Oxyplasma meridianum gen. nov., sp. nov., an extremely acidophilic organotrophic member of the order Thermoplasmatales

Golyshina, Olga V; Lunev, Evgenii A; Distaso, Marco A; Bargiela, Rafael; Gaines, Matthew C; Daum, Bertram; Ferrer, Manuel; Bale, Nicole J; Koenen, Michel; Damsté, Jaap S Sinninghe; Yakimov, Mikhail M; Golyshin, Peter N

International Journal of Systematic Evolutionary Microbiology

DOI:
10.1099/ijsem.0.006499

Published: 27/08/2024

Peer reviewed version

Oxyplasma meridianum gen. nov. sp. nov., an extremely acidophilic organotrophic member of the order *Thermoplasmatales*

Olga V. Golyshina[1], Evgenii A. Lunev[1], Marco A. Distaso[1], Rafael Bargiela[1,2], Matthew C. Gaines[3], Bertram Daum[3], Manuel Ferrer[2], Nicole J. Bale[4], Michel Koenen[4], Jaap S. Sinninghe Damsté[4], Mikhail M. Yakimov[5], Peter N. Golyshin[1]

[1]Centre for Environmental Biotechnology, School of Environmental and Natural Sciences, Bangor University, Bangor, UK

[2]Instituto de Catalisis y Petroleoquimica (ICP), CSIC, Madrid, Spain

[3]Living Systems Institute and Department of Biosciences, Faculty of Health and Life Sciences, University of Exeter, Exeter, UK

[4]Department of Marine Microbiology and Biogeochemistry, NIOZ Royal Netherlands Institute for Sea Research, Texel, Netherlands

[5]Institute of Polar Sciences, CNR, Messina, Italy

Correspondence: Olga V Golyshina, o.golyshina@bangor.ac.uk

Abstract

A mesophilic, hyperacidophilic archaeon, strain M1^T, was isolated from a rock sample from Vulcano Island, Italy. Cells of this organism were cocci with an average diameter of 1 µm. Some cells possessed filaments. The strain grew in the range of temperatures between 15 and 52°C and pH 0.5-4.0 with growth optima at 40°C and pH 1.0. Strain M1^T was aerobic and chemoorganotrophic, growing on complex substrates, such as casamino acids, trypticase, tryptone, yeast and beef extracts. No growth at expenses of oxidation of elemental sulfur or reduced sulfur compounds, pyrite, or ferrous sulfate was observed. The core lipids were glycerol dibiphytanyl glycerol tetraether lipids (membrane spanning) with 0 to 4 cyclopentane moieties and archaeol, with trace amounts of hydroxy archaeol. The dominant quinone was MK-7:7. The genome size of M1^T was 1.67 Mbp with a G+C content of 39.76 mol%, and both characteristics were well within the common range for *Thermoplasmatales*. The phylogenetic analysis based on 16S rRNA gene

sequence placed the strain M1^T within the order *Thermoplasmatales* with sequence identities of 90.9, 90.3 and 90.5% to the closest SSU rRNA gene sequences from organisms with validly published names, *Thermoplasma acidophilum*, *T. volcanium* and *Thermogymnomonas acidicola*, respectively. Based on the results of our genomic, phylogenomic, physiological and chemotaxonomic studies, we propose strain M1^T (=DSM 116605 =JCM 36570) to represent a new genus and species, *Oxyplasma meridianum* gen. nov., sp. nov., within the order *Thermoplasmatales*.

Keywords: *Oxyplasma*, *Thermoplasmatales*, geothermal environments, acidic environments, acidophilic archaea

The GenBank accession number for the 16S rRNA gene strain of strain M1^T is OR949061. The GenBank accession number for complete genome sequence of strain M1^T is CP133772.

Introduction

Archaea of the order *Thermoplasmatales* are ubiquitous across most terrestrial acidic environments of various scale and origin (geothermal and anthropogenic sulfide-ores-containing mining biotopes with temperatures between 10-60°C) [1-3]. These archaea occur in a variety of sites in significant abundance, suggesting they contribute substantially to element cycling and community composition [4-6]. However, most of these archaea were detected by obtaining metagenomes worldwide and, thus, remain uncultured. Up to now, there are six genera with validly published names within the order *Thermoplasmatales*: *Thermoplasma*, *Picrophilus*, *Ferroplasma*, *Thermogymnomonas*, *Acidiplasma* and *Cuniculiplasma* [7]. The scarcity of isolated and described members limits our knowledge about the ecological, physiological, morphological, and chemotaxonomic properties of these organisms. The phylogenetic position of the order *Thermoplasmatales* has been recently changed. Originally, the order was affiliated with the Phylum *Euryarchaeota* [2]. However, an updated phylogenetic reconstruction from Genome Taxonomy Database (GTDB) firmly placed these organisms as a separate Phylum *Thermoplasmatota* [8, 9]. According to this classification, the Phylum *Thermoplasmatota* comprises classes "*Ca.* Poseidonia" and *Thermoplasmata*, with the latter containing multiple orders along with *Thermoplasmatales*. The tight clustering of *Thermoplasmatales* together with other orders into a separate phylum point at a distinct evolutionary trajectory for this group of organisms. There are also other factors, making these archaea particularly attractive for further isolation efforts and study. Archaea of the order

Thermoplasmatales are the most acidophilic organisms among prokaryotes, able to survive at pH values lower than 0 [10]. Another hallmark is the lack of cell walls in most cultured members, leading to a pleomorphic morphology, which is unusual for archaea [2]. Finally, these hyperacidophilic archaea are an attractive target for bioprospecting of enzymes and metabolites of biotechnological relevance [11-15].

In this study, we describe a new member of the order *Thermoplasmatales* isolated from a rock sample of Vulcano Island, Italy. Based on the morphological, physiological, chemotaxonomic, and phylogenetic characteristics, the organism represents a new genus and species within the order *Thermoplasmatales*.

