

Bacillus natronophilus sp. nov., an alkaliphilic bacterium isolated from a soda lake

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Abstract

An alkaliphilic, moderately halophilic, heterotrophic, rod-shaped, spore-forming bacterium (M30^T) was isolated from a sediment sample collected from a soda lake (Lake Magadi, Tanzania). Strain M30^T was strictly aerobic, catalase-positive, oxidase-negative and non-motile. Growth occurred at 12–43 °C (optimum, 25–30 °C), at pH 8.0–12 (optimum, pH 9.5–10) and at salinities of 0.5–15% (w/v) NaCl (optimum 5%). It utilized various sugars and organic acids as sole carbon sources and was positive for amylase, cellulase, gelatinase, protease and xylanase activities. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid and the polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified lipid and one unidentified phospholipid. The DNA G+C content was 48.9 mol%. The predominant menaquinone was MK-7 and the major fatty acids (>10%) comprised anteiso-C_{15:0}, iso-C_{15:0}, and anteiso-C_{17:0}. Phylogenetic analysis based on 16S rRNA gene sequence affiliated M30^T to the genus *Bacillus* and showed the highest similarities to *Bacillus populi* FJAT-45347^T (96.4%) and *Bacillus aurantiacus* K1-5^T (96.2%). Based on the data from the current polyphasic study, M30^T represents a novel species of the genus *Bacillus*, for which the name *Bacillus natronophilus* sp. nov. is proposed. The type strain is M30^T (=JCM 32118^T=CGMCC 1.16739^T=MCC 3010^T).

Bacillus is a genus of rod-shaped endospore-forming cells with a wide diversity of physiological abilities, ranging from psychrophilic to thermophilic, acidophilic to alkaliphilic and salt tolerant to halophilic [1]. At the time of writing, there were 372 species of the genus *Bacillus* recorded on LPSN (www.bacterio.net/bacillus.html; May 2019). Soda lakes are naturally occurring alkaline environments, with pH values around 10 and above, that contain large amounts of sodium carbonate (or complexes of this salt), formed by evaporative concentration. Other salts, especially sodium chloride, may also concentrate leading to the formation of alkaline saline lakes [2, 3]. With the aim of studying the diversity of cultivable alkaliphilic bacteria from some soda lakes in the Great Rift Valley region of Africa, dilutions of sediment samples of Lake Magadi were plated on several alkaliphilic solid media and incubated for obtaining isolated colonies. One of the colonies, formed by Gram-stain-negative rods, designated strain M30^T, was purified and selected for further characterization.

Samples were collected in April 2006 from the upper 3–5 cm sediment layers of Lake Magadi (3.1833° S, 35.5333° E; Arusha region, Tanzania), and isolation was performed as described previously [4].

The strain was routinely grown on modified tryptic soy agar (mTSA), modified nutrient agar, (mNA) or modified nutrient broth [mNB; all media supplemented with 2% NaCl, and after sterilization, with 100 ml of 10% (w/v) Na₂CO₃ solution per litre of medium, final pH around 10]. The strain was maintained at 4 °C for short-term preservation and lyophilized for long-term storage.

Morphological, physiological and biochemical characterization of strain M30^T was performed with cultures grown on mNA at 30 °C for 2 days. Tests were incubated at 30 °C and checked daily for up to 10 days, unless otherwise stated. Motility and shape were observed under phase contrast microscopy Eclipse E200, Nikon) in cultures at the exponential phase of growth cultivated in mNB. Anaerobic growth

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The 16S rRNA gene and genome sequences were deposited at DDBJ/EMBL/GenBank under accession numbers MG189964 and VCR001000000, respectively.

