Natranaerobius thermophilus gen. nov., sp. nov., a halophilic, alkalithermophilic bacterium from soda lakes of the Wadi An Natrun, Egypt, and proposal of *Natranaerobiaceae* fam. nov. and *Natranaerobiales* ord. nov.

Noha M. Mesbah,¹ David B. Hedrick,² Aaron D. Peacock,² Manfred Rohde³ and Juergen Wiegel¹

Correspondence
Juergen Wiegel
jwiegel@uga.edu

¹Department of Microbiology, University of Georgia, Athens, GA 30602, USA

²University of Tennessee, Knoxville, TN 37932, USA

³Department of Microbial Pathogenicity, Helmholtz Center for Infection, Research, Inhoffenstrasse 7, D-38124 Braunschweig, Germany

Novel halophilic, alkalithermophilic, Gram-type-positive bacterial strains were isolated from sediment of alkaline, hypersaline lakes of the Wadi An Natrun, Egypt. Cells of strain JW/NM-WN-LF^T were rod-shaped, non-spore-forming and non-motile. Strain JW/NM-WN-LF^T grew (at pH^{55 °C} 9.5) between 35 and 56 °C, with an optimum at 53 °C. The pH^{55 °C} range for growth was 8.3–10.6, with an optimum at pH^{55 °C} 9.5 and no growth at pH^{55 °C} 8.2 or below, or at pH^{55 °C} 10.8 or above. At the optimum pH and temperature, the strain grew in the Na⁺ range of 3.1–4.9 M (1.5–3.3 M added NaCl) and optimally between 3.3 and 3.9 M Na⁺ (1.7–2.3 M added NaCl). Strain JW/NM-WN-LF^T utilized fructose, cellobiose, ribose, trehalose, trimethylamine, pyruvate, Casamino acids, acetate, xylose and peptone as carbon and energy sources. Fumarate (20 mM), S₂O₃²⁻ (20 mM), NO₃⁻ (20 mM) and iron(III) citrate (20 mM) were utilized as electron acceptors. During growth on sucrose, the isolate produced acetate and formate as major fermentation products. Main cellular fatty acids were iso-branched 15:0, i17:0 dimethylacetal and 16:0 dimethylacetal. The G + C content of genomic DNA was 40.4 mol% (HPLC). On the basis of genotypic and phenotypic characteristics, it is proposed that strain JW/NM-WN-LF^T represents a novel genus and species, *Natranaerobius thermophilus* gen. nov., sp. nov. The type strain is JW/NM-WN-LF^T (=DSM 18059^T=ATCC BAA-1301^T). Based on 16S rRNA gene sequence analysis, the strain forms a novel lineage within the class 'Clostridia' and clusters with uncultivated bacteria and unidentified strains retrieved from alkaline, hypersaline environments. The phylogenetic data suggest that the lineage represents a novel family, *Natranaerobiaceae* fam. nov., and order, *Natranaerobiales* ord. nov.

Extremophiles are micro-organisms that are well adapted to one or two extreme environmental conditions. Halophilic alkalithermophiles are a novel physiological group that require high salt concentrations, alkaline pH values and elevated temperatures for growth. Halophilic

alkalithermophiles must possess special adaptive mechanisms for survival under these three extreme conditions. As a result of their unique and extreme growth conditions, halophilic alkalithermophiles are of considerable commercial and biotechnological significance. Halophilic alkalithermophiles are also of evolutionary significance, as they represent model organisms for evaluating theories on the origin of life. These include the hypothesis that life evolved in shallow, heated saline and alkaline pools (Baross, 1998; Zavarzin, 1993). We describe in this report the characterization of, to our knowledge, the first true anaerobic, halophilic alkalithermophile isolated from sediments of the solar-heated, alkaline, hypersaline soda lakes of the Wadi An Natrun, Egypt [criteria for defining halophiles,

Abbreviations: DMA, dimethylacetal; PLFA, phospholipid fatty acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JW/NM-WN-LF^T is DQ417202.

A supplementary table showing the PLFA composition of strain JW/NM-WN-LF^T and supplementary figures showing the dependence of growth of strain JW/NM-WN-LF^T on temperature and medium pH and a Fitch–Margoliash tree based on 16S rRNA gene sequences are available with the online version of this paper.

alkaliphiles and thermophiles have been described by Oren (2000) and Wiegel (1998a)]. On the basis of the physiological and phylogenetic evidence presented, we propose a novel genus, *Natranaerobius* gen. nov., to accommodate this micro-organism. Further, the order *Natranaerobiales* ord. nov., consisting of the family *Natranaerobiaceae* fam. nov., is proposed to encompass *Natranaerobius* gen. nov.