Methods

Sampling, isolation and cultivation conditions

The sampling was conducted in the Levante Bay of Vulcano Island (Aeolian archipelago, Italy; (38.416115° N, 14.96035° E) in October 2012. The collected sample was a soft biofilm on the surface of the rock. For cultivation, the modified medium DSMZ 88 was used, which contained (g l^{-1}): $(NH_4)_2SO_4$, 1.3; KH_2PO_4, 0.28; $MgSO_4 \cdot 7H_2O$, 0.25; $CaCl_2 \cdot 2H_2O$, 0.07; $FeCl_3 \cdot 6H_2O$, 0.02. Additionally, the medium was amended with the trace element solution SL-10 from DSMZ medium 320 in proportion 1:1000 (v/v), betaine at 0.06% (w/v) and vitamin solution Kao and Michayluk (Sigma-Aldrich, Gillingham, UK) at 1:100 (v/v). Beef extract and tryptone (both ThermoFisher Scientific, Paisley, UK), were added at final concentration 1 g l^{-1}. Casamino acids, trypticase, yeast extract, amino acids, casein, chitin, cellulose, sucrose, lactose, raffinose, xylose, glucose and galactose (all from ThermoFisher Scientific, Paisley, UK) were individually tested at a concentration of 1 g l^{-1}. The pH of the medium was adjusted to 1.0-1.2 with concentrated H_2SO_4. The oxidation of $FeSO_4 \cdot 7H_2O$ (25 g/l) and pyrite (Kremer Pigments, Aichstetten Germany; 1 g l^{-1}) were tested in the medium DSMZ 874, as described previously, in the presence of yeast extract (ThermoFisher Scientific Paisley, UK), 0.02% (w/v)) [16]. Reduced sulfur compounds, $K_2S_4O_6$ and $K_2S_2O_3$ (both 5 mM), and elemental sulfur (Sigma Aldrich, Gillingham, UK, 1 g l^{-1}) were tested separately in the DSMZ medium 88 in the presence of beef extract and tryptone (1 g l^{-1}). Anaerobic growth with $Fe_2(SO_4)_3$ (10 mM) with and without yeast extract addition (0.02% w/v) was studied in DSMZ media 88 and 874. Furthermore, the consumption of H_2 (1 and 5 ml taken by syringe from gas phase) was tested with and without $Fe_2(SO_4)_3$ (10 mM) under anaerobic conditions in the DSMZ medium 88. The growth under anaerobic conditions was studied also in the presence of elemental sulfur (1 g l^{-1}), ferric citrate (1 mM) and KNO_3 (10 mM), and fermentative growth was tested in the

DSMZ medium 88 with addition of beef extract and tryptone (both in concentrations 1 g l^{-1}). The gas mixture used for anaerobic atmosphere was N_2:H_2:CO_2, in proportion 80:10:10. The anaerobic growth was studied in 18 ml Hungate type culture tubes with butyl rubber stoppers (Glasgerätebau Ochs, Bovenden, Germany) with the volume of cultures between 5-15 ml and with the gas headspace varied 3-13 ml. The pure culture was isolated by serial dilution to extinction method and monitored by PCR amplification with universal bacterial (F27 and R1492) and archaeal (AF23 and R1492) primers. The purified cultures were grown aerobically in 100 ml Erlenmeyer borosilicate Duran® flasks (DWK Life Sciences GmbH, Mainz, Germany) for ca. 5 days with 25 ml of medium with shaking at 120 rpm. The growth was studied at temperatures between 5 and 55°C and pH between 0 and 4.5. The modified DSMZ Medium 88 with amendments described above and organic substrates (beef extract and tryptone (both in concentrations 1 g l^{-1})) with various pH values was used for determining the optimal pH for growth. The growth was estimated spectrophotometrically at the wavelength 600 nm in a BioPhotometer Plus (Eppendorf, Hamburg, Germany), and direct cell counts in a Thoma chamber for anaerobic cultivation conditions.

Cell morphology

Cell morphology was investigated with the use of transmission electron microscopy (TEM). For TEM, 5 µL of liquid cell culture was pipetted onto Cu 400 mesh Negative stain grids (Agar Scientist AGS160-4). These grids had been glow discharged at 20 mA for 1 min and the biological sample applied within 15 min after glow discharging. The sample was left on the grid for 2 min, before excess liquid was blotted off from the periphery of the grid using filter paper (Whatman, Grade 1). Three wash steps using Milli-Q water were performed, whereby 5 µL of Milli-Q was pipetted onto the grid before being immediately removed using filter paper via the same blotting method. 1% uranyl acetate stain (dissolved in Milli-Q) was then applied to the grid, before being immediately blotted off. Finally, a second application of 1% uranyl acetate stain was pipetted onto the grid and left for 30 sec before blotting. The grids were then left to dry on filter paper for 20 min before being investigated in a Thermo Fisher Tecnai Spirit TEM, operating at 120 kV. Images were recorded using a OneView CMOS detector (Gatan).

Chemotaxonomic characterisation

The intact polar lipids (IPLs) and quinones were extracted from freeze-dried biomass with methanol, dichloromethane (DCM) and phosphate buffer (2:1:0.8, v:v:v) using an ultrasonic bath (2 x 10 min). The extracts were phase-separated by adding DCM and buffer to a final solvent ratio of 1:1:0.9 (v:v:v). The IPL-containing organic phases were re-extracted twice with DCM. All steps of the extraction were then repeated on the freeze-dried biomass with a solvent mixture of methanol,

DCM and trichloroacetic acid pH 2-3 (2:1:0.8, v:v:v). Finally, the combined extract was dried under a stream of N_2 gas [17]. For analysis, the extracts were redissolved in MeOH:DCM (9:1, v:v) and filtered through 0.45 µm cellulose syringe filters (4 mm diameter; Grace Alltech, Deerfield, IL, United States). Analysis was carried out using an Ultra High-Pressure Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS^n) [17]. Identification was carried out by comparison of accurate masses and mass spectral fragmentation with published data for IPLs and for quinones [18-20].