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One supplementary table and four supplementary figures are available with the online version of this article.

was checked in mRNA incubated in an anaerobic atmosphere using the Anaerocult A Mini (Merck) gas generator system. Production of spores was assayed in mRNA supplemented with $0.005 \text{ g l}^{-1} \text{ MnSO}_4 \cdot 7\text{H}_2\text{O}$. The biomass obtained was fixed at 4°C overnight in 2.5% glutaraldehyde (with 0.1 M phosphate buffer pH 7.2), washed in the same buffer and post-fixed in 1% OsO_4 for 1 h, dehydrated in an ascending gradient of ethanol, followed by anhydrous acetone and then impregnated in successive passages of mixtures of araldite/acetone of increasing concentration. Finally, the sample was embedded in Araldite resin. Thin and ultrathin sections were cut with an ultramicrotome RMC-MT-X and stained with 1% borax methylene blue. Ultrathin sections were contrasted with 4% aqueous uranyl acetate followed by lead citrate in CO_2 -free chamber and observed in a JEOL JEM-1010 transmission electron microscope.

To determine the range of substrates used as carbon and energy sources or as carbon, nitrogen and energy sources, a basal medium (BM) with the following composition was employed: $50 \text{ g l}^{-1} \text{ NaCl}$; $2 \text{ g l}^{-1} \text{ KCl}$; $0.2 \text{ g l}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$; $1.0 \text{ g l}^{-1} \text{ KNO}_3$; $1.0 \text{ g l}^{-1} (\text{NH}_4)_2\text{HPO}_4$; $0.5 \text{ g l}^{-1} \text{ KH}_2\text{PO}_4$; 0.2 g l^{-1} yeast extract (Oxoid). When amino acids were used as substrates, the BM contained neither KNO_3 nor $(\text{NH}_4)_2\text{HPO}_4$. BM was dispensed in 5 ml aliquots, autoclaved and, after sterilization, 0.25 ml of 10% (w/v) Na_2CO_3 sterile solution (final pH around 9.5) and filter-sterilized stock substrates were added to each tube at a final concentration of 0.1% (w/v), except 1% (w/v) for carbohydrates. To study different electron acceptors, BM was prepared anaerobically (with oxygen free nitrogen) in Hungate tubes, supplemented as described before, and glucose 1% (w/v) and electron acceptors (nitrite 5 mM, nitrate 10 mM, fumarate 10 mM) were added from anaerobic stock solutions. Growth was checked by measuring OD at 660 nm. Tubes inoculated with strain M30^T, but without substrates, were used as blank controls. The utilization of glucose by oxidation or fermentation was checked in modified basal OF medium described previously [4]. Other biochemical tests were conducted using the API 20NE, API 50CH and API ZYM strips, according to the manufacturer's instructions (bioMérieux), except that cell suspensions for carbon assimilation tests for API 20NE were prepared using AUX medium supplemented with NaCl solution (2% final concentration), 0.05% (w/v) yeast extract (Oxoid) and 5% (v/v) of 10% (w/v) Na_2CO_3 solution. For API 50CH cell suspensions were prepared using the modified basal OF medium described previously [4] without agar. Catalase activity was tested in a slide with 3% hydrogen peroxide solution and oxidase activity with Bactident oxidase (Merck). The following phenotypic characteristics were performed in media prepared with 5% NaCl and supplemented with 15 ml of 10% (w/v) Na_2CO_3 solution per litre of medium unless indicated. ONPG (o-nitrophenyl- β -D-galactopyranoside), pigments from tyrosine, haemolysis of red blood cells [with 2% NaCl and pH adjusted to 8.0 with 10% (w/v) Na_2CO_3 solution], Tween 20, Tween 60, Tween 80 and tyrosine hydrolysis tests were performed according to Barrow and Felthman [5]. Aesculin, casein, DNA (pH adjusted to 8.5 with 10% (w/v) Na_2CO_3

