Methylohalomonas lacus gen. nov., sp. nov. and *Methylonatronum kenyense* gen. nov., sp. nov., methylotrophic gammaproteobacteria from hypersaline lakes

Dimitry Yu. Sorokin,^{1,2} Yuri A. Trotsenko,³ Nina V. Doronina,³ Tatjana P. Tourova,¹ Erwin A. Galinski,⁴ Tatjana V. Kolganova⁵ and Gerard Muyzer²

Correspondence

Dimitry Yu. Sorokin
soroc@inmi.host.ru
or
D.Y.Sorokin@tnw.tudelft.nl

¹Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-let Otyabrya 7/2, 117811 Moscow, Russia

²Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

³G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia

⁴Institute of Microbiology and Biotechnology, Rheinische Friedrich-Wilhelms University, Meckenheimer Allee 168, 53115 Bonn, Germany

⁵Center Bioengineering, Russian Academy of Sciences, Prospect 60-let Otyabrya 7/1, 117312 Moscow, Russia

Aerobic enrichment at 4 M NaCl, pH 7.5, with methanol as carbon and energy source from sediments of hypersaline chloride–sulfate lakes in Kulunda Steppe (Altai, Russia) resulted in the isolation of a moderately halophilic and obligately methylotrophic bacterium, strain HMT 1^T. The bacterium grew with methanol and methylamine within a pH range of 6.8–8.2 with an optimum at pH 7.5 and at NaCl concentrations of 0.5–4 M with an optimum at 2 M. In addition to methanol and methylamine, it can oxidize ethanol, formate, formaldehyde and dimethylamine. Carbon is assimilated via the serine pathway. The main compatible solute is glycine betaine. 16S rRNA gene sequence analysis placed the isolate as a new lineage in the family *Ectothiorhodospiraceae* (*Gammaproteobacteria*). It is proposed, therefore, to accommodate this bacterium within a novel genus and species, *Methylohalomonas lacus* gen. nov., sp. nov., with HMT 1^T (=DSM 15733^T =NCCB 100208^T =UNIQEM U237^T) as the type strain. Two strains were obtained in pure culture from sediments of soda lake Magadi in Kenya and the Kulunda Steppe (Russia) on a mineral medium at pH 10 containing 0.6 M total Na⁺ using methanol as a substrate. Strain AMT 1^T was enriched with methanol, while strain AMT 3 originated from an enrichment culture with CO. The isolates are restricted facultative methylotrophs, capable of growth with methanol, formate and acetate as carbon and energy sources. With methanol, the strains grew within a broad salinity range from 0.3 to 3.5–4 M total Na⁺, with an optimum at 0.5–1 M. The pH range for growth was between 8.3 and 10.5, with an optimum at pH 9.5, which characterized the soda lake isolates as obligate haloalkaliphiles. Carbon is assimilated autotrophically via the Calvin–Benson cycle. Sequence analysis of the gene coding for the key enzyme RuBisCO demonstrated that strain AMT 1^T possessed a single *cbbL* gene of the ‘green’ form I, clustering with members of the family *Ectothiorhodospiraceae*. Analysis of the 16S rRNA gene sequence showed that strains AMT 1^T and AMT 3 belong to a single species that forms a separate lineage within the family

Abbreviation: EPS, exopolysaccharide.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains HMT 1^T, AMT 1^T and AMT 3 are DQ834966, DQ789390 and EU006088, respectively. Those for the *cbbL* gene from AMT 1^T and for the *mxoF* gene from HMT 1^T are respectively EF152335 and EF152336.

Fatty acid compositions of isolates AMT 1^T and HMT 1^T and results of PCR amplifications of *mxoF* with four different primer sets from (halo)alkaliphilic methylotrophs are available as supplementary material with the online version of this paper.

Ectothiorhodospiraceae. On the basis of phenotypic and genetic data, the novel haloalkaliphilic methylotrophs are described as representing a novel genus and species, *Methylonatronum kenyense* gen. nov., sp. nov. (type strain AMT 1^T = DSM 15732^T = NCCB 100209^T = UNIQEM U238^T).