Isolation and cultivation of strain JW/NM-WN-LF^T

Strain JW/NM-WN-LF^T was isolated from a mixed water-sediment sample collected from the sediment of Lake Fazda, Wadi An Natrun, Egypt, during May 2005. At the time of collection, the lake water had a salinity of 4.7 M and pH^{25 °C} 9.8. For initiating enrichment cultures, 5 g soil was inoculated into 80 ml carbonate-buffered medium consisting of (g l⁻¹): KH₂PO₄, 0.2; MgCl₂, 0.1; KCl, 0.2; NH₄Cl, 0.5; NaCl, 100; Na₂CO₃, 68; NaHCO₃, 38; cysteine.HCl, 0.7; yeast extract, 5; tryptone, 5; sucrose, 5; and trace element solution, 1 ml (Kevbrin & Zavarzin, 1992); vitamin solution, 10 ml (Wolin *et al.*, 1963). The pH^{55 °C} was adjusted to 9.5 with anaerobic 5 M HCl. The enrichment cultures became turbid after 48 h growth at 55 °C. Pure cultures were obtained in dilution rows in agar (1 %, w/v) shake-roll tubes (Ljungdahl & Wiegel, 1986). To ensure that colonies were derived from a single cell, the isolate was purified by four successive rounds of single-colony isolation. The isolates were maintained in the carbonate-buffered medium at pH^{55 °C} 9.5 and 55 °C under anaerobic conditions (100 % N₂) by using a modified Hungate technique (Ljungdahl & Wiegel, 1986).

Colony and cell morphology

Colonies of strain JW/NM-WN-LF^T appeared in agar shake-roll tubes after 3–4 days and were 1–2 mm in diameter, circular to irregularly shaped and opaque. Cell morphology was observed via light microscopy (Olympus VANOX phase-contrast microscope) and electron microscopy. Cells in liquid culture in the exponential-growth phase were straight to curved rods, 0.2–0.4 µm in diameter

and 3–5 µm in length. Cells either were single or formed chains. No active motility was observed under phase-contrast microscopy; accordingly, flagella were absent in negatively stained samples (2 % uranyl acetate). Cells exhibited a rod-like appearance with variable length, as shown by field emission scanning electron microscopy (Fig. 1a; taken with a Zeiss DSM982 Gemini). Ultrathin sections exhibit a Gram-type-positive cell wall and no endospores were observed either with a Zeiss EM910 (Fig. 1b) or by light microscopy after heat treatment (10 min at 80 °C) (Fig. 1). Cells stained Gram-positive in both the early exponential- and stationary-growth phases (Doetsch, 1981).

Cultural and physiological characteristics

The optimal conditions for growth of strain JW/NM-WN-LF^T were tested in carbonate-buffered medium with 0.3 % yeast extract and tryptone, 640 mM Na₂CO₃ and 320 mM NaHCO₃ (before pH adjustment, yielding a base Na⁺ concentration of 1.6 M). By using a temperature-gradient incubator (Scientific Industries, Inc.), the temperature range for growth (at pH^{55 °C} 9.5) was 35–56 °C with an optimum at 53 °C, and no growth was observed at 34 °C or below, or at 57 °C or above after 2 weeks.

The growth-temperature profile revealed a broken Arrhenius plot with two peaks and an intermediate plateau [see Supplementary Fig. S1(a), available in IJSEM Online]. The initial peak in growth rate occurred at 37 °C (doubling time, 6 h); the second, and larger, peak in growth occurred at 53 °C (doubling time, 3.2 h). Such a pattern has been observed with other thermophiles (Wiegel, 1990, 1998b). Dilution to extinction, microscopy and 16S rRNA gene sequence analysis all confirmed that the isolate was pure and was not contaminated with another mesophilic or thermotolerant micro-organism that could have caused the initial growth peak at 37 °C.

