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Cyclonatronaceae

Zhilina et al. 2023, VL211

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

The family *Cyclonatronaceae* is a member of the order *Balneolales*, class *Rhodothermia*, and phylum *Bacteroidota*. It includes heterotrophic bacteria with either aerobic or fermentative metabolism that utilize peptides or sugars as growth substrates. The known members of the family are mesophilic, moderately salt-tolerant, chloride-independent, and obligate alkaliphiles found in saline soda lakes in Central Asia. The family currently includes two genera, the type genus *Cyclonatronum* and the genus *Natronogracidivirga*, both represented by a single species.

DNA G+C content (mol%): 49.7–51.5 (whole-genome sequences).

Type genus: **Cyclonatronum** Zhilina et al. 2023, VL211.

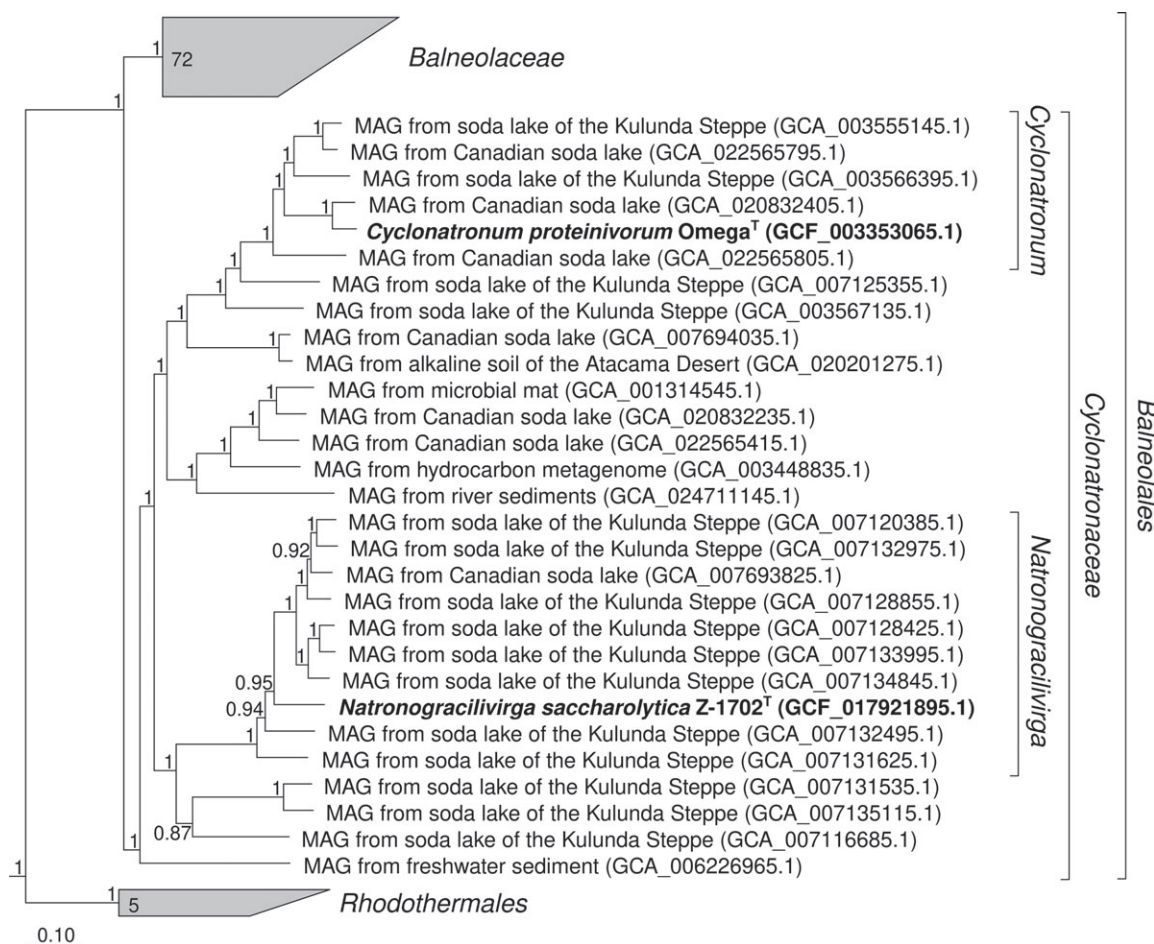
The family *Cyclonatronaceae* includes two genera. The type genus *Cyclonatronum* currently has a single species, *C. proteinivorum*, which was proliferating as an aerobic heterotrophic satellite in a culture of benthic filamentous cyanobacteria enriched from a soda lake in Altai Region (Russia). It is an obligately aerobic proteolytic bacterium