Hypersaline inland lakes occur as two major types: chloride–sulfate lakes, with a neutral to slightly alkaline pH (6–8.5), and soda lakes, where the presence of free carbonate/bicarbonate can reach molar concentrations, resulting in the accumulation of highly alkaline brines with a pH from 9.5 to 11. Both lake types are populated almost entirely by prokaryotes belonging to specialized (halo)alkaliphilic ecotypes. Most of these lakes are highly productive, with nitrogen-fixing (halo)alkaliphilic cyanobacteria as dominant primary producers. Various types of (halo)alkaliphilic anaerobic prokaryotes, including fermentative bacteria, methanogens and sulfate-reducing bacteria, are responsible for the complete degradation of organic matter, resulting in the formation of sulfide and methane as final products (Oren, 2002; Zavarzin *et al.*, 1999). Methanotrophic and methylotrophic bacteria utilize the C₁ compounds produced from anaerobic degradation of organic matter, thus closing the carbon cycle. Furthermore, in hypersaline habitats, the total flux of methylated compounds is enhanced by their formation as a degradation product from osmolytes, such as glycine betaine and dimethyl sulfoniopropionate. Also, haloalkaliphilic methanotrophic bacteria are known to excrete substantial amounts of C₁ intermediates during methane oxidation (Trotsenko & Khmelenina, 2002a, b). Measurements with ¹⁴C₁ compounds have demonstrated low but detectable rates of degradation in sediments of hypersaline lakes, indicating the presence of methano- and methylotrophic bacteria active under high salt conditions (Oremland *et al.*, 1982; Sokolov & Trotsenko, 1995; Namsaraev *et al.*, 1999; Joye *et al.*, 1999; Sorokin *et al.*, 2004). Nevertheless, the (halo)alkaliphilic methano- and methylotrophic bacteria isolated so far in pure culture from saline lakes all belong to a low-salt-tolerant type (Trotsenko & Khmelenina, 2002a, b; Heyer *et al.*, 2005; Doronina *et al.*, 2000, 2001, 2003a, b). The only exception is a halophilic methanotroph, *Methylohalobius crimeensis* 10Ki^T, capable of growth with up to 15 % NaCl (Heyer *et al.*, 2005).

In this paper, we describe the isolation and properties of two novel species of methylotrophic gammaproteobacteria from hypersaline lakes capable of growth with C₁ compounds up to salt-saturating conditions.

The Kulunda Steppe (Altai, Russia) harbours numerous salt lakes with a total salt content from 10 to 38 % and a pH of 7.0–8.5, with Na⁺, Mg²⁺, Cl[−] and SO₄^{2−} as the dominant ions in the brines. Samples of the top 10 cm of sediment with overlying brine water from ten lakes were mixed and used as an inoculum to enrich for halophilic methylotrophic bacteria. A sediment sample from the

hypersaline soda lake Magadi (Kenya), pH 10.5 and total salt content of 22 %, was used to enrich for haloalkaliphilic methylotrophs.

The mineral base medium used for enrichment of halophilic methylotrophs included 4 M NaCl, 10 mM K₂HPO₄ and 5 mM (NH₄)₂SO₄, final pH 7.2. The mineral base for haloalkaliphiles contained sodium carbonate/bicarbonate buffer, pH 10, containing 0.6–4 M total Na⁺, 0.1 M NaCl, 10 mM K₂HPO₄ and 5 mM KNO₃. After sterilization, the media were supplemented with 1 mM MgSO₄·7H₂O, 1 ml trace metal solution l^{−1} (Pfennig & Lippert, 1966), 100 µg vitamin B₁₂ l^{−1} and 10 mM NaHCO₃. Methanol (25 mM) served as the electron donor and carbon source. In an enrichment with CO as the only carbon and energy source from Kulunda Steppe lakes, methanol was replaced with 5 % CO in the gas phase (10 mM). After several consecutive additions of CO, the culture was transferred into medium with methanol. The incubation temperature was 30 °C. The isolation strategy included several 1:100 transfers to stabilize the cultures, serial dilutions and plating onto solid medium prepared by mixing at 50 °C of equal volumes of the liquid mineral base medium, indicated above, and 4 % (w/v) Noble agar (Difco). The plates were incubated in closed jars at 5 % O₂. Autotrophic growth with hydrogen as electron donor was studied at optimal pH and salt using 100 ml serum bottles with 20 ml medium under an atmosphere of 50 % air and 50 % H₂. To test for anaerobic growth with methanol (25 mM) and nitrate (20 mM), 80 ml medium was used in 100 ml serum bottles with argon in the gas phase.