The pH range for growth was determined at 55 °C in the above-mentioned carbonate-buffered medium. All pH measurements were performed as described previously (Mesbah & Wiegel, 2006) with a microelectrode (Accumet

Fig. 1. Electron microscopic images of strain JW/NM-WN-LF^T. (a) Field emission scanning electron micrograph of aldehyde-fixed, acetone-dehydrated and critical point-dried cells of strain JW/NM-WN-LF^T, revealing a rod-like appearance with variable length of single cells. Bar, 5 µm. (b) Ultrathin section (embedded in LR White and counterstained with uranyl acetate), exhibiting the Gram-positive-like cell structure (cm, cell membrane; cw, cell wall); furthermore, no endospores were detectable within the cytoplasm. Bar, 0.25 µm.

combination microelectrode with calomel reference; Cole-Parmer), calibrated at 55 °C with pH standards preheated to the same temperature. The pH of the medium was adjusted by addition of sterile, anaerobic HCl or Na₂CO₃. The pH^{55 °C} range for growth was 8.3–10.6, with an optimum at pH^{55 °C} 9.5. There was no growth at pH^{55 °C} 8.2 or below, or at pH^{55 °C} 10.8 or above [Supplementary Fig. S1(b)].

The salinity range for growth was determined in carbonate-buffered medium at pH^{55 °C} 9.5. Strain JW/NM-WN-LF^T grew over total Na⁺ concentrations (which includes 1.5–3.3 M added NaCl) of 3.1–4.9 M [corresponding to 18.0–28.5 % (w/v) NaCl]. No growth occurred when the total Na⁺ concentration was below 3.0 M. Optimal growth occurred at Na⁺ concentrations between 3.3 and 3.9 M. Maximum Na⁺ tolerance of strain JW/NM-WN-LF^T was not increased or decreased by addition of 500 mM KCl to the growth medium. Strain JW/NM-WN-LF^T did not grow when equimolar amounts of K₂CO₃ and KHCO₃ were substituted for Na₂CO₃ and NaHCO₃, even in the presence of 1.7–3.1 M NaCl. The doubling time at the optimal conditions, i.e. 3.5 M Na⁺, pH^{55 °C} 9.5 and 53 °C, was 3.5 h.

For substrate-utilization tests, cultures were incubated for up to 5 days and growth was judged positive if, in the third successive transfer, the OD₆₀₀ of the culture was twice that of a control culture incubated with only 0.2 % yeast extract and tryptone. Utilization of possible substrates (0.5 %, w/v) was tested in the presence of 0.2 % yeast extract and tryptone. Strain JW/NM-WN-LF^T used fructose, cellobiose, ribose, trehalose, trimethylamine, pyruvate, Casamino acids, acetate, xylose and peptone as carbon and energy sources. No growth was observed with glucose, mannose, formate, glycine betaine, ethanol, n-propanol or ribitol as carbon or energy sources. The use of electron acceptors was determined by measuring growth (increase in OD₆₀₀), production of sulfide, ammonium and nitrate, and colour change. In the presence of 0.2 % yeast extract, strain JW/NM-WN-LF^T utilized the following as electron acceptors: fumarate (20 mM), S₂O₃²⁻ (20 mM), NO₃⁻ (20 mM) and iron(III) citrate [20 mM, determined by A₅₆₂ of Fe(II)–ferrozine complex (Stookey, 1970)]. None of the following electron acceptors was utilized: SO₄²⁻ (20 mM), SO₃²⁻ (20 mM) or MnO₂ (10 mM). The main organic fermentation products from 20 mM sucrose were acetate (17 mM) and formate (10 mM), and minor amounts of lactate (2.5 mM) were also produced.

Strain JW/NM-WN-LF^T was negative for catalase and oxidase, gelatin liquefaction and casein degradation. Strain JW/NM-WN-LF^T was obligately anaerobic. Negative results were obtained in API ZYM enzyme assay (bioMérieux) for alkaline phosphatase, esterase C-4, esterase lipase, leucine arylamidase, valine arylamidase and cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-α,β-phosphohydrolase, α- and β-galactosidase, α- and β-glucosidase, β-glucouronidase, α-mannosidase, α-fucosidase and N-acetyl-β-glucosaminidase.

Chemotaxonomic characteristics

Attempts to purify peptidoglycan from cells of strain JW/NM-WN-LF^T failed, and no isomer of diaminopimelic acid was detected in the strain. It was concluded that the amount of peptidoglycan in the strain is below detectable amounts (Peter Schumann, personal communication).