Genome sequencing, annotation and comparative genomic analysis

The DNA was isolated using a QIAGEN DNeasy PowerLyzer PowerSoil Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol from 25-100 ml cultures and quantified using Qubit^{TM} dsDNA BR Assay kit and Qubit fluorometer (Invitrogen, Carlsbad, CA, UK). The genome was sequenced using in-house Illumina MiSeq and Nanopore platforms. Pre-processing of Nanopore reads were conducted by porechop (https://github.com/rrwick/Porechop) and filtlong (https://github.com/rrwick/Filtlong). The assembly was performed using the "zga" pipeline (https://github.com/laxeye/zga) with Unicycler version 0.4.4. Genome annotation was performed using PGAP v2022-12-13.build6494 [21] and the genome was submitted to GenBank with accession number CP133772. Comparative genomic analysis was done using a dDDH (digital DNA-DNA hybridisation) calculation, formula *d4* and Average Nucleotide Identity (ANIb and ANIm), utilising the DSMZ platform (https://tygs.dsmz.de) and JSpeciesWS web server (https://jspecies.ribohost.com/jspecieswsw/#analyse), respectively [22, 23].

Phylogenetic analysis

For the phylogenetic analysis based on 16S rRNA gene sequence, reference sequences have been downloaded from the NCBI nucleotide database and aligned using MAFTT v7 [24]. Multiple alignment was trimmed using TrimAL 1.2rev59 [25] and the tree was performed by maximum likelihood method with a bootstrap based on 1,000 replicates using the R package phangorn [26]. Graphical representation for both phylogenetic trees was developed using R programming language [27] and the package ape [28]. Phylogenetic analysis based on 122 proteins alignment was performed using GTDB-tk tool v2.1.1 [29].

Results and Discussion

Phenotypic properties

Growth of the isolate M1^T occurred between 15 and 52°C with the optimum at 40°C (doubling time 19.2 h), reaching up to 7 x 10^8 cells/ml. Growth occurred in a pH range between 0.5 and 4 with a pH 1 being the optimal value. The highest growth rate as determined by optical density measurements was detected with a mixture of beef extract and tryptone in a concentration of 1 g l^{-1} each. The following substrates (1 g l^{-1} each) were stimulating growth at optimal temperature and pH: casamino acids, trypticase, yeast extract, with weak growth detected with amino acids. No growth stimulation was observed with casein, chitin, cellulose, sucrose, lactose, raffinose, xylose, glucose and galactose. No growth or stimulation of growth was observed with addition of elemental sulfur or reduced sulfur compounds (tetrathionate or thiosulfate). No growth on ferrous iron sufate or pyrite was detected in the presence or absence of yeast extract. No fermentative growth, and no anaerobic growth with any acceptors tested was observed.

Cell Morphology

Transmission electron micrographs revealed that cells had slightly irregular coccoid morphology with an average diameter of ~1 µm (Fig. 1). The cells extended on average up to 3 surface filaments per cell in early log phase. This value then increased to 8 surface filaments per cell in the late log to stationary growth phases. These filaments closely resembled pili or archaella. In accordance with previous observations of *Thermoplasmatales* species [2], no canonical S-layer was evident. Notably, the cells were speckled with small (10-20 nm) globular surface structures (Fig. 1). Close inspection of these extensions did not reveal any similarity to viruses and indeed, viral DNA was absent in the culture.

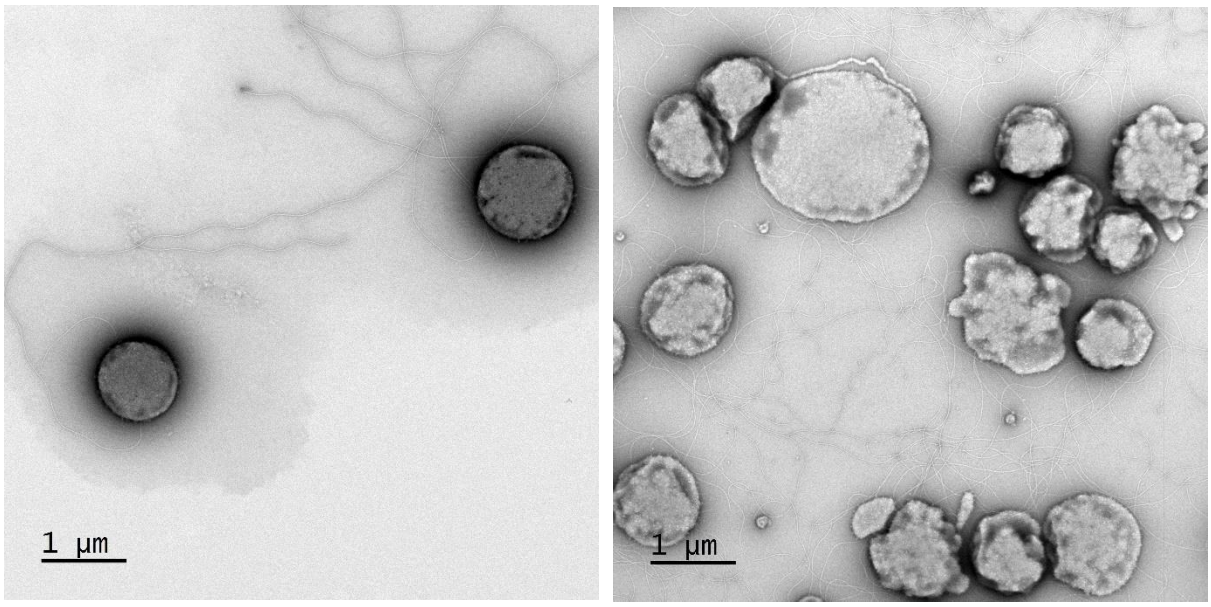

Fig. 1 (a and b)

Chemotaxonomy

For lipid analysis three separate culture of strain M1^T were grown and harvested at the late log phase. The most abundant core lipids (ca. 80%) were glycerol dibiphytanyl glycerol tetraether lipids (GDGTs, membrane spanning) with 0 to 4 cyclopentane moieties (GDGT-0 – GDGT-4). The most dominant GDGTs were GDGT-2 and GDGT-4 (Table 1). A similar GDGT distribution was observed for the (hyper)acidophiles "*Ferroplasma acidarmanus*" [30], *Thermogymnomonas acidicola* [31], *Thermoplasma acidophilum* [32], *Acidiplasma aeolicum* [16], *C. divulgatum* [7], all also belonging to the order *Thermoplasmatales*. The majority of GDGT IPLs had a phosphoglycerol (PG) head group at one glycerol moiety, with predominantly 1, and to a minor extent, 2-3 hexose sugar(s) (glycosyl; gly) at the other glycerol moiety (Table 2). Archaeol (AR) and minor amounts of hydroxy archaeol (OH-AR) represented the other 20% of the core lipids. PG was the only polar head group of AR detected, whilst OH-AR was only detected with a DiGly head group. The dominant (ca. 94%) quinone present in M1^T was MK-7:7 (Table 2).

