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Genus

Firmicutes D/Natranaerobiia/Natranaerobiales/Natranaerofabaceae/

Natranaerofaba

Sorokin et al. 2021, VL202

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Natr.an.ae.ro.fa'ba. N.L. neut. n. *natron*, derived from Arabic *natrun* soda (sodium carbonate); Gr. pref. *an-*, not; Gr. masc. n. *aêr*, air; L. fem. n. *faba*, bean; N.L. fem. n. *Natranaerofaba*, bean-shaped soda loving anaerobe.

The genus *Natrabaerofaba* forms a deep-branching lineage in the order *Natranaerobiales*, class *Natranaerobiia*. It includes obligately anaerobic bacteria utilizing CO and pyruvate during acetogenic growth and formate and lactate in the presence of thiosulfate, fumarate, or nitrate as electron acceptors. Nitrate is ammonified. They are extremely halophilic and obligately alkaliphilic, moderate thermophiles. The polar lipids are dominated by diethers, which is rare in bacteria. Currently includes a single species *Natranaerofaba carboxydovora*.

DNA G + C content (%): 35.3 (genome sequence).

Type species: **Natranaerofaba carboxydovora** Sorokin et al. 2021, VL202.

Cells of *Natranaerofaba* are bean-shaped rods, 0.4 × 3–6 µm, motile by peritrichous flagella and with the **Gram-positive type of cell wall.** Endospore formation is not observed. Polar lipids include **phosphocholines** and **phosphoglycerols**, with the dominant core in the form of **ether-bound** $\mathbf{C_{14:0}}$ and **plasmalogen-derived anteiso-**$\mathbf{C_{17:0}}$. The **respiratory lipoquinones** are **not detected**. Has a genetic potential to produce **glycine betaine for osmoprotection**. The bacterium is an **obligately anaerobic heterotroph utilizing pyruvate and CO** during **acetogenic growth**. Furthermore, it is **capable of anaerobic respiration using CO, pyruvate, formate, and lactate as the electron donors and fumarate and thiosulfate** (2-electron reduction) as the electron acceptors. Lactate is converted to acetate. **Dissimilatory reduction of nitrate to ammonia is also possible with formate** as the electron donor. **Growth** in any condition **depends on yeast extract** serving as the **carbon source**. The bacterium is an **obligately alkaliphilic, extremely salt-tolerant, moderate thermophile**, growing optimally at pH 9.5–9.7, 3.5–4 M of total Na^+, and 48–50°C. Found in **anaerobic sediments of a hypersaline soda lake** in southwestern Siberia (Sorokin et al., 2021).

DNA G + C content (%): 35.3 (genome sequence).

Type species: **Natranaerofaba carboxydovora** Sorokin et al. 2021, VL202.

Number of species with validly published name: 1.

Family classification: The genus *Natranaerofaba* is classified within the family *Natranaerofabaceae*.

Further descriptive information

The cells are mostly curved rods of variable length with a thin monolayer cell wall (monoderm) of the Gram-positive type (Figure 1). The colonies formed in soft agar are lens-shaped, slightly yellowish, up to 1 mm.

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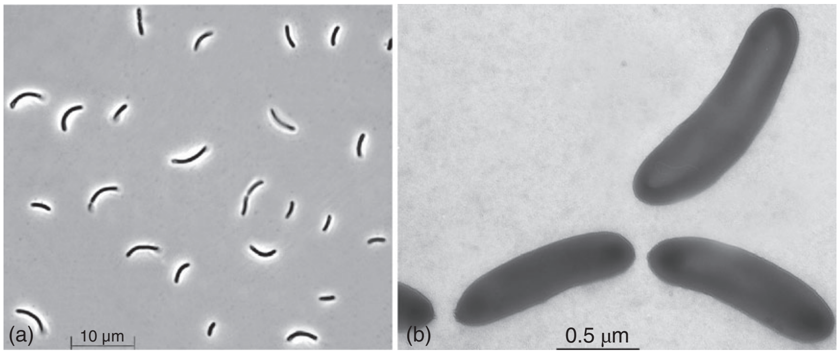
FIGURE 1. Cell morphology of *Natranaerophaga carboxydovora* ANCO1^T grown anaerobically at 4 M total Na^+, pH 9.7, and 48°C with CO + yeast extract. (a) Phase contrast and (b) electron microphotograph of whole cells.