Phospholipid fatty acid (PLFA) analysis was performed on cells that had been grown at 53 °C, pH^{55 °C} 9.5, 1.7 M NaCl, 640 mM Na₂CO₃ and 320 mM NaHCO₃. Lyophilized cell material was extracted by using a chloroform/methanol/water solvent system (Bligh & Dyer, 1959) with the modification of Peacock *et al.* (2001). The total lipid extract obtained was then fractionated into neutral lipid, glycolipid and polar lipid fractions by silicic acid column chromatography (Guckert *et al.*, 1985). The polar lipid fraction was prepared for gas chromatography/mass spectroscopy by transesterification to fatty acid methyl esters by mild alkaline hydrolysis (Guckert *et al.*, 1985). The resulting mixed fatty acid methyl esters and dimethylacetals (DMAs) were separated and quantified by using a Hewlett Packard 5890 series 2 gas chromatograph interfaced with a Hewlett Packard 5971 mass-selective detector. The chromatographic column was a 50 m non-polar column (0.2 mm i.d., 0.11 mm film thickness). The amount of PLFA + DMA (g cells)⁻¹ was 13.6 nmol (g dry weight cell material)⁻¹. PLFA composition of strain JW/NM-WN-LF^T was dominated by branched-chain fatty acids (i15:0, i17:0), which formed 29 % of total PLFAs. PLFA analysis also showed a unique pattern of DMAs, which were predominated by a branched-chain DMA (i17:0DMA, 27.4 % of total PLFA) and an unbranched DMA (16:0DMA, 16.4 % of total PLFA). Small amounts of unsaturated PLFAs and unbranched DMAs were also present (see Supplementary Table S1, available in IJSEM Online).

The DNA G + C content of strain JW/NM-WN-LF^T was determined by HPLC according to Mesbah *et al.* (1989) with the modification of Lee *et al.* (2005), using S1 nuclease and 0.3 M sodium acetate (pH 5.0). The G + C content of genomic DNA was 40.4 mol% (mean of six replicate analyses).

Phylogenetic analysis

The nearly complete 16S rRNA gene sequence for strain JW/NM-WN-LF^T was determined by Macrogen, Inc. (Seoul, Korea), and compared with all GenBank entries by BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST>). The partial 16S rRNA gene sequence of strain JW/NM-WN-LF^T was located in a phylogenetic cluster consisting of uncultured bacterial clones from sediments of the alkaline, hypersaline lakes of the Wadi An Natrun, Egypt (Mesbah *et al.*, 2007). The pH in these sediments ranged from 9 to 11 and the NaCl concentration at the time of sampling was approximately 5 M. Strain JW/NM-WN-LF^T was also related closely (93–95 % 16S rRNA gene sequence

similarity) to unpublished bacterial strains isolated from the alkaline soda lakes in the Kenyan–Tanzanian Rift (Jones *et al.*, 1998; Owenson, 1997). No effective description of these isolates exists in the literature describing the temperature range. These isolates were retrieved from mixed water–sediment samples and grown at 37 °C (Owenson, 1997). The soda lakes of the Kenyan–Tanzanian Rift are reported to have pH values ranging between 10 and 12 and salinity levels greater than 2.5 M. The lakes are solar-heated; no source of geothermal heating has been reported. The three corresponding strains mentioned by Owenson (1997) were Gram-staining-variable, anaerobic, heterotrophic rods of various sizes, able to use a variety of heterotrophic substrates including glucose, and formed acetate and isovalerate as major fermentation products. The NaCl range is given as 12–26 % (w/v) NaCl and the pH optimum as >9.5. However, the strains are not presently available for comparison.

Among species with validly published names, the highest 16S rRNA gene sequence similarity levels were with members of the family *Peptococcaceae*, namely *Desulfotomaculum geothermicum* (approx. 85 % similarity) and the type species *Desulfotomaculum nigrificans* (84 % similarity) (Fig. 2). Strain JW/NM-WN-LF^T clearly belongs to the class ‘*Clostridia*’, but is not affiliated closely with any of the described lineages (Supplementary Fig. S2, available in IJSEM Online, shows type genus *Natranaerobius* gen. nov. for the proposed novel family and order in a tree with the type species of the type genera of the orders and classes in the phylum *Firmicutes*).