Table 1. Intact polar lipids identified in *Oxyplasma meridianum* strain M1^T and their relative abundance (in percent of lipid peak area). Columns 1-3 represent the data of the three parallel cultures**.**

Polar Headgroup 1	**Polar Headgroup 2**	**Core**	**[M+H]$^+$**	**AEC**	**Δ mmu**	**Relative abundance (%) 1**	**2**	**3**
None		AR	653.680	$C_{43}H_{89}O_3$	0.3	6.3	6.6	8.8
PG		AR	807.683	$C_{46}H_{96}O_8P$	0.9	9.3	9.4	11.3
DiGly		OH-AR	993.781	$C_{55}H_{109}O_{14}$	0.0	3.1	3.2	3.6
PG		GDGT-0	1456.325	$C_{89}H_{180}O_{11}P$	1.0	0.5	1.4	0.4
		GDGT-1	1454.309	$C_{89}H_{178}O_{11}P$	0.8	0.3	0.8	0.3
		GDGT-2	1452.293	$C_{89}H_{176}O_{11}P$	1.5	6.6	8.8	5.8
		GDGT-3	1450.278	$C_{89}H_{174}O_{11}P$	1.3	1.9	2.0	1.5
		GDGT-4	1448.262	$C_{89}H_{172}O_{11}P$	1.5	5.6	4.1	4.4
		Total				**15**	**17**	**13**
PG	**Gly**	GDGT-0	1618.378	$C_{95}H_{190}O_{16}P$	0.9	0.3	1.1	0.4
		GDGT-1	1616.352	$C_{95}H_{188}O_{16}P$	12	2.3	3.7	2.4
		GDGT-2	1614.348	$C_{95}H_{186}O_{16}P$	0.2	11.2	15.8	10.3
		GDGT-3	1612.323	$C_{95}H_{184}O_{16}P$	9	6.8	7.9	6.2
		GDGT-4	1610.317	$C_{95}H_{182}O_{16}P$	0.7	42.9	32.6	41.0
		Total				**64**	**61**	**60**
PG	**diGly**	GDGT-0	1780.429	$C_{101}H_{200}O_{21}P$	2.2	0.1	0.2	0.2
		GDGT-1	1778.412	$C_{101}H_{198}O_{21}P$	3.4	0.0	0.1	0.1
		GDGT-2	1776.399	$C_{101}H_{196}O_{21}P$	0.7	1.5	1.5	1.8
		GDGT-3	1774.382	$C_{101}H_{194}O_{21}P$	2.8	0.2	0.2	0.3
		GDGT-4	1772.367	$C_{101}H_{192}O_{21}P$	2.2	0.6	0.3	0.7
		Total				**2**	**2**	**3**
PG	**triGly**	GDGT-0	1942.479	$C_{107}H_{210}O_{26}P$	4.9	0.1	0.2	0.2
		GDGT-1	1940.468	$C_{107}H_{208}O_{26}P$	0.6	0.0	0.1	0.1

GDGT-2	1938.451	$C_{107}H_{206}O_{26}P$	2.1	0.5	0.5	0.6
GDGT-3	1936.434	$C_{107}H_{204}O_{26}P$	2.9	0.1	0.1	0.2
GDGT-4	1934.420	$C_{107}H_{202}O_{26}P$	1.5	0.2	0.2	0.4
Total				**1**	**1**	**1**
Sum AR				**16**	**16**	**20**
Sum OH-AR				**3**	**3**	**4**
GDGT-0				**1**	**3**	**1**
GDGT-1				**3**	**5**	**3**
GDGT-2				**20**	**27**	**19**
GDGT-3				**9**	**10**	**8**
GDGT-4				**49**	**37**	**46**

Δ mmu = (measured mass – calculated mass) x 1000 as calculated for strain 1; AEC = assigned elemental composition; PG = phosphatidylglycerol; Gly = monoglycosyl; diGly = diglycosyl; triGly = triglycosyl; AR = archaeol; OH-AR = hydroxy archaeol, GDGT = glycerol dibiphytanyl glycerol tetraether.

Table 2. Menaquinones identified in *Oxyplasma meridianum* M1^T. 1-3 are data of three parallel cultures of strain M1^T**.**

	[M+H]$^+$	**AEC**	**Δ mmu**	**Relative abundance (%)**		
				1	**2**	**3**
MK-8:8	717.560	$C_{51}H_{73}O_2$	0.2	1.1	1.1	1.1
MK-7:7	649.498	$C_{46}H_{65}O_2$	0.3	94.2	93.2	94.6
MK-7:6	651.514	$C_{46}H_{67}O_2$	0.2	4.7	5.6	4.3

Δ mmu = (measured mass – calculated mass) x 1000 as calculated for strain 1; AEC = assigned elemental composition.

Phylogenetic analysis

Based on its 16S rRNA gene sequence, the strain M1^T clusters together with other archaea of the order *Thermoplasmatales*, class *Thermoplasmata*, phylum *Thermoplasmatota*. The nearest phylogenetic neighbour of the strain M1^T is *Thermoplasma acidophilum* (90.9%), followed by *Thermogymnomonas acidicola* (90.5%) and *Thermoplasma volcanium* (90.3%) (Fig. 2). Therefore, according to the accepted boundaries for a genus (<94.5% for 16S rRNA gene sequence identity) [33] M1^T represents a new genus.