solution), gelatin (methods 2 and 3) and starch hydrolysis, citrate utilization (method 1 visualized only by growth), hydrogen sulfide production from cysteine, indole production (revealed with Kovács' reagent), lipase (method 1), lecithinase, phenylalanine deaminase, phosphatase (method 2), urease activity (method 3), Voges–Proskauer reaction, nitrate reduction, nitrite reduction and denitrification tests were carried out according to Tindall *et al.* [6]. Cellulase, xylanase and polygalacturonases activities were carried out in plates of BM previously described, supplemented with 1.5% agar and 0.5% methyl-cellulose, 0.5% xylan and 0.5% polygalacturonic acid, respectively, and with 15 ml of 10% (w/v) Na_2CO_3 solution per litre of medium. For inoculation, wells of 6 mm were cut in the solid medium and filled with broth cultures (previously grown at 30°C for 48 h in mNB). Plates were incubated and revealed after 48 h by flooding with Gram's iodine solution. The enzyme activity was observed by detecting a clear zone around the well. Growth at different temperatures (from 5 to 45°C at intervals of around 5°C) was performed in mNB. The pH range for growth (pH 6.0–12.0, at intervals of 0.5 pH units) was determined in mNB without 10% Na_2CO_3 solution and supplemented with 100 mM K_2HPO_4 – KH_2PO_4 buffer (for pH 6.0–8.0), 100 mM NaHCO_3 / Na_2CO_3 buffer (pH 8.5–10.5), 100 mM NaHCO_3 –NaOH buffer (pH 11.0–11.5) and 100 mM KCl/NaOH buffer (pH 12.0). Initial pH was carefully adjusted with HCl or NaOH as required, and media was sterilized by filtration. pH changes above 0.2 units were observed for some pH values after 48 h, corresponding to the exponential growth period (pH 10.5–9.9, pH 11.0–10.1, pH 11.5–10.3, pH 12.0–10.1). Growth at different NaCl concentrations was tested in mNB prepared without added NaCl (containing 0.5% NaCl), and with 1, 2, 5, 10, 15, and 20% (w/v) final NaCl concentration. Growth was monitored by measuring the OD_{660} . Slopes of OD_{660} versus time were plotted and averaged from triplicate runs at each pH, NaCl concentration and temperature for the determination of optimal growth rate at each condition. Antimicrobial susceptibility tests were determined with MicroScan Pos Combo panel type 33 (Siemens). The panels were inoculated according to the manufacturer's protocol using the turbidity method except that mNB was used for inoculation but the pH adjusted to 8.5 with 10% (w/v) Na_2CO_3 solution, incubated at 30°C for 48 h and read on a MicroScan autoSCAN-4 instrument.

Cells of strain M30^T were Gram-stain-negative rods, single or in pairs, or short chains, non-motile and spore-forming (Fig. S1, available in the online version of this article), and measured 0.5 – $0.8 \mu\text{m}$ in diameter and 1.5 – $2.5 \mu\text{m}$ long. Cells formed central, ellipsoidal endospores in unswollen sporangia only in the presence of manganese salts after 10 days of incubation. Strain M30^T did not grow in mineral minimal media BM supplemented with vitamins [7], but it grew in mineral minimal media supplemented with yeast extract. It was strictly aerobic, positive for catalase activity, but negative for oxidase activity. Acid was produced aerobically from D-glucose in OF modified media. The detailed phenotypic characteristics of strain M30^T are listed in the species description and the list of tested antibiotics and minimum inhibitory concentration are shown in Table S1.

Biomass for fatty acid analyses of strains were harvested from cultures grown on TSA supplemented with 5% NaCl, and after sterilization, with 100 ml of 10% (w/v) Na₂CO₃ solution per litre of medium and incubated at 30 °C for 3 days. Fatty acid composition, polar lipids, respiratory quinones and diaminopimelic acid analyses, were carried out by the Identification Service of DSMZ (Braunschweig, Germany). Fatty acid methyl esters obtained from freeze-dried cells were prepared and analysed [8], followed by gas chromatography (Agilent 5890). The microbial identification standard software package MIDI Sherlock (version 6.1) was used to automatically integrate the peaks, annotate the fatty acids and determine the relative percentages using TSBA40 and TSBA6 databases. Respiratory lipoquinones were extracted from freeze-dried cell material with methanol/hexane, separated into their functional classes by thin-layer chromatography (TLC) and analysed by reverse-phase HPLC as described previously [9, 10]. Polar lipids were extracted from the methanolic/aqueous phase and cell material remaining after extraction of the respiratory lipoquinones, separated by two-dimensional TLC and identified as described by Tindall *et al.* [6]. Whole-cell hydrolysates (4N HCl, 100 °C, 16 h) were examined by TLC on cellulose plates for the presence of isomers of 2,6-diaminopimelic acid and of 2,6-diamino-3-hydroxypimelic acid.