Growth was monitored by measuring the OD₆₀₀ and by cell protein analysis with the Lowry method. Respiration rates of washed cells were measured in mineral buffers corresponding to the growth medium composition without nitrogen sources using a Biological oxygen monitor (Yellow Spring Inc.). Activity of the key methylotrophic enzymes in cell-free extracts and the composition of membrane lipids and fatty acids were analysed as described previously (Doronina *et al.*, 1995, 2003a). The composition of compatible solutes in the halophilic isolate HMT 1^T was analysed by an HPLC/¹³C-NMR method according to Galinski & Herzog (1990). Phase-contrast photomicrographs were obtained using a Zeiss Axioplan Imaging 2 microscope. For electron microscopy, cells were fixed with glutaraldehyde (final, 3 % v/v) and positively contrasted with 1 % (w/v) uranyl acetate. For thin sectioning, the cells were fixed in 1 % (w/v) OsO₄ solution containing 0.5–1.0 M NaCl, dehydrated, embedded in resin and stained with uranyl acetate and lead citrate after sectioning. The

isolation of DNA and subsequent determination of the G+C content and DNA–DNA hybridization were performed by the thermal denaturation/reassociation technique (Marmur, 1961; De Ley *et al.*, 1970).

For molecular analysis, genomic DNA was extracted from cells using the UltraClean Soil DNA extraction kit (Mo Bio Laboratories), following the manufacturer's instructions. Nearly complete 16S rRNA genes were amplified by PCR from pure cultures using bacterial primers GM3F and GM4R (Schäfer & Muyzer, 2001). To amplify the *cbbL* gene, coding for the RuBisCO large subunit, a specially designed primer pair and protocol was employed (Spiridonova *et al.*, 2004). Amplification of the *mxsF* gene, coding for methanol dehydrogenase, was performed with four different primer sets (McDonald & Murrell, 1997; Dedysh *et al.*, 2005). The sequences obtained in this study were first compared to sequences stored in GenBank using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/BLAST>). The nucleotide and inferred amino acid sequences were aligned with sequences from GenBank using CLUSTAL W. Phylogenetic trees were reconstructed using the TREECONW (Van de Peer & De Wachter, 1994) and PHYLIP 3.5c (Felsenstein, 1993) program packages with four different algorithms: neighbour-joining, maximum-parsimony, distance matrix and maximum-likelihood.

The enrichment with methanol at 4 M NaCl using a mixture of sediment samples from hypersaline Siberian lakes resulted in the domination of rod-shaped bacteria, which were further purified by serial dilutions and eventually isolated in pure culture from a single colony on plates containing 2 M NaCl and methanol as strain HMT 1^T. Among the enrichments from Lake Magadi at pH 10 with methanol as substrate, positive results were obtained only at low salt (0.6–1.0 M total Na⁺, but not at 3–4 M), resulting in isolation of strain AMT 1^T. Another haloalkaliphilic methylotroph, strain AMT 3, was obtained from an enrichment inoculated with a mixed sediment sample from Kulunda Steppe hypersaline soda lakes where CO served as the only substrate. AMT 3 was a minor satellite component of that enrichment, but it became dominant after transfer into the medium with methanol.

Cells of all three isolates were short, non-motile rods with a Gram-negative type of cell wall (Fig. 1). Cells of strain HMT 1^T were covered with an exopolysaccharide (EPS)-like substance.

