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Sorokin, Dimitry Y.; Merkel, Alexander Y.

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Genus

Proteobacteria/Gammaproteobacteria/Nitrococcales/Aquisalimonadaceae/

Natronocella

Sorokin et al. 2007, VL116

Dimitry Y. Sorokin, *Winogradsky Institute of Microbiology, Federal Research Centre of Biotechnology, Russian Academy of Sciences, Moscow, Russia and Department of Biotechnology, Delft University of Technology, Delft, The Netherlands*

Alexander Y. Merkel, *Winogradsky Institute of Microbiology, Federal Research Centre of Biotechnology, Russian Academy of Sciences, Moscow, Russia*

Edited by: William Whitman, *University of Georgia, Athens, GA, USA*

Na.tro.no.cel'la; N.L. neut. n. *natron* (arbitrarily derived from the Arabic n. natrun or natron), soda, sodium carbonate; N.L. pref. *natrono-*, pertaining to soda; L. fem. n. *cella*, a room, a cell in biology; N.L. fem. n. *Natronocella*, a soda-loving cell.

The genus *Natronocella* comprise aerobic heterotrophs that utilizes short-chain nitriles and their corresponding fatty acids and amides as energy, carbon, and nitrogen sources. *Natronocella* is a moderately salt-tolerant, chloride-independent obligate alkaliphile inhabiting soda lakes in Central Asia. The genus currently includes a single species *N. acetinitrilica*. It was originally classified as a member of the family *Ectothiorhodospiraceae*, order *Chromatiales*, class *Gammaproteobacteria*, but more recent phylogenomic analyses classify it in the proposed order "*Nitrococcales*" and family "*Aquisalimonadaceae*."

DNA G+ C content (%): 63.0 (genome sequence).

Type species: **Natronocella acetinitrilica** Sorokin et al. 2007, VL116.

Cells of *Natronocella* are **straight rods**, 0.4–0.5 × 1.5–4.0 µm, **motile** with a **single polar flagellum.** The **cell wall** is of the **Gram-negative type**. Produces membrane-bound **yellow ketocarotenoids.** It is strictly **aerobic heterotroph** specialized in the **utilization of aliphatic nitriles and their amides** for growth. It is a **moderately salt-tolerant alkaliphile** optimally growing in sodium carbonate buffer containing 0.6 M total Na^+ at pH between 9.5 and 10. Found in Central Asia steppe soda lakes. The genus includes a single species *N. acetinitrilica* and two closely related strains: a type strain ANL 6-2^T and strain ANL 1 (Sorokin et al., 2007). The dominant **respiratory quinone is ubiquinone Q8.** The **membrane polar lipids** include **phosphatidylglycerol (PG)** and **phosphatidylserine (PS)** esterified mostly with **$C_{18:1}$ ω7*c*** fatty acid. *Natronocella* forms a distinct lineage in the ***Gammaproteobacteria***.

DNA G + C content (%): 63.0 (genome).

Type species: **Natronocella acetinitrilica** Sorokin et al. 2007, VL116.

Number of species with validly published name: 1.

Family classification: The genus *Natronocella* is classified within the family "*Aquisalimonadaceae*."

Further descriptive information

The cells are short motile rods with a single polar/subpolar flagellum. The cells have an extended periplasm and Gram-negative type of cell wall (Figure 1). Colonies on acetonitrile-containing haloalkaline agar are yellowish due to the presence of membrane-associated carotenoids largely composed of zeaxanthin (Sorokin et al., 2007).

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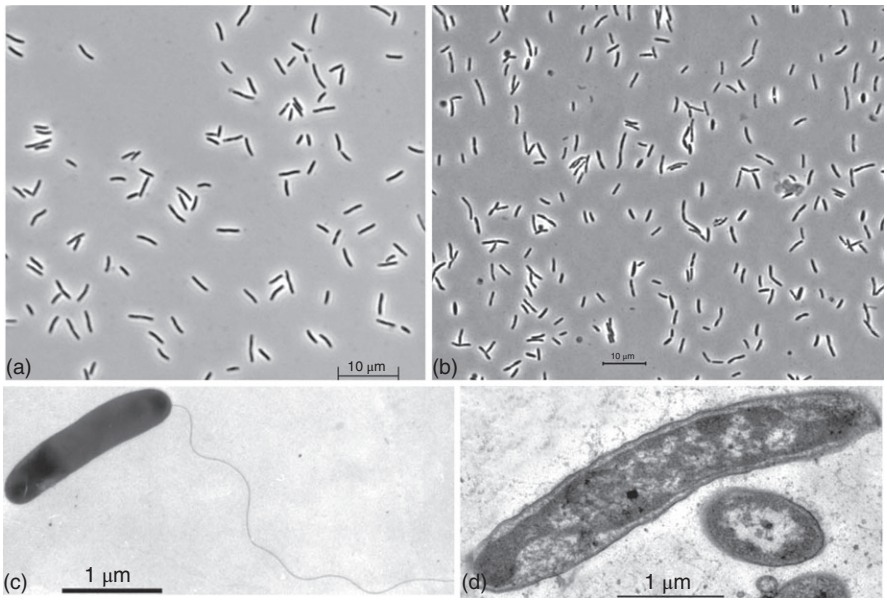
FIGURE 1. Morphology of *Natronocella acetinitrilica* grown at 0.6 M total Na^+ and pH 10 with acetonitrile. (a, b) Phase contrast microphotographs of cells of strain ANL6-2^T and ANL1, respectively; (c) electron microphotograph of positively stained cell of ANL6-2^T showing a single polar flagellum; (d) thin-section electron microphotograph of ANL6-2^T cells showing the Gram-negative type of cell wall.



