.

FIGURE 2. Phylogenetic position of *Cyclonatronum* (in bold) within the *Balneolales* order based on sequence analyses of concatenated alignment of 120 single-copy conserved bacterial protein markers [according to the Genome Taxonomy DataBase (GTDB) taxonomy] (Parks et al., 2020). The tree was built using the IQ-TREE 2 program (Minh et al., 2020) with fast model selection via ModelFinder (Kalyanamoorthy et al., 2017) and ultrafast bootstrap approximation (Minh et al., 2013) as well as approximate likelihood-ratio test for branches (Anisimova and Gascuel, 2006). The bootstrap consensus tree is shown with values placed at the nodes. Bar, 0.10 changes per position.



Acknowledgment

The work was supported by the Gravitation-SIAM Program of the Dutch Ministry of Education and Science (grant 24002002).

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