Strain HMT 1^T belonged to the obligate methylotrophs, utilizing only methanol and methylamine as growth substrates. In contrast, the soda lake isolates, in addition to utilizing methanol (AMT 1^T, AMT 3) and formate (AMT 3), also grew on acetate and ethanol and therefore

Fig. 1. Cell morphology of haloalkaliphilic strain AMT 1^T (a, c) and halophilic strain HMT 1^T (b, d) from hypersaline lakes. (a, b) Phase-contrast photomicrographs; (c, d) thin sections. Bars, 10 μm (a, b) and 1 μm (c, d).

can be considered as restricted methylotrophs. According to its pH and salt response, the salt lake isolate HMT 1^T is a neutrophilic, extremely salt-tolerant moderate halophile. To our knowledge, it represents the first halophilic, methylotrophic bacterium capable of growth with C₁ compounds up to 4 M NaCl. The soda lake isolates are typical haloalkaliphiles, with a relatively narrow alkaline pH range for growth and an optimum at pH 10. Although isolated at low salt, in pure culture, strains AMT 1^T and AMT 3 grew with methanol and acetate in saturated soda brines (3.5–4 M total Na⁺). This makes these strains unique among the haloalkaliphilic methylotrophs obtained from soda lakes so far. It needs to be mentioned, however, that, for all isolates, the growth rate at 3.5–4 M Na⁺ was only 5–10 % of the maximum ($\mu_{\max}$ with methanol 0.045 and 0.02–0.035 h⁻¹ for halophilic strain HMT 1^T and haloalkaliphilic strains AMT 1^T and AMT 3, respectively), and the growth yield decreased by a factor of 2–2.5. This indicates highly stressed growth conditions. Autotrophic growth was not observed either with hydrogen or thiosulfate as electron donor, and anaerobic growth was not observed with nitrate. Respiration activity tests using washed cells demonstrated that, apart from methanol and its oxidation products (i.e. formate and formaldehyde), all three isolates can also oxidize ethanol. Furthermore, cells of HMT 1^T induced methyl- and dimethylamine-oxidizing capacity when grown with methylamine, while still keeping the potential to oxidize methanol. On the other hand, growth of strains AMT 1^T and AMT 3 with acetate led to repression of the methanol dehydrogenase system and induction of acetate-dependent respiration, while retaining high activities of formaldehyde oxidation (Table 1). The maximum rate of methanol-dependent respiration activity for halophilic strain HMT 1^T was observed at pH 8 and 2 M NaCl and at pH 10 and 0.5–0.75 M total Na⁺ for the haloalkaliphilic strains AMT 1^T and AMT 3.

Measurements of the activity of key enzymes of C₁ metabolism (Table 2) identified the presence of methanol-catabolizing dehydrogenases in strains HMT 1^T and

AMT 1^T. However, their carbon assimilation pathways were different. In halophilic strain HMT 1^T, the key enzymes of the serine pathway (hydroxypyruvate reductase and serine-glyoxylate aminotransferase) were detected. In contrast, the haloalkaliphilic strain AMT 1^T is an autotroph, with the Calvin–Benson cycle and RuBisCO and phosphoribulokinase as the key enzymes.

Lipid analysis revealed the presence of cardiolipin, phosphatidylethanolamine and phosphatidylserine as the major membrane phospholipids in strains HMT 1^T and AMT 1^T. However, the cellular fatty acid compositions were very different in the two strains (Supplementary Table S1, available in IJSEM Online). The fatty acid profile in the haloalkaliphilic methylotroph AMT 1^T was very similar to that of the low-salt-tolerant, alkaliphilic, sulfur-oxidizing autotroph *Thioalkalimicrobium aerophilum*, isolated from the same habitats (Banciu *et al.*, 2005), with an absolute dominance of C_{18:1}ω7. In the neutrophilic halophile HMT 1^T, saturated species were dominant, with C_{16:0} as a predominant component and cyc C_{17:0} and 10-methyl C_{16:0} as secondary dominant components. While the former is quite common for halophiles (Vargas *et al.*, 2005), the abundance of cyc C_{17:0} and complete absence of C_{18:1} species is certainly unusual. As for the third abundant species, 10-methyl C_{16:0}, our recent work with two new groups of extremely halophilic, sulfur-oxidizing gamma-proteobacteria obtained from the same habitats (Sorokin *et al.*, 2006) identified it as a dominant fatty acid in their polar membrane lipids. Analysis of organic compatible solute composition accumulated by the cells of halophilic strain HMT 1^T grown with methanol at 2 M NaCl demonstrated the presence of glycine betaine as the dominant species, constituting approximately 17 % of the total cell mass. This solute and its specific content are typical for high-salt-tolerant (halo)alkaliphilic gammaproteobacteria (Galinski & Trüper, 1994).