The major fatty acids (>10%) comprised anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0} (Table 1), the predominant quinones were MK-7 (88%) and MK-6 (12%). The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified lipid and one unidentified phospholipid (Fig. S2). The diamino acid of peptidoglycan was *meso*-diaminopimelic acid.

Extraction of genomic DNA was carried out with the QIAamp DNA Mini Kit (Qiagen) and amplification of nearly full-length 16S rRNA gene fragments was performed as described previously [4]. The sequence was compared to other released sequences in GenBank against a database containing the type strains with validly published prokaryotic names. The pairwise sequence similarity was calculated by using the global alignment algorithm, which was implemented at the EzBioCloud server [11]. Phylogenetic trees were inferred by three different methods: neighbour-joining [12], maximum-likelihood [13] and maximum-parsimony [14]. The trees were rooted using *Halobacillus halophilus* NBRC 102448^T (AB681790) as the outgroup, and 1369 positions were used for the phylogenetic analysis. Trees were generated by using the MEGA version 7.0 software package [15]. Kimura's two-parameter model [16] was used to calculate evolutionary distance matrices of the neighbour-joining and maximum-likelihood phylogenetic trees. Bootstrap analyses (1000 replicates) were performed in order to assess the reliability of the inferred branches [17].

Analysis of an almost-complete (1399 bp) 16S rRNA gene sequence of strain M30^T in the EzBioCloud server revealed that it shared the highest sequence similarities with *Bacillus populi* FJAT-45347^T (96.4%) and *Bacillus aurantiacus* K1-5^T (96.2%) and below 96.0% with other type strains of validly published species. The phylogenetic tree reconstructed from

Table 1. Cellular fatty acid composition (%) of strain M30^T and closely related species

Strains: 1, M30^T; 2, *Bacillus aurantiacus* K1-5^T; 3, *Bacillus populi* FJAT-4^T. Major components (>10%) are shown in bold type. Fatty acids representing less than 0.5% are not shown.

Fatty acid	1	2	3
iso-C _{14:0}	2.0	1.1	1.0
C _{14:0}	–	0.8	–
iso-C _{15:0}	16.4	21.6	10.5
anteiso-C _{15:0}	49.8	38.8	52.1
iso-C _{16:0}	5.9	5.9	7.0
C _{16:0}	3.5	2.9	6.0
C _{16:0} N alcohol	–	–	2.0
C _{16:1} ω7c alcohol	1.5	–	–
C _{16:1} ω11c	0.6	–	–
iso-C _{17:0}	3.8	3.3	3.5
anteiso-C _{17:0}	14.3	11.6	16.1
anteiso-C _{17:1} A	–	3.0	–
iso-C _{17:1} ω5c	–	1.7	–
C _{18:0}	–	0.5	0.8
C _{18:1} ω9c	–	–	0.8
Summed feature 2*	–	1.1	–
Summed feature 3*	–	5.7	–
Summed feature 4*	0.9	–	–

*Summed features comprise a group of two or more fatty acids that could not be separated under the given conditions. Summed feature 2 comprises C_{12:0} aldehyde and/or unknown; summed feature 3 comprises: iso-C_{15:0} 2OH and/or C_{16:1} ω7c and/or C_{16:1} ω6c; summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B.

evolutionary distances of Kimura's two-correction parameter by the neighbour-joining method (Fig. 1) revealed clear affiliations of the novel isolate with the genus *Bacillus* and clustered with *Bacillus aurantiacus* K1-5^T which was supported with a high bootstrap value. The phylogenetic position was also confirmed by the trees generated using the methods of maximum-likelihood (Fig. S3) and maximum-parsimony (Fig. S4) with identical topologies.