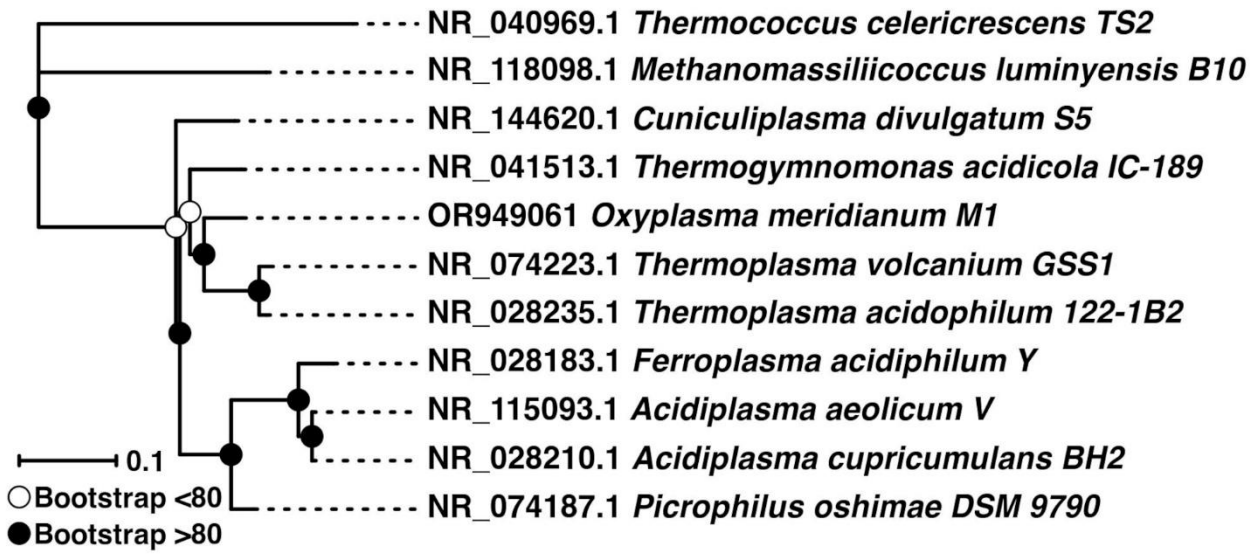

Fig. 2

Genome properties

About 239.2-fold genome coverage by Illumina reads and 23.4-fold coverage by Oxford Nanopore reads were obtained. The genome assembly resulted in one circular chromosome of 1.67 Mbp with a G+C content 39.76%. The genome annotation revealed 1725 genes with 1679 protein-coding sequences and 43 genes encoding tRNA. Analysis of the genome revealed the presence of all enzymes required for glycolysis. Genes encoding the non-phosphorylative Entner-Doudoroff pathway and the non-oxidative pentose phosphate pathway were detected. We also identified all genes encoding the TCA cycle in the genome with alpha-ketoglutarate dehydrogenase as the only exception (Table S1). Likely, the function of this enzyme is performed by 2-oxoacid:acceptor oxidoreductase. Multiple copies of genes are present in the genome, including a location in close proximity to the TCA enzymes-encoding genes. This possibility was previously considered for other organisms, including a phylogenetic neighbour of the strain M1^{T}, *P. torridus* [34]. Aerobic respiration was backed up by the presence of genes encoding a NADH dehydrogenase complex, a cupredoxin-domain-containing (plastocyanin) protein, a cytochrome *cbb3*-type cytochrome C oxidase subunits I and II, and a polyferredoxin NapH superfamily (OXIME_000996 – OXIME_001001). Furthermore, cytochrome ubiquinol/*bd* terminal oxidase subunits I and II and cytochrome bc complex cytochrome b subunit encoding genes (OXIME_001706 - OXIME_001707 and OXIME_000377) were identified in the genome. Moreover, we detected V-type ATP synthase subunits A, B, C, D, F, E and H in the genome of the strain M1^{T}. Genes for proteolytic proteins affiliated to peptidase families M50, M13, M19, and S49, a trypsin-like peptidase, an archaeal Lon

protease, a tricorn protease, and a thermopsin were found in the genome and reflect the organotrophic lifestyle of the strain M1^{T}. We detected genes encoding a mevalonate 3-kinase, a mevalonate 3-phosphate 5-kinase and mevalonate biphosphate decarboxylase proteins, confirming the route III of the mevalonate pathway, characteristic for *Thermoplasmatales* archaea [35-37]. Interestingly, we also identified genomic loci for hercynine oxygenase/ergothioneine biosynthesis protein EgtB (OXIME_001566) and a L-histidine N(alpha)-methyltransferase (OXIME_001567), both being involved into the ergothioneine pathway. Ergothioneine is a low molecular weight thiol, a derivative of histidine with a sulfur atom containing imidazole ring and was previously predicted in some archaeal genomes considering that it might be synthesised in archaea [38]. The organism encodes the CRISPR-Cas (Clusters of Regularly Interspaced Short Palindromic Repeats)-associated proteins, namely co-localised genes encoding for endoribonucleases Cas2 and Cas6, an endonuclease Cas2, type I_D protein Cas5/Csc1, Csc2, Cas4 and two copies of endonuclease Cas1 genes. To summarise, physiological, morphological and genomic features of the strain M1^{T} suggest this organism to be a typical member of the order *Thermoplasmatales* (Table 3).

The phylogenetic tree based on 122 concatenated proteins revealed that the M1^{T} strain is the closest to *Thermogymnomonas acidicola* among organisms with validly published names and most similar metagenome assembled genomes (Fig. 3).