The most characteristic feature of the genus is its acetogenic growth with CO as the electron donor. Combined with its triple extremophily, *N. carboxydovora* is a truly unique representative of hypersaline soda lake microbial communities. Furthermore, its ability for anaerobic respiration using thiosulfate and nitrate as the electron acceptors is also rare among lithotrophic acetogens. It also has unusual membrane polar lipids, sometimes reported in extremophilic, particularly thermophilic, bacteria (Grossi et al., 2015). They are mostly ether-based, instead of acyl-based common in bacteria (Sorokin et al., 2021). Especially interesting are two variants of macrocyclic diethers analogous to archaeal macrocyclic archaeol. Such membrane lipids might offer this bacterium a better potential to cope with the combination of extreme alkalinity, salinity, and high temperature (Hamilton-Brehm et al., 2013).

Habitat, enrichment, and isolation

Genus *Natranaerofaba* was found in anaerobic sediments of a hypersaline soda lake in Kulunda Steppe (Altai, Russia) and originated from an anaerobic enrichment in a medium containing 4 M total Na^+ as carbonates and chloride in equimolar proportion at 50°C with CO and formate as electron donors. The pure culture isolation was achieved by several rounds of dilutions under anaerobic conditions with CO as the electron donor and yeast extract as the carbon source, and the final purity of the isolate was confirmed by 16S rRNA gene and genome sequencing.

Genome features

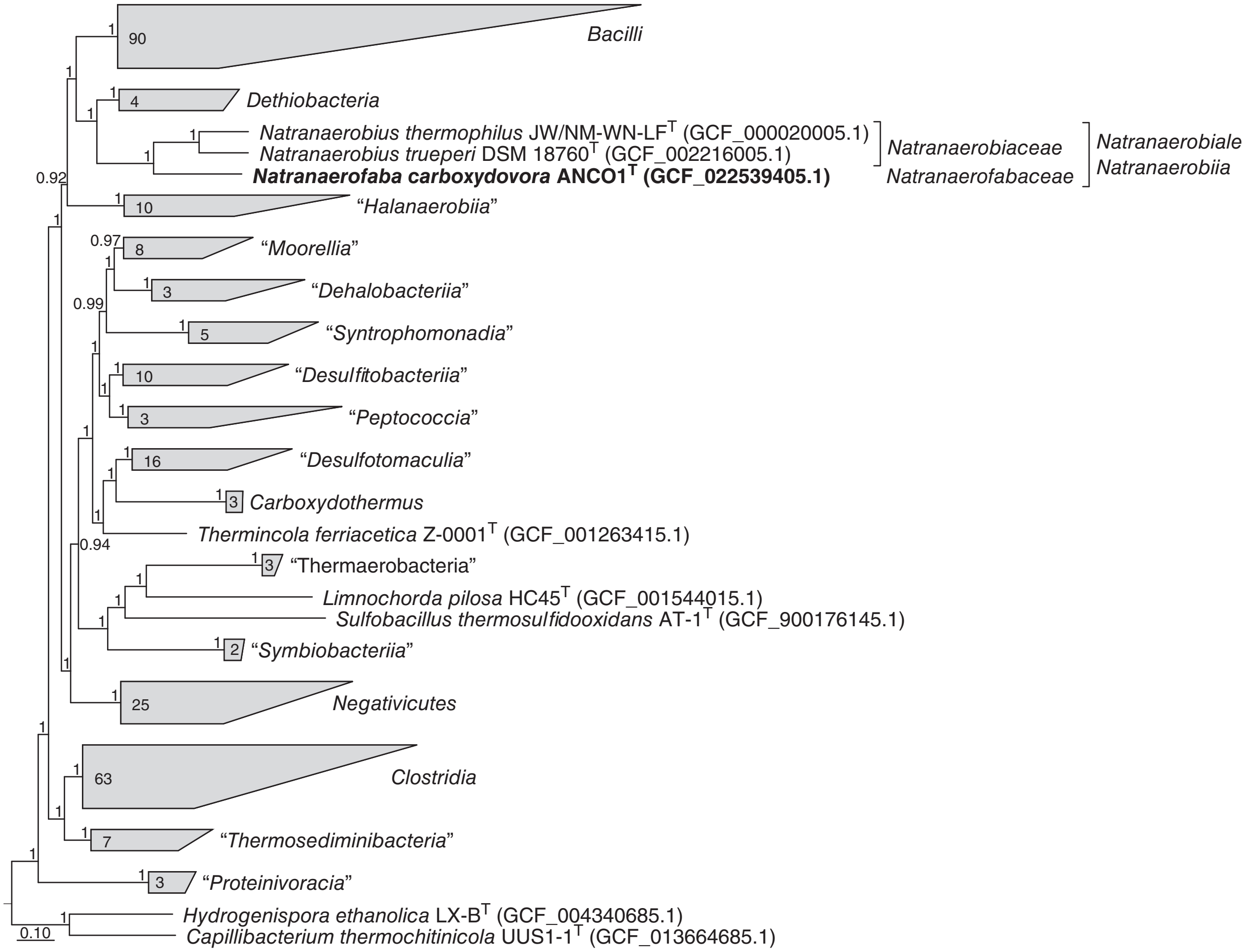
The genome of *N. carboxydovora* ANCO1^T (CP054394) was assembled in a single contig of 3.26 Mb and consists of 3,123 genes encoding 3,040 proteins. The detailed genome analysis was published previously (Sorokin et al., 2021). Here are the most important functional features (confirmed physiologically) encoded in the genome:

1. Full array of the Wood–Ljungdahl pathway genes in a single locus (CooC/AcsABCDE/MetF).
2. Three copies of genes encoding the anaerobic Ni, Fe-CODH (CooS), one in the Wood–Ljungdahl cluster, one in a Ech hydrogenase cluster, and one as a singleton.
3. Three multisubunit membrane Na^+-pumps typically involved in sodium-dependent bioenergetics, including a Na^+-Rnf complex, a Na^+-ATPase, and two Na^+-pumping decarboxylases (methylmalonyl-CoA and glucatonyl-CoA specific).
4. For anaerobic respiration, the bacterium uses thiosulfate reductase PhsABCD, fumarate-reductase FrdAB (two copies of the genes), and NrfA-NapFDAHG for dissimilatory nitrate reduction to ammonia (although NrfH is missing).
5. Osmoprotection is probably based either on the *de-novo* synthesis of glycine betaine using two-step methylation of glycine or on export (multiple copies of glycine betaine transporters are present in the genome). The alkaliphilic pH-Na homeostasis is achieved via a multisubunit complex MnhBCDEFG (two copies lacking subunit A but containing two to three copies of subunits B and D).

Maintenance and preservation

Active liquid cultures of *Natranaerofaba* remain viable at 4°C for up to 3 months. Long-term preservation can be achieved by deep freezing with 15% (v/v) glycerol as a cryoprotectant.

FIGURE 2. Phylogenetic position of *Natranaerofaba* in the *Firmicutes* 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). The tree was 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 placed at the nodes. Bar, 0.10 changes per position.



Taxonomy

According to the results of phylogenomic analyses based on the 120 conserved single-copy bacterial protein markers, genus *Natranaerofaba* forms a deeply branching phylogenetic lineage at the genus and family level and is classified in accordance to the Genome Taxonomy DataBase taxonomic designation within the order *Natranaerobiales*, class *Natranaerobiia*, phylum *Firmicutes D*. (Sorokin et al., 2021) (Figure 2).

List of species of the genus *Natranaerofaba*

Natranaerofaba carboxydovora Sorokin et al. 2021

car.bo.xy.do.vo'ra. N. L. neut. adj. *carboxydum*, carbon monoxide; L. v. *voro*, to devour, consume; N. L. fem. adj. *carboxydovora*, consuming carbon monoxide.

Cells are motile, bean-shaped rods, 0.4 × 3–6 μm with a Gram-positive type of cell wall. Endospore formation is not observed, but the potential is encoded in the genome. Polar lipids include phosphocholines and phosphoglycerols, mostly based on the diether-bound $C_{14:0}$ and plasmalogen-derived anteiso-$C_{17:0}$. The respiratory lipoquinones are lacking. It is a strictly anaerobic and heterotrophic acetogen capable of anaerobic respiration. Acetogenic growth with pyruvate and CO as the electron donors produces acetate and formate as the major and minor products, respectively. Fumarate and thiosulfate serve as the electron acceptors for anaerobic respiration with CO, pyruvate, formate, and lactate as the electron donors, while only formate can support ammonifying nitrate respiration. Growth under all conditions depends on yeast extract as the carbon source. The bacterium is obligately alkaliphilic with a pH range for growth between 9 and 10.5 (optimum at pH 9.5–9.7), extremely salt-tolerant with a total Na^+ range from 2.5 to 4.5 M (optimum 3.5–4 M), and moderately thermophilic with a temperature range of 35–56 (optimum at 48–50°C). The type strain was obtained from anaerobic sediments of a hypersaline soda lake in Kulunda Steppe (Altai region, Russia).

DNA G+ C content (%): 35.3 (genome sequence).

Type strain: ANCO1 (=DSM 108926 =UQM 41457).

EMBL/GenBank accession (16S rRNA gene): DQ834966 (type strain).

The EMBL/GenBank genome accession number: CP054394 (type strain).

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Validation List No. 202 (2021) List of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol* **71**: 5096. Ⓑ