The most characteristic feature of the genus is its ability to utilize short-chain aliphatic nitriles and their corresponding amides via the nitrile hydratase pathway. While growth is only possible with aceto- and propionitrile, the cells grown with those nitriles were also able to hydrolyze acrylonitrile (Sorokin et al., 2007).

Habitat, enrichment, and isolation

N. acetinitrilica ANL 1 was enriched from a mixture of brines/surface sediments from several soda lakes in the Kulunda Steppe (Altai, Russia), while the type strain ANL 6-2^T was obtained from soda lakes in northeastern Mongolia. The enrichment medium was based on a sodium carbonate buffer at pH 10 containing 0.6 M total Na^+ supplemented with 10 mM acetonitrile. After several dilution-to-extinction series, the final positive dilutions were plated on agar medium with the same composition, and only the yellow-orange colonies (among many others) were finally able to grow on liquid medium with acetonitrile as the only substrate.

Genome analysis

The draft genome of *N. acetonitrilica* was sequenced in the Joint Genome Institute and the assembly is available in the GenBank under the number GCA_024170285.1. It is 4.57 Mb and comprises 4,494 genes of which 4,391 are protein-coding. Below the main functional content of the genome is described.

1. The main functional property of *N. acetonitrilica* is its ability to use aliphatic nitriles as carbon, energy, and the nitrogen source. The primary nitrile degradation in this bacterium proceeds via nitrile hydratase, and the formed amide is further degraded to ammonia and carboxylic acid via amidase. The genome contains three putative operons encoding [Co]-nitrile hydratases NthAB, one of which also includes an aliphatic amidase. In the three NthAB loci, the catalytic [Co]-subunits NthA are not closely related, indicating independent origins. Furthermore, 12 additional amidases are encoded elsewhere on the genome. Apart from amides released from nitriles, *Natronocella* stains can use free ammonium and urea as N-sources while growing on carboxylic acids. The ability to degrade urea is also confirmed by the presence of a full urease operon *ureABCDGJ* in the genome.
2. *Halophilic adaptation*: De novo organic osmolyte biosynthesis includes the genes for ectoine biosynthesis (EctABC) and glycine-betaine production by *S*-adenosylmethionine-dependent dimethylation of sarcosine with the sarcosine-dimethylglycine methyl-transferase [first characterized

in the gammaproteobacterium *Halorhodospira halophila* (Nyyssölä et al., 2001)]. Import of the glycine betaine and choline by the BCC-type transporters (four copies) is also possible. Furthermore, a genomic island encoding oxidation of choline into glycine betaine and its further oxidative demethylation to glycine is present, including choline dehydrogenase BetA [choline→glycine betaine (Landfald and Strøm, 1986)]; dimethylglycine demethylase DgcAB (DMG→sarcosine) plus two copies of sarcosine oxidase SoxABCD [sarcosine→glycine (Wargo, 2013)].

3. *Alkaliphilic adaptation includes*: Two operons encoding multisubunit sodium:proton antiporters, MrpBCD1D2D3EFG (lacking the subunit A) and MrpABCDEFG; three copies of the monosubnit proton/sodium-potassium antiporter NhaP, two copies of the Ca/Na antiporter, and KefB type potassium/proton antiporter.
4. Respiratory complexes include proton-pumping NADH dehydrogenase NuoABCDEFGHIJKLMN, F_0F_1 ATP synthase AtpABCαβγΔε, and *cbb*$_3$ type cytochrome *c* oxidase. An operon is present encoding a membrane-bound dissimilatory nitrate reductase NarGHIJKL, but the both strains tested negative for anaerobic growth with acetate and nitrate as the electron acceptor.