The G + C contents in the genomic DNA of strains AMT 1^T, AMT 3 and HMT 1^T were 59.6, 59.0 and 62.9 mol% (T_m), respectively. Phylogenetic analysis based on sequencing of

Table 1. Respiration activity of washed cells of (halo)alkaliphilic methylotrophic isolates

Cells were incubated under the same conditions of pH and salt composition with the C₁ compound shown. Strains AMT 1^T and AMT 3 were grown at pH 10 and 0.6 M Na⁺, while strain HMT 1^T was grown at pH 7.5 and 2 M NaCl. Values are nmol O₂ (mg protein)⁻¹ min⁻¹.

Substrate	AMT 1 ^T		AMT 3			HMT 1 ^T	
	Methanol	Acetate	Methanol	Formate	Acetate	Methanol	Methylamine
Methanol	70	0	85	0	23	55	56
Formaldehyde	380	350	320	200	250	60	80
Formate	150	80	210	250	92	15	8
Ethanol	80	25	70	0	25	45	64
Acetate	15	120	25	0	130	0	0
Methylamine	10	0	40	0	0	0	56
Dimethylamine	0	0	0	0	0	0	136
Trimethylamine	0	0	0	0	0	0	4

Table 2. Enzyme activities in cell-free extracts of methylotrophic extremophilic bacteria

The growth substrate was methanol. Cells were incubated under the same conditions of pH and salt as in the growth media. Activities are given as nmol (mg protein)⁻¹ min⁻¹. Strain AMT 1^T was grown at pH 10 and 0.6 M Na⁺ and strain HMT 1^T was grown at pH 7.5 and 2 M NaCl. GSH, Reduced glutathione; PMS, phenazine methosulfate.

Enzyme (cofactor)	AMT 1 ^T	HMT 1 ^T
Methanol dehydrogenase (PMS)	124	180
Formaldehyde dehydrogenase (PMS)	176	85
(NAD ⁺ , GSH)	0	0
Formate dehydrogenase (PMS)	95	15
(NAD ⁺)	0	10
Hydroxypyruvate reductase (NADH)	0	120
(NADPH)	0	22
Serine-glyoxylate aminotransferase (NADH)	0	107
(NADPH)	0	10
3-Hexulose-phosphate synthase	0	0
Phosphoribulokinase	63	0
RuBisCO	79	0

the 16S rRNA gene placed the extremophilic methylotrophs into the family *Ectothiorhodospiraceae*. According to this analysis, the AMT strains clearly belonged to the same species (99.7 % sequence similarity). The group was loosely associated with sequences from non-phototrophic members of the *Ectothiorhodospiraceae*, such as the *Alkalispirillum*–*Alkalilimnicola* group, *Aquisalina* and *Arhodomonas aquaeolei* (Fig. 2). The nearest culturable relative of the halophilic strain HMT 1^T is the haloalkaliphilic sulfur-oxidizing bacterium *Thioalkalispira microaerophila* ALEN 1^T, isolated from a soda lake (Sorokin *et al.*, 2002). It must be noted that HMT 1^T represents the first example of a methylotroph with the serine pathway of carbon assimilation within the *Gammaproteobacteria*. In both cases, the level of 16S rRNA gene sequence similarity with the nearest relatives was below 95 %.