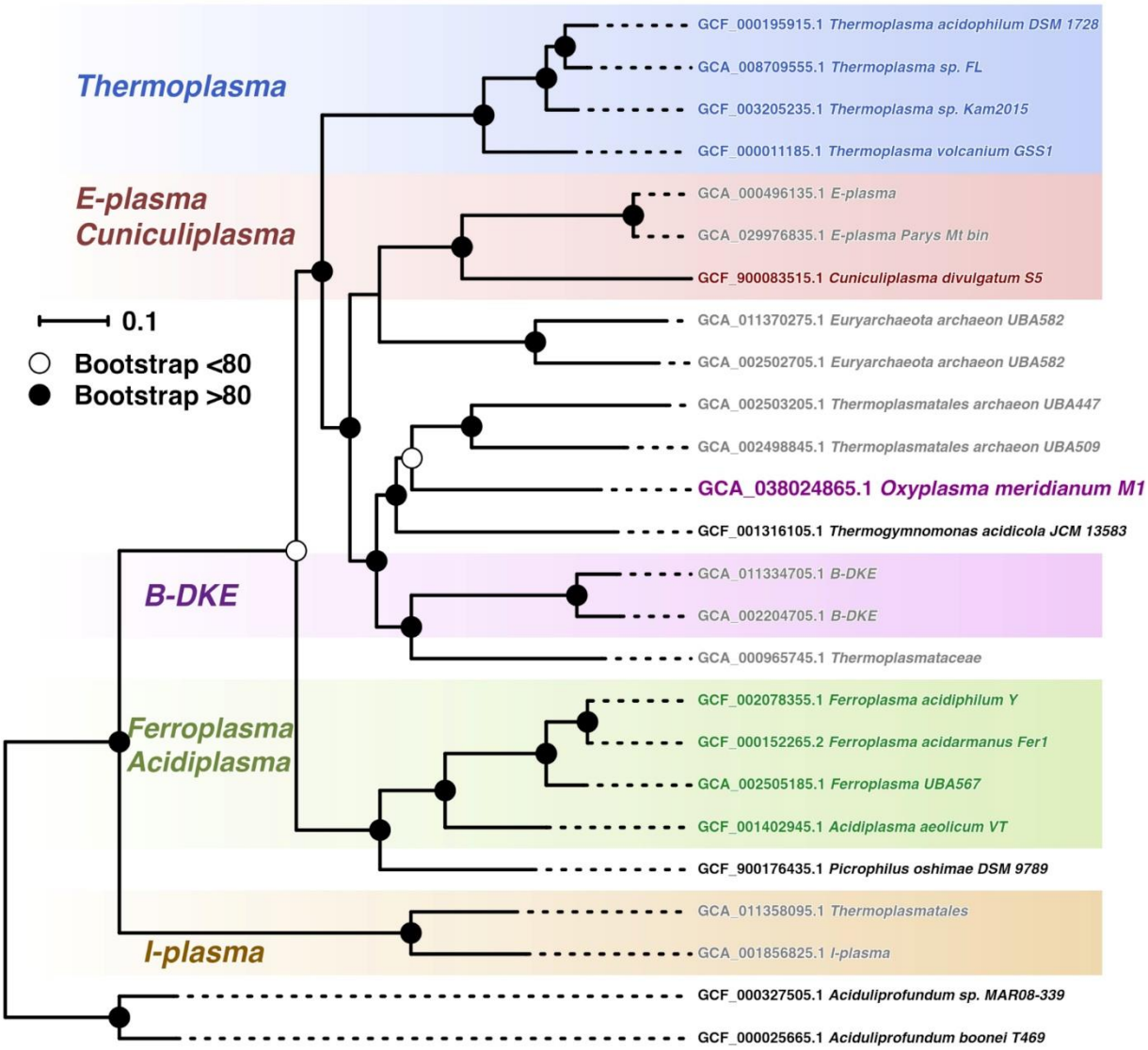

Fig. 3

dDDH (formula *d4*) showed values significantly below the threshold level of 70% and ANI calculations produced indices lower than 95-96% for strains M1 and *Thermoplasmatales* archaea with validly published names (Tables S2 and S3), recommended for species delineation [39].

Table 3. Main characteristics of genera of the order *Thermoplasmatales* with validly published names and strain M1^{T}.

Characteristic	*Thermoplasma* 1	*Picrophilus* 2	*Ferroplasma* 3	*Acidiplasma* 4	*Thermogymnomonas* 5	*Cuniculiplasma* 6	M1^{T}

Cell wall/S-layer	-	+	-	-	-	-	-
Growth temperature, °C Range Optimum	33-69 67	47-65 60	15-45 35-37	15/22-65 45-53.5	38-68 60	10-48 37-40	15-52.5 40
Growth pH Range Optimum	0.5-4 1-2	0-3.5 0.7	1.3-2.2 1.7	0/0.4-1.8/4 1-1.6	1.8-4 3	0.5-4 1-1.2	0.5-4 1
Fe^{2+} oxidation	-	-	+	+	-	-	-
Anaerobic growth	+	-	±	+	-	+	-
DNA G+C content (mol%)	38-46	36	37	34-36	56	37	40

Data taken from: [7, 10, 16, 31, 40-42].

Conclusion

The strain M1^T is a mesophilic, thermotolerant, hyperacidophilic, aerobic, organotrophic and cell-wall lacking organism. The physiology of the organism is comparable to representatives of all genera of *Thermoplasmatales* with validly published names characterised up to date, which reflects the adaptation to specific physicochemical conditions of indigenous environments. The inability of anaerobic metabolism resembles that of *Picrophilus* and *Thermogymnomonas*, both of which were isolated from geothermal settings as well [10, 31]. Electron microscopy suggested the lack of cell wall, which is also common in *Thermoplasmatales* [2, 7]. Interestingly, the majority of cells had relatively small sizes (<1 µm) and possessed multiple (up to 8) filaments. The membrane lipid composition of M1^T was found to be rather characteristic for this group of archaea, with core lipids (GDGTs and archaeol) content being generally similar to that in *C. divulgatum*, which has a similar pH and temperature range, and both having menaquinone MK 7:7 as the main quinone [7]. The genome of the strain M1^T had size and G+C molar content rather typical for all known *Thermoplasmatales* and encoded proteins essential for aerobic and peptidolytic lifestyle. It should be noted that the GenBank records on 16S rRNA sequences of organisms with identities >98% to the strain M1^T are represented by acidic environments of geothermal origin and mining regions, e.g. GenBank accession numbers, AF544219 (Iron Mountain acid mine drainage site, California, USA), KJ907756 (Michoacan, Los Azufres thermal and acidic green biofilms from a fumarole, Mexico), DQ303253 and EF441883 (floating microscopic filaments from Rio Tinto and endolithic

community in the basin of Rio Tinto, Spain), and KM410353 (biofilm from subsurface sulfidic cave stream, Italy). These results imply that representatives of the *Oxyplasma* genus similarly to phylogenetic neighbours forming the same order, are distributed across the globe in ecological niches with low pH and diverse temperatures and might be both non-thermophilic and moderately thermophilic organisms, the known property for other *Thermoplasmatales* [7]. Based on the polyphasic (genomic and phylogenomic, chemotaxonomic and physiological) analysis, strain M1^T is proposed to represent a novel genus and species with the name *Oxyplasma meridianum* gen. nov., sp. nov. within the family *Thermoplasmataceae,* order *Thermoplasmatales*.