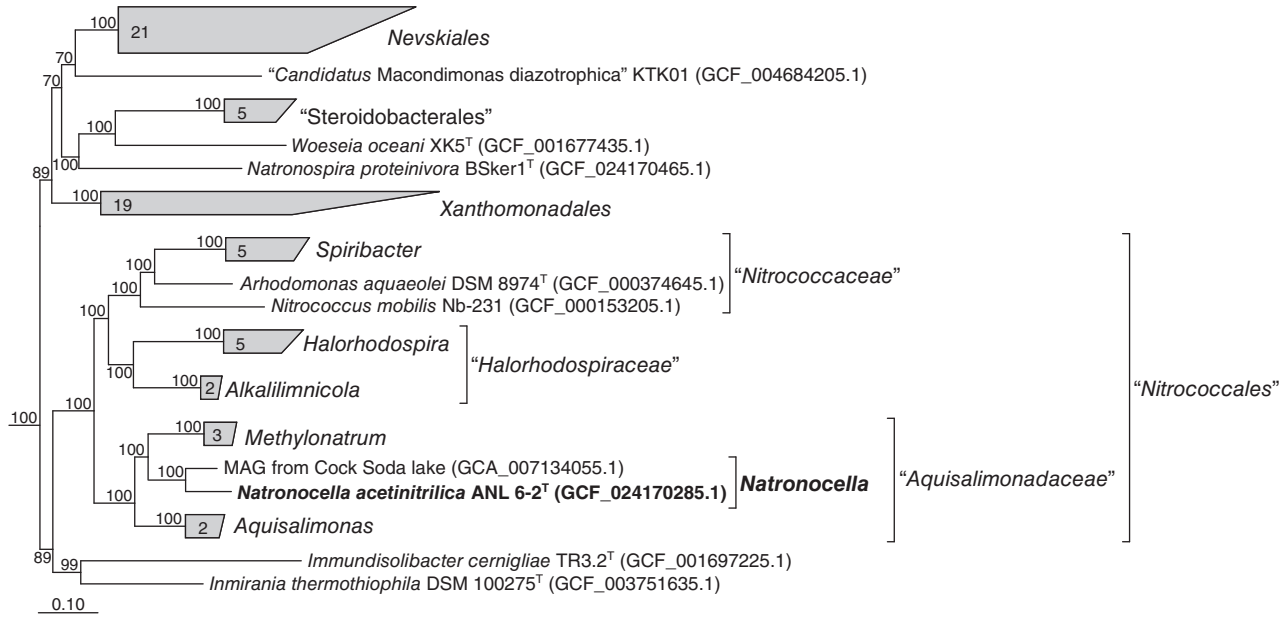
Maintenance and preservation

Active liquid cultures of *N. acetinitrilica* remain viable at 4°C for up to 6 months. Long-term preservation by deep freezing is possible with 15% glycerol as a cryoprotectant.

Taxonomy

The genus *Natronocella* was originally classified as a member of the family *Ectothiorhodospiraceae*, order *Chromatiales*, *Gammaproteobacteria*, based on 16S rRNA gene phylogenetic analysis with the ubiquitous soda lake genera *Alcalilimnicola* and *Alkalispirillum* as the nearest relatives (Sorokin et al., 2007). However, more advanced phylogenetic reconstructions based on 120 single-copy bacterial conserved protein markers place *Natronocella* in a new family "*Aquisalimonadaceae*" and a new order "*Nitrococcales*" in the *Gammaproteobacteria* (according to the Genome Taxonomy DataBase classification, https://gtdb.ecogenomic.org/) (Figure 2). Furthermore, two metagenomic assembled genomes (MAGs) apparently

FIGURE 2. Phylogenetic position of *Natronocella acetinitrilica* ANL 6-2^T (in bold) within the *Gammaproteobacteria* based on analysis of concatenated alignment of 120 single copy conserved bacterial protein markers (according to the Genome Taxonomy DataBase 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 (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.



closely related to *Natronocella* have been recovered from Cock Soda lake—a southwestern Siberian moderately saline soda lake (Vavourakis et al., 2018, 2019). Only the high-quality MAG (GCA_007134055.1; completeness > 90%) is shown in Figure 2.

List of species of the genus *Natronocella*

Natronocella acetinitrilica Sorokin et al. 2007, VL116

a.ce.ti.ni.tri'li.ca N.L. neut. n. *acetinitrilum*, acetinitrile; L. adj. suff. *-icus -a -um*, suffix used with the sense of belonging to; N.L. fem. adj. *acetinitrilica*, pertaining to the ability to utilize acetonitrile.

Cells are rods, 1.5–4.0 × 0.4–0.5 µm, motile with a single polar or subpolar flagellum. Yellow-colored due to the presence of ketocarotenoids, dominated by zeaxanthin. Identified membrane polar phospholipids include phosphatidylglycerol (PG) and phosphatidylserine (PS). The dominant polar lipid fatty acids are $C_{18:1}$ ω7*c* and $C_{16:0}$. Major respiratory lipoquinone is ubiquinone Q8. Has a genetic potential to synthesize the organic osmolyte ectoine and to import and metabolize precursors of glycine betaine. Utilizes acetonitrile and propionitrile as the carbon, energy, and nitrogen sources via [Co]-nitrile hydratase/amidase enzyme tandem. Furthermore, ammonium and urea can serve as the nitrogen source. Obligately alkaliphilic with a pH range for growth from 8 to 10.5 (optimum at 9.5–9.8). Can grow in saturated soda brines containing up to 4 M total Na^+ with an optimum at 0.6 M. Includes two strains isolated from soda lakes of southwestern Siberia and northeastern Mongolia.

DNA G+C content (%): 63.0 (genome sequence of the type strain).

Type strain: ANL 6-2 (=NCCB 100123=UNIQEM U236).

EMBL/GenBank accession (16S rRNA gene): EF103128.

Assembly sequence ID of *ANL 6-2^T in GenBank*: GCA_024170285.1.

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