The 16S rRNA gene sequence of strain JW/NM-WN-LF^T was aligned with those of representatives of the class ‘*Clostridia*’ (Fig. 2). Multiple sequence alignments were created with the CLUSTAL_X program (<http://bips.u-strasbg.fr/fr/Documentation/ClustalX/>). Trees were constructed by using the PHYLIP software package (<http://evolution.genetics.washington.edu/phylip.html>). Distances were calculated by using the Jukes–Cantor algorithm of

DNADIST, and branching order was determined via the neighbour-joining algorithm of NEIGHBOR. Each tree was a consensus of 1000 replicate trees. Strain JW/NM-WN-LF^T and the African rift isolates and environmental clones formed a strongly supported cluster, with similarity between gene sequences ranging from 92 to 96 %. Interestingly, strain JW/NM-WN-LF^T was only 93 % similar to uncultured clone WN-FSB-108, which was retrieved from sediment of the same lake, indicating that the genus *Natranaerobius* is represented by several species in the lakes that may even be members of closely related genera. The *Natranaerobius* cluster forms a separate lineage within the class ‘*Clostridia*’. Additional phylogenetic analyses performed with 16S rRNA gene sequences of the type genera of the *Firmicutes* and a different treeing method (Fitch–Margoliash) confirmed the divergence of strain JW/NM-WN-LF^T and related sequences from representatives of described families and orders of the class ‘*Clostridia*’ (Supplementary Fig. S2).

Taxonomic conclusions

Phylogenetic analysis indicates that strain JW/NM-WN-LF^T belongs to the class ‘*Clostridia*’ of the phylum *Firmicutes*. Table 1 shows the phenotypic characteristics of strain JW/NM-WN-LF^T and the two most closely related species with validly published names, *D. geothermicum* and *D. nigrificans*, belonging to the clostridial family *Peptococcaceae*. Similar to strain JW/NM-WN-LF^T, *D. geothermicum* has a growth temperature optimum of 53 °C (temperature range, 37–57 °C), and also has i15:0 as a major cellular fatty acid. However, it clearly differs in its NaCl requirement for growth (0.0–0.7 M) and lack of DMAs in the PLFA profile. *D. geothermicum* is also neutrophilic; it does not tolerate pH values greater than 8.5. *D. nigrificans* is distinguished from strain JW/NM-WN-LF^T in that it is motile and has a different fatty acid content and different NaCl, pH and temperature ranges and optima (Table 1).

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences, showing the position of strain JW/NM-WN-LF^T in relation to its closest relatives within the phylum *Firmicutes*, unidentified strains from the Kenyan–Tanzanian Rift and uncultured clones from sediments of Wadi An Natrun lakes. GenBank accession numbers for the sequences are shown in parentheses. The tree was rooted with the 16S rRNA gene sequence of *Escherichia coli* DSM 30083^T as the out-group. Numbers at nodes are bootstrap values based on 1000 replicates; only values greater than 500 are shown. Bar, one nucleotide substitution per 100 nt.

Table 1. Differential characteristics of strain JW/NM-WN-LF^T and closely related species

Strains: 1, JW/NM-WN-LF^T; 2, *Desulfotomaculum geothermicum* DSM 3669^T [data from Daumas *et al.* (1988)]; 3, *Desulfotomaculum nigrificans* ATCC 19998^T [data from Nazina *et al.* (2005)]; 4, African rift isolate G-M14CH-4 (Owenson, 1997). +, Positive, –, negative; ND, not determined.

Characteristic	1	2	3	4
Length of rods (μm)	3–5	2–3	3–6	0.6–10
Motility	–	+	+	–
pH range (optimum)	8.3–10.6 (9.5)	6.5–8.5 (7.3–7.5)	6.0–8.5 (7.0)	9.5–10.5 (>9.5)
NaCl range (optimum) (% w/v)	9–19 (10–14)	0–4 (2.5–3.5)	0–6 (1)	12–26 (20)
Temperature range (optimum) (°C)	35–56 (53)	37–57 (54)	45–75 (60–65)	ND
Major fatty acids (>8%)	i15:0 (23%); i17:0DMA (27.4%); 16:0DMA (16.4%)	i15:0 (32.8%); 16:0 (10.1%)	i15:0 (29.6%); 16:0 (26.6%); i17:0 (14.2%); 18:0 (16.7%)	ND
DNA G + C content (mol%)	40.4	50.4	50.8	ND

Altogether, phylogenetic and physiological data indicate that strain JW/NM-WN-LF^T is sufficiently divergent from all known bacterial species to be described as a novel genus and species, *Natranaerobius thermophilus* gen. nov., sp. nov.