DNA, extracted as described previously, was sequenced at MacroGen (Republic of Korea) using the Illumina HiSeq-2000 platform. A total of 17664596 high quality pair-ends reads were produced with an average insertion size of 573. *De novo* assembly was performed with SPAdes assembler version 3.9.1 [18], using a pre-assembly approach with Velvet 1.2.10 [19]. 99.5% of the generated reads were mapped resulting in a mean nucleotide coverage of 435×, as reported by QUAST [20]. Corrected reads showed an average length of 98 bp. The final assembly was composed of 43 contigs, 19 of which were

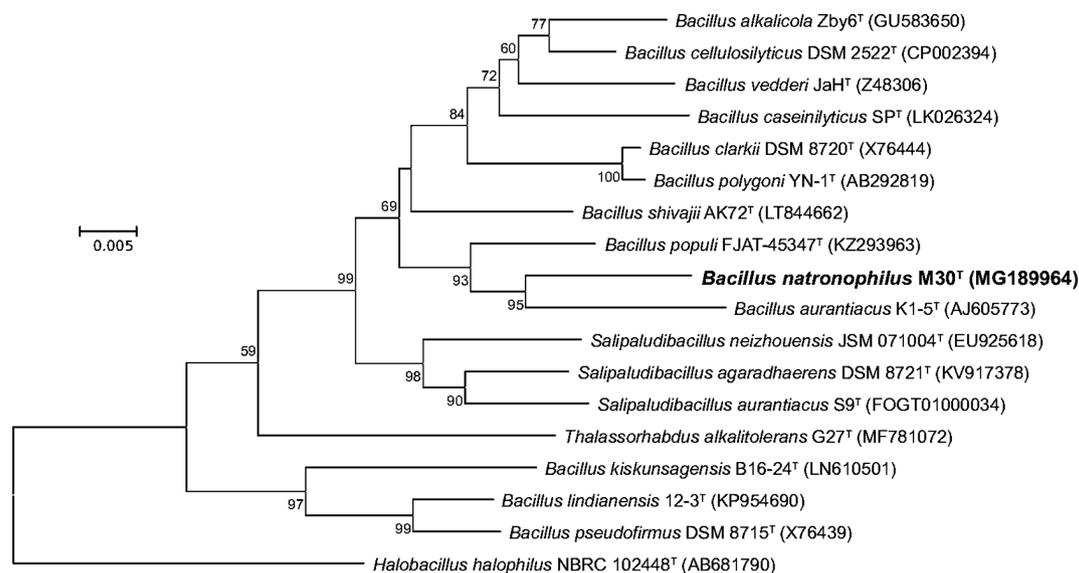


Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relation of strain M30^T and closely related species. *Halobacillus halophilus* NBRC 102448^T (AB681790) was used as the outgroup. Bootstrap values (above 70%) based on 1000 resamplings are listed at the nodes. Bar, 0.005 substitutions per nucleotide position.

above 500 bp long. In total, these contigs were 3 969 381 bp with an *N50* contig size of 651850 (max length 1 422 034) and had a DNA G+C content of 48.89mol%. The genome was annotated using the RAST server with default parameters [21]. There were 4137 protein-coding genes predicted in the genome of strain M30^T, 73 putative tRNAs genes and one complete ribosomal cluster plus nine 5S rRNA coding genes.

The average nucleotide identity (ANI) value, belonging to the overall genome related index, was calculated to estimate relatedness using the OrthoANI algorithm [22] available at the EzBioCloud-e server. The ANI calculated values of strain M30^T and the related species *B. aurantiacus* K1-5^T and *B. populi* FJAT-45347^T were 71.3 and 70.4%, respectively, which were lower than the cut-off value (95–96%) for species demarcation [23].