Since both the alkaliphilic and halophilic isolates formed deep lineages with uncertain branching, key functional genes *cbbL* (RuBisCO) for the alkaliphilic strain AMT 1^T and *mxoF* (methanol dehydrogenase) for both groups were also analysed. A single *cbbL* gene of the 'green-like' form I RuBisCO was detected in AMT 1^T. Phylogenetic analysis placed the sequence within the *Thioalkalivibrio* cluster in the *Ectothiorhodospiraceae*, consistent with the 16S rRNA gene phylogeny (Fig. 3a). The *mxoF* gene was easily detectable in the halophilic isolate HMT 1^T, but all attempts to amplify the gene from the AMT strains, using four different primer sets, failed (Supplementary Fig. S1). This might indicate that the enzyme in this autotrophic

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic affiliations of (halo)alkaliphilic methylotrophic isolates from hypersaline lakes within the *Gammaproteobacteria*. Bar, 5 % sequence variation. Bootstrap values indicated at nodes are presented only when they exceed 70 %.

methylotroph is different from the classical methanol dehydrogenase. Phylogenetic analysis of the partial *mxoF* sequence from HMT 1^T placed it between beta- and gammaproteobacterial methylotrophs as a separate branch, which confirmed its separate position among known methylotrophs (Fig. 3b).

On the basis of phenotypic and genetic properties, the novel (halo)alkaliphilic methylotrophic gammaproteobacteria isolated from hypersaline lakes are proposed to be assigned into two new genera and species. For the halophilic neutrophilic strain HMT 1^T, the name *Methylohalomonas lacus* gen. nov., sp. nov. is proposed, and the name *Methylohalomonas kenyaense* gen. nov., sp. nov. is proposed for the haloalkaliphilic strains AMT 1^T and AMT 3. Their essential properties in comparison with known (halo)alkaliphilic methylotrophs are presented in Table 3.

Description of *Methylohalomonas* gen. nov.

Methylohalomonas [Me.thy'lo.ha.lo.mo'nas. N.Gr. n. methyl (from Gr. n. methu wine and Gr. n. hulê wood) the methyl radical; Gr. n. hals, halos salt; Gr. fem. n. monas a unit, monad; N.L. fem. n. *Methylohalomonas* salt (-tolerant), methyl-group-utilizing monad].

Cells are Gram-negative rods. Obligately methylotrophic. Utilize C₁ compounds as carbon and energy sources using the serine cycle for carbon assimilation. Halophilic and

Fig. 3. Phylogenetic positions of methylotrophic isolates based on translated amino acid analysis of functional genes. (a) Position of haloalkaliphilic isolate AMT 1^T based on *cbbL* gene sequence analysis (large subunit of RuBisCO); (b) position of halophilic strain HMT 1^T based on sequence analysis of the *mxhF* gene (methanol dehydrogenase). Tree topologies and evolutionary distances are given by the neighbour-joining method with Poisson corrections. Numbers at nodes indicate percentage bootstrap values from 1000 replications. Bars, 10 % (a) and 5 % (b) sequence variation.

neutrophilic. C_{16:0}, cyc C_{17:0} and 10-methyl C_{16:0} are the dominant cellular fatty acids. The genus belongs to the *Gammaproteobacteria*. Known habitat is hypersaline chloride-sulfate lakes. *Methylohalomonas lacus* is the type species.

Description of *Methylohalomonas lacus* sp. nov.

Methylohalomonas lacus (la'cus. L. gen. n. *lacus* of a lake).

Displays the following properties in addition to those described for the genus. Cells are non-motile (0.5–0.6 × 1–

Table 3. Comparative properties of (halo)alkaliphilic methylotrophic bacteria from salt lakes

Strains: 1, HMT 1^T (data from this study); 2, AMT 1^T and AMT3 (this study); 3, *Methylophaga alcalica* M39^T (Doronina *et al.*, 2003a); 4, '*Methylophaga natronica*' Bur 2 (Doronina *et al.*, 2003b); 5, '*Ancylobacter natronum*' Bur 3 (Doronina *et al.*, 2001); 6, *Methylarcula terricola* h37^T (Doronina *et al.*, 2000). RuMP, Ribulose monophosphate pathway; ND, no data available.