At present, the class ‘Clostridia’ is represented by three orders, *Clostridiales*, *Halanaerobiales* and ‘*Thermoanaerobacteriales*’. Based on the distinct phylogenetic position of *Natranaerobius thermophilus* gen. nov., sp. nov. within the class ‘Clostridia’ and the differences observed in physiological and cultural characteristics, a novel order, *Natranaerobiales* ord. nov., represented by the single family *Natranaerobiaceae* fam. nov., is proposed.

Description of *Natranaerobius* gen. nov.

Natranaerobius [Natr.an.ae.ro'bi.us. N.Gr. n. *natron* derived from Arabic *natrun* soda (sodium carbonate); Gr. pref. *an* not; Gr. n. *aer* air; Gr. masc. n. *bios* life; N.L. masc. n. *Natranaerobius* a soda-requiring anaerobe].

Cells are Gram-type-positive; endospores are not observed. Obligately halophilic (growth requires at least 3 M Na⁺); obligately alkaliphilic (no growth below pH 8.3). Thermophilic. Fatty acid profile is dominated by branched fatty acids with odd numbers of carbons; dimethylacetals are also present. The DNA G + C content is approximately 40 mol%. Strictly anaerobic chemo-organotrophs. The type species is *Natranaerobius thermophilus* sp. nov.

Description of *Natranaerobius thermophilus* sp. nov.

Natranaerobius thermophilus (ther.mo'phi.us. Gr. n. *therme* heat; Gr. adj. *philos* friendly, loving; N.L. masc. adj. *thermophilus* heat-loving, referring to its growth temperature).

Cells form irregularly shaped to circular, opaque colonies with a white colour (when grown inside 1 % agar). Cells are 3–5 × 0.2–0.4 μm in size, non-motile and catalase- and oxidase-negative. Cells are Gram-staining and Gram-type-positive (Wiegel, 1981). Extremely halophilic: optimal

growth occurs between 3.3 and 3.9 M Na⁺ (1.7–2.3 M added NaCl); no growth occurs at Na⁺ concentrations below 3.0 M or greater than 5 M. Obligately alkaliphilic: pH^{55 °C} range, 8.3–10.6, with an optimum at pH^{55 °C} 9.5. Thermophilic: temperature range for growth is 34–57 °C (at pH^{55 °C} 9.5), with an optimum at 53 °C. Obligately anaerobic. When 0.2 % yeast extract and tryptone are present, fructose, cellobiose, ribose, trehalose, trimethylamine, pyruvate, Casamino acids, acetate, xylose and peptone are used as carbon and energy sources. The main organic fermentation products from 0.5 % sucrose are formate and acetate. Fumarate (20 mM), S₂O₃^{2–} (20 mM), NO₃[–] (20 mM) and iron(III) citrate (20 mM) are utilized as electron acceptors. Major cellular fatty acids include i15:0, i17:0DMA and 16:0DMA. The type strain lacks significant amounts of murein and *meso*-diaminopimelic acid in the cell wall. The DNA G + C content of genomic DNA is 40.4 mol% (HPLC).

The type strain, JW/NM-WN-LF^T (=DSM 18059^T=ATCC BAA-1301^T), was isolated from sediment of Lake Fazda, Wadi An Natrun, Egypt.

Description of *Natranaerobiales* ord. nov.

Natranaerobiales (Natr.an.ae.ro.bi.a'les. N.L. masc. n. *Natranaerobius* type genus of the order; *-ales* ending to denote an order; N.L. fem. pl. n. *Natranaerobiales* the order of the genus *Natranaerobius*).

Description is as for the family. The type genus is *Natranaerobius*.

Description of *Natranaerobiaceae* fam. nov.

Natranaerobiaceae (Natr.an.ae.ro.bi.a'ce.ae. N.L. n. *Natranaerobius* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Natranaerobiaceae* the family of the genus *Natranaerobius*).

Cells are Gram-staining and Gram-type-positive; endospores are not observed. Straight or slightly curved, slender rods. Non-motile. Strictly anaerobic. Members are halophilic and

alkaliphilic. Chemolitho- or organoheterotrophic. Phylogenetically, the family belongs to the order *Natranaerobiales*. The type genus is *Natranaerobius*.

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