Growth characteristics (temperature, pH and salinity ranges) of strain M30^T were consistent with the environmental parameters of its habitat. Phenotypic and phylogenetic properties of this strain support its inclusion in the genus *Bacillus*. Differentiating characteristics of strain M30^T and closely related type strains are shown in Table 2. Strain M30^T differed from *B. aurantiacus* K1-5^T and *B. populi* FJAT-45347^T by its colony colour, β-galactosidase activity, production of pigments from tyrosine, utilization of D-galactose, maltose, D-mannitol and D-xylose, hydrolysis of casein and starch, and the DNA G+C content. Strain M30^T and *B. aurantiacus* K1-5^T differed in β-haemolysis and lipase activities, and hydrolysis of aesculin, DNA (data not shown), lecithin, polygalacturonic acid, Tween 20, Tween 60, Tween 80 and xylan. Strain M30^T differed from *B. populi* FJAT-45347^T by citrate utilization, results for

Voges–Proskauer reaction, utilization of D-glucose, glycerol and melibiose, and hydrolysis of urea.

The fatty acid profiles of the three strains were similar (Table 1), however anteiso-C_{17:1} A (3.0%) was detected in *B. aurantiacus* K1-5^T but not in M30^T and *B. populi* FJAT-45347^T, and C_{16:1} ω7c alcohol was detected in M30^T (1.5%) but not in *B. aurantiacus* K1-5^T and *B. populi* FJAT-45347^T.

Therefore, based on its distinctive phenotypic, chemotaxonomic and genotypic characteristics, strain M30^T represents a novel species within the genus *Bacillus*, for which the name *Bacillus natronophilus* sp. nov. is proposed.

DESCRIPTION OF *BACILLUS NATRONOPHILUS* SP. NOV.

Bacillus natronophilus [na.tro.no'phi.lus. N.L. n. *natron* (arbitrarily derived from the Arabic n. *natrun* or *natron*), soda; N.L. pref. *natrono-*, pertaining to soda; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos*) friend, loving; N.L. masc. adj. *natronophilus* soda-loving].

Cells of strain M30^T are Gram-stain-negative rods, single or in pairs, or short chains, non-motile and spore-forming, and measure 0.5–0.8 μm in diameter and 1.5–2.5 μm long. Cells form central, ellipsoidal endospores in unswollen sporangia. Does not grow on trypticase soy agar, nutrient agar, plate count agar, Reasoner's 2A agar or nutrient broth without added NaCl and pH 7.0–7.5. Colonies on trypticase soy agar plus 2% NaCl and pH 10 are around 2.0–3.0 mm in diameter, beige, shiny, smooth, convex and circular with entire margins after incubation at 30 °C for

Table 2. Differential characteristics of strain M30^T and closely related type strains

Strains: 1, M30^T; 2, *Bacillus aurantiacus* K1-5^T; 3, *Bacillus populi* FJAT-45347^T. All data, unless otherwise indicated, are from this study. All strains are positive for catalase, gelatin hydrolysis, and negative for anaerobic growth, H₂S production, indole production, methyl red test, motility, nitrate and nitrite reduction, oxidase, and phenylalanine deaminase activity. ND, not determined; w, weak reaction.

Characteristic	1	2	3
Colony colour	Beige	Orange	White
NaCl range for growth (% w/v)	0.5–15	0–15*	0–20†
pH range for growth	8–12	8–12*	7.5–12†
Temperature range for growth (°C)	12–43	10–45*	15–35†
Alkaline phosphatase	+	–	†
Citrate utilization	–	–	+
β-Galactosidase	+	–	–
β-Haemolysis	–	+	ND
Lipase	–	+	–
Voges–Proskauer	–	–	+
Pigments from tyrosine	+	–	–
Carbon source utilization:			
D-Galactose	+	–	–
D-Glucose	+	+	–
Glycerol	+	+	–
Maltose	+	–	–
D-Mannitol	+	–	–
Melibiose	+	+	–
D-Xylose	+	–	–
Hydrolysis of:			
Aesculin	+	–	+
Casein	+	–	–
Lecithin	–	+	–
Polygalacturonic acid	+	–	ND
Starch	+	–	–
Tyrosine	w	–	–
Tween 20	–	+	–
Tween 60	–	+	–
Tween 80	–	+	–
Urea	–	–	+
Xylan	+	–	ND
Major menaquinones	MK7, MK6	MK7*	MK7†
DNA G+C content (% or mol%)	48.9	42.9*	40.6†