Description of *Oxyplasma* gen. nov.

Oxyplasma (O.xy.plas'ma. Gr. masc. adj. *oxys*, acid; Gr. neut. n. *plasma*, something shaped or moulded, N.L. neut.n.).

Oxyplasma a form living in acid.

Cells are lacking cell walls. Aerobic, mesophilic/thermotolerant. Organotrophic. Hyperacidophilic.

The core lipids: archaeol with trace amounts of hydroxy archaeol and glycerol dibiphytanyl glycerol tetraether lipids. The dominant Quinone MK-7:7.

The type species is *Oxyplasma meridianum*.

Description of ***Oxyplasma meridianum* sp. nov.**

Oxyplasma meridianum (me.ri.di.a'num L.neut.adj. *meridianum,* southern, isolated from the South of Italy).

Cells are regular and irregular cocci about 1 µm in diameter. The temperature range for growth (15-52.5°C), the optimum at 40°C. The pH range for growth (0.5-4), with an optimum at pH 1. Grows organotrophically with tryptone, beef and yeast extracts, casamino acids, trypticase. Lipids represented mostly by archaeol with trace amounts of hydroxy archaeol and glycerol dibiphytanyl glycerol tetraether lipids (GDGT) with 0 to 4 cyclopentane moieties (GDGT-0 – GDGT-4). The main respiratory quinone represented by menaquinone.

The type strain is M1^T (DSM 116605^T=JCM 36570^T), isolated from rock sample of Vulcano Island, Italy. The DNA G+C content of type strain is 39.76 mol%. Accession numbers of the strain M1^T 16S rRNA gene is OR949061 and of the complete genome is CP133772.

Funding information

PNG is indebted to the Era-Net Project 'MetaCat' funded through BBSRC, Contract Nr BB/M029085/1. RB, PNG and OVG also acknowledge the support of the Centre for Environmental Biotechnology Project N 810280, funded by the European Regional Development Fund (ERDF) via the Welsh Government. MF acknowledges Ministerio de Ciencia e Innovación, AEI (DOI 10.13039/501100011033), FEDER and NextGenerationEU/PRTR (PID2020-112758RB-I00, PDC2021-121534-I00, TED2021-130544B-I00).

Conflicts of interest

The authors declare no conflicts of interest.

Data availability statement

The GenBank accession number for complete genome sequence of *Oxyplasma meridianum* M1^T is CP133772.

References

1. **Golyshina OV.** Environmental, biogeographic, and biochemical patterns of archaea of the family *Ferroplasmaceae*. *Appl Environ Microbiol* 2011;77(15):5071-5078.
2. **Huber H, Stetter KO**. Thermoplasmatales. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (eds) *The Prokaryotes*. New York; Springer, 2006. https://doi.org/10.1007/0-387-30743-5_7.
3. **Mendez-Garcia C, Pelaez AI, Mesa V, Sanchez J, *et al.*** Microbial diversity and metabolic networks in acid mine drainage habitats. *Front Microbiol* 2015;6:475.
4. **Arce-Rodríguez A, Puente-Sánchez F, Avendaño R, Martínez-Cruz M, *et al.*** *Thermoplasmatales* and sulfur-oxidizing bacteria dominate the microbial community at the surface water of a CO_2-rich hydrothermal spring located in Tenorio Volcano National Park, Costa Rica. *Extremophiles* 2019;23(2):177-187.
5. **Korzhenkov AA, Toshchakov SV, Bargiela R, Gibbard H** *et al*. Archaea dominate the microbial community in an ecosystem with low-to-moderate temperature and extreme acidity. *Microbiome* 2019;7(1):11.
6. **Distaso MA, Bargiela R, Brailsford FL, Williams GB, *et al.*** High representation of archaea across all depths in oxic and low-pH sediment layers underlying an acidic stream. *Front Microbiol* 2020;11:576520.
7. **Golyshina OV, Lünsdorf H, Kublanov IV, Goldenstein NI, *et al.*** The novel extremely acidophilic, cell-wall-deficient archaeon *Cuniculiplasma divulgatum* gen. nov., sp. nov.

represents a new family, *Cuniculiplasmataceae* fam. nov., of the order *Thermoplasmatales*. *Int J Syst Evol Microbiol* 2016;66(1):332-40.

8. **Chuvochina M, Mussig AJ, Chaumeil PA, Skarshewski, A *et al.*** Proposal of names for 329 higher rank taxa defined in the Genome Taxonomy Database under two prokaryotic codes. *FEMS Microbiol Lett* 2023;17:370:fnad071.
9. **Oren A, Göker M**. Validation List no. 215. Valid publication of new names and new combinations effectively published outside the IJSEM. *Int J Syst Evol Microbiol* 2024;74(1):doi: 10.1099/ijsem.0.006173.
10. **Schleper C, Puehler G, Holz I, Gambacorta A, *et al.*** *Picrophilus* gen. nov., fam. nov.: a novel aerobic, heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. *J Bacteriol* 1995;177:7050-7059.
11. **Rossoni L, Hall SJ, Eastham G, Licence P**, **Stephens G**. The putative mevalonate diphosphate decarboxylase from *Picrophilus torridus* is in reality a mevalonate-3-kinase with high potential for bioproduction of isobutene. *Appl Environ Microbiol* 2015;81(7):2625-2634.
12. **Murphy J, Walsh G**. Purification and characterization of a novel thermophilic β-galactosidase from *Picrophilus torridus* of potential industrial application. *Extremophiles* 2019;23(6):783-792.
13. **Nagy I, Knispel RW, Kofler C, Orsini M, *et al.*** Lipoprotein-like particles in a prokaryote: quinone droplets of *Thermoplasma acidophilum*. *FEMS Microbiol Lett* 2016;363(18):fnw169.
14. **Lund S, Courtney T, Williams GJ**. Probing the substrate promiscuity of isopentenyl phosphate kinase as a platform for hemiterpene analogue production. *Chembiochem*. 2019;20(17):2217-2221.
15. **Rennella E, Huang R, Yu Z, Kay LE.** Exploring long-range cooperativity in the 20S proteasome core particle from *Thermoplasma acidophilum* using methyl-TROSY-based NMR. *Proc Natl Acad Sci USA* 2020;117:5298-5309.
16. **Golyshina OV, Yakimov MM, Lünsdorf H, Ferrer M, *et al.*** *Acidiplasma aeolicum* gen. nov., sp. nov., a euryarchaeon of the family *Ferroplasmaceae* isolated from a hydrothermal pool, and transfer of *Ferroplasma cupricumulans* to *Acidiplasma cupricumulans* comb. nov. *Int J Syst Evol Microbiol* 2009;59:2815-2823.
17. **Bale NJ, Ding S, Hopmans EC, Arts MGI, *et al.*** Lipidomics of environmental microbial communities. I: visualization of component distributions using untargeted analysis of high-resolution mass spectrometry data. *Front Microbiol* 2021;12:659302.