with nonmotile polymorphic cells, from semicircles to long spirilla or whip-like flexible rods, depending on the growth conditions. Cells produce pink carotenoids and an active sodium-translocating proteorhodopsin. The dominant polar lipid fatty acids include iso-C₁₅ and C_{16:1} ω7c, and the major respiratory lipoquinone is MK-7 (Sorokin et al., 2018; Zhilina et al., 2023).

The second described genus in this family, *Natronogracidivirga*, also includes a single species, *N. saccharolytica*, enriched from a soda lake Tanatar-3 in the same area as *C. proteinivorum*. It was present in a coculture with a peptolytic iron-reducing bacterium *Isachenkonkia alkalipectolytica* (Zavarzina et al., 2020). This bacterium is a strictly anaerobic fermentative saccharolytic organoheterotroph with long, flexible, motile, and rod-shaped cells when cultured under optimal growth conditions. Utilizes a range of sugars and several soluble alpha-glucans as growth substrates. The dominant polar lipid fatty acids include iso-C₁₅, iso-C₁₆, and C₁₆. The respiratory lipoquinones were not detected (Zhilina et al., 2023).

The genera of the family described so far accommodate mesophilic, moderately salt-tolerant and obligate alkaliphilic, and chloride-independent bacteria with a maximum tolerance of up to 3.2 M total Na⁺ (in the form of sodium carbonate/bicarbonate) and the pH range from 7.5 to 10.2 (optimum at 9–9.5). The family is a member of the order *Balneolales* within the class *Rhodothermia* of the phylum

FIGURE 1. Phylogenetic position of the family *Cyclonatraceae* within the order *Balneolales* (class *Rhodothermia*) 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 above 85% placed at the nodes. Bar, 0.10 changes per amino acid position.



Bacteroidota, which mostly contains extremophilic species, particularly extreme halophiles and haloalkaliphiles. The closest related family is *Balneolaceae* (Figure 1). Apart from the two cultured species, the phylogenetic clade of the family *Cyclonatraceae* seems to include multiple environmental metagenome-assembled genomes (MAGs) obtained from hypersaline soda lakes in the Kulunda Steppe (Altai region, Russia) from which both cultured species of the family originated and from the chemically/climatically similar lakes in Cariboo Plateau (British Columbia (Canada)), whereby the microbial communities are very similar to the Kulunda Steppe counterparts (Zorz et al., 2019). Four out of the nine MAGs clustering around the genus *Cyclonatrum* most likely belong to the same genus or a closely related sister

genus. A brief look at the translated proteomes of three of those MAGs retrieved from the Canadian soda lakes and alkaline soil in Atacama desert showed that all of them have a rhodopsin protein highly similar to the Na^+ -proteorhodopsin of *Cyclonatrum*. Also, similar to *Cyclonatrum*, the translated proteomes feature multiple excreted peptidases (especially abundant are serine proteases from the S9 and S8 families) and also have cytochrome *c* oxidases (*cbb₃* and *aa₃* types). These potentially indicate a similar aerobic proteolytic lifestyle for the whole *Cyclonatrum*-affiliated phylogenetic clade.

DNA G + C content (mol%): 49.7–51.5 (genome sequences).

Type genus: *Cyclonatrum* Zhilina et al. 2023, VL211.

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