*Data from Borsodi *et al.* [24].

†Data from Liu *et al.* [25].

48 h. Grows at pH 8.0–12.0 (optimum, pH 9.5–10), in the presence of 0.5–15% NaCl (optimum, 5%) and at 12–43 °C (optimum, 25–30 °C). Requires yeast extract for growth. Catalase-positive but oxidase-negative. Strictly aerobic. Acid is produced from D-glucose in OF modified media. Aesculin, casein, gelatin and starch are hydrolysed, but DNA, lecithin, Tween 20, Tween 60, Tween 80 and urea are not. Tyrosine is weakly hydrolysed. Alkaline phosphatase, cellulase, esterase lipase (C8), leucine arylamidase, α -chymotrypsin, β -galactosidase, α -glucosidase, polygalacturanase and xylanase are produced. Test results with acid phosphatase, cysteine arylamidase, esterase (C4), esterase lipase (C14), α -fucosidase, α -galactosidase, β -glucosidase, β -glucuronidase, lipase (egg yolk), naphthol-AS-BI-phosphohydrolase, *N*-acetyl- β -glucosaminidase and α -mannosidase are negative. Pigments are produced from tyrosine. ONPG-positive. Red blood cells are not haemolysed. Citrate (Simmons), H₂S from cysteine, indole production, methyl red test, nitrate and nitrite reduction, denitrification, phenylalanine deaminase and Voges-Proskauer test are negative. Grows aerobically on the following substrates as a sole carbon source: D-galactose, D-glucose, glutarate, glycerol, glycogen, maltose, malate, D-mannitol, melibiose, trehalose and D-xylose; and in L-alanine, L-arginine, L-asparagine, L-aspartate, L-serine, L-tryptophan, L-isoleucine, L-ornithine, L-phenylalanine and L-valine as sole carbon and nitrogen sources. No growth on acetate, *N*-acetyl glucosamine, L-arabinose, D-arabitol, butyrate, cellobiose, citrate, α -ketoglutarate, formate, D-fructose, propionate, raffinose or L-rhamnose as sole carbon sources; and on L-cysteine, L-glutamine, glycine and L-lysine and as sole carbon and nitrogen sources. Does not grow anaerobically with glucose as substrate and nitrate, nitrite or fumarate as electron acceptors.

Acid is produced from D-galactose, D-glucose, glycerol, 5-ketogluconate, maltose, D-mannitol, D-mannose, melibiose, starch, sucrose, trehalose and hydrolysed aesculin, but no reactions are observed with *N*-acetyl glucosamine, D-adonitol, amygdaline, D-arabinose, L-arabinose, L-arabitol, D-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fructose, D-fucose, L-fucose, gentiobiose, gluconate, glycogen, inositol, inulin, 2-ketogluconate, D-lactose, D-lyxose, methyl α -D-glucoside, methyl α -D-mannoside, methyl β -D-xyloside, melezitose, raffinose, L-rhamnose, D-ribose, D-turanose, salicin, D-sorbitol, L-sorbose, D-tagatose, D-xylose, L-xylose or xylitol. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified lipid and one unidentified phospholipid. The predominant quinones are MK-7 (88%) and MK-6 (12%). The major fatty acids are anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid.

The type strain, M30^T (=JCM 32118^T=CGMCC ^T=MCC 3010^T), was isolated from sediment sampled at Lake Magadi (