18. **Yoshinaga MY, Kellermann MY, Rossel PE, Schubotz F, *et al.*** Systematic fragmentation patterns of archaeal intact polar lipids by high-performance liquid chromatography/electrospray ionization ion-trap mass spectrometry. *Rapid Commun Mass Spectrom* 2011;25:3563–3574.
19. **Bale NJ, Sorokin DY, Hopmans EC, Koenen M, *et al.*** New insights into the polar lipid composition of extremely halo(alkali)philic euryarchaea from hypersaline lakes. *Front Microbiol* 2019;10:377.
20. **Elling FJ, Becker KW, Könneke M, Schröder JM, *et al.*** Respiratory quinones in Archaea: phylogenetic distribution and application as biomarkers in the marine environment. *Environ Microbiol* 2016;18:692–707.
21. **Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, *et al.*** NCBI prokaryotic genome annotation pipeline. *Nucl Acids Res* 2016;44(14):6614-6624.
22. **Meier-Kolthoff JP, Göker M**. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182.
23. **Richter M, Rosselló-Móra R, Glöckner FO, Peplies J**. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2015; pii: btv681.
24. **Katoh K, Standley DM**. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;30(4):772-80.
25. **Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T**. trimAl: a tool for automated alignment trimming. *Bioinformatics* 2009;25:1972-1973.
26. **Schliep K**. phangorn: phylogenetic analysis in R. *Bioinformatics* 2011;27(4):592–593.
27. **R Core Team**. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, URL: https://www.R-project.org/.
28. **Paradis E, Schliep K**. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 2019;35:526-528.
29. **Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH**. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. *Bioinformatics* 2022;38:5315-5316.
30. **Macalady JL, Vestling MM, Baumler D, Boekelheide N, *et al.*** Tetraether-linked membrane monolayers in *Ferroplasma* spp: a key to survival in acid. *Extremophiles* 2004;8:411-419.
31. **Itoh T, Yoshikawa N, Takashina T**. *Thermogymnomonas acidicola* gen. nov., sp. nov., a novel thermoacidophilic, cell wall-less archaeon in the order *Thermoplasmatales,* isolated from a solfataric soil in Hakone, Japan. *Int J Syst Evol Microbiol* 2007;57:2557-2561.

32. **Shimada H, Nemoto N, Shida Y, Oshima T, Yamagishi A**. Effects of pH and temperature on the composition of polar lipids in *Thermoplasma acidophilum* HO-62. *J Bacteriol* 2008;190(15):5404-5411.
33. **Yarza P, Yilmaz P, Pruesse E, Glöckner FO, *et al.*** Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12(9):635-645.
34. **Fütterer O, Angelov A, Liesegang H, Gottschalk G, *et al.*** Genome sequence of *Picrophilus torridus* and its implications for life around pH 0. *Proc Natl Acad Sci USA* 2004;101(24):9091-9096.
35. **Azami Y, Hattori A, Nishimura H, Kawaide H, *et al.*** (R)-mevalonate 3-phosphate is an intermediate of the mevalonate pathway in *Thermoplasma acidophilum*. *J Biol Chem* 2014;89(23):15957-15967.
36. **Vinokur JM, Cummins MC, Korman TP, Bowie JU**. An Adaptation to life in acid through a novel mevalonate pathway. *Sci Rep* 2016;6:39737.
37. **Hoshino Y, Villanueva L**. Four billion years of microbial terpenome evolution. *FEMS Microbiol Rev* 2023;47(2):1-39.
38. **Rawat M, Maupin-Furlow JA**. Redox and thiols in archaea. *Antioxidants* (Basel) 2020;9(5):381.
39. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, *et al.*** DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81-91.
40. **Darland G, Brock TD, Samsonoff W, Conti SF**. A thermophilic acidophilic *Mycoplasma* isolated from a coal refuse pile. Science 1970;170:1416-1418.
41. **Segerer A, Langworthy TA, Stetter KO**. *Thermoplasma acidophilum* and *Thermoplasma volcanium* sp. nov. from solfatara fields. *Syst Appl Microbiol* 1986;10:161-171.
42. **Golyshina OV, Pivovarova,TA, Karavaiko GI, Kondratéva TF, *et al.*** *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the *Archaea*. *Int J Syst Evol Microbiol* 2000;50:997-1006.

Figure legends:

Figure 1 (a and b). Negative stain transmission electron microscopy of strain M1^{T}. Micrographs of the cellular periphery of strain M1^{T} cells imaged at different magnifications.

Figure 2. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences of *Oxyplasma meridianum* M1^{T} and its closest phylogenetic neighbours with validly published names. Bootstrap values are based on 1,000 replicates and those <80 are shown as open circles, values >80 as closed circles. Sequences were previously aligned using MAFTT v7 and the resulting multiple alignment was trimmed using TrimAl 1.2rev59. The tree was constructed and decorated under R programming environment using the package phangorn for the tree calculations, selecting TIM3+I+G as best substitution model (using ModelTest pluggin within phangorn) and stochastic algorithm for tree rearrangement.

Figure 3. Phylogenetic tree based on 122 concatenated proteins. Tree calculation was performed using the GTDB-tk tool focusing exclusively on the *Thermoplasmatales* order, using genus *Aciduliprofundum* as an outgroup. Bootstrap values are highlighted as closed circles (values > 80), and open circles (bootstrap values <80).