Characteristic	1	2	3	4	5	6
Cell morphology	Rods	Rods	Rods	Rods	Bean-shaped	Rods
Motility	–	–	+	+	–	–
Electron donors*	M, MM	M, formate, acetate, ethanol	M, MM	M, MM, TM, fructose	M, H ₂ , sugars	MM, DM, acetate, sugars
Carbon assimilation	Serine cycle	CO ₂	RuMP	RuMP	CO ₂	Serine cycle
pH range (optimum)	6.5–8.2 (7.5)	8.3–10.5 (10.0)	7.0–11.0 (9.0–9.5)	7.0–11.0 (8.5–9.0)	6.5–9.0 (8.0–8.5)	5.5–10.0 (7.5–8.5)
Salt range (optimum) (M Na ⁺)	0.5–4.0 (2.0)	0.3–4.0 (0.5)	0–1.6 (0.55)	0–1.6 (0.5)	<0.3	0.1–2.4 (0.5–1.0)
Dominant fatty acids	C _{16:0} , cyc C _{17:0}	C _{18:1} ω7, C _{16:0}	C _{16:0} , C _{16:1} , C _{18:1}	C _{16:0} , C _{16:1}	ND	C _{18:1} ω7, C _{18:0}
DNA G + C content (mol%)	59.6	62.9	48.3	45.0	66.2	57.1
Isolation source	Hypersaline neutral lake	Soda lake	Soda lake	Soda lake	Hypersaline alkaline lake	Saline soil
Proteobacterial class	Gamma	Gamma	Gamma	Gamma	Alpha	Alpha

*DM, Dimethylamine; M, methanol; MM, methylamine; TM, trimethylamine.

3 µm), occurring singly or in short chains, and covered with a layer of EPS-like material. Strictly aerobic, utilizing methanol and methylamine as carbon and energy sources. With methanol, grows at pH 6.5–8.2 (optimum pH 7.5). Extremely salt-tolerant, moderate halophile with a NaCl range for growth between 0.5 and 4 M and an optimum at 2 M. Unable to grow autotrophically with H₂ or thiosulfate as the energy source. Ammonium serves as a nitrogen source. The G + C content in the DNA of the type strain is 59.6 mol%.

The type strain, HMT 1^T (=DSM 15733^T =NCCB 100208^T =UNIQEM U237^T), was isolated from hypersaline inland lakes in south-western Siberia (Altai, Russia).

Description of *Methylnatronum* gen. nov.

Methylnatronum [Me.thy'lo.na.trum. N.Gr. n. *methyl* (from Gr. n. *methu* wine and Gr. n. *hulê* wood) the methyl radical; N.Gr. n. *natron* arbitrarily derived from the Arabic n. *natrun* or *natron* soda; N.L. neut. n. *Methylnatronum* methyl-group-utilizing, soda (-loving bacterium)].

Cells are Gram-negative, short rods. Obligately aerobic, restricted methylotrophs. Autotrophic Calvin–Benson cycle is used for carbon assimilation during methylotrophic growth. Moderately salt-tolerant and obligately alkaliphilic. C_{18:1ω7} is the dominant cellular fatty acid. The genus belongs to the *Gammaproteobacteria*. Known habitat is soda lakes. *Methylnatronum kenyense* is the type species.

Description of *Methylnatronum kenyense* sp. nov.

Methylnatronum kenyense (ken.yen'se. N.L. neut. adj. *kenyense* pertaining to Kenya, where the type strain was isolated).

Displays the following properties in addition to those described for the genus. Cells are short, coccoid, non-motile rods (0.5–0.7 × 1–1.2 µm), occurring singly or in pairs. Utilizes methanol, formate, ethanol and acetate as carbon and energy sources. With methanol, grows at pH 8.3–10.5 (optimum pH 10). Extremely salt-tolerant, growing at salt contents between 0.3 and 4 M total Na⁺ with an optimum at 0.5–1.0 M. Can not grow autotrophically with H₂ or thiosulfate as the energy source. Utilizes ammonium and nitrate as nitrogen sources. G + C content in the DNA is 62–62.9 mol%.

The type strain, AMT 1^T (=DSM 15732^T =NCCB 100209^T =UNIQEM U238^T), was isolated from the soda lake Magadi in Kenya. The closely related strain AMT 3 (=NCCB 100206) originated from soda lakes in the Kulunda Steppe (Altai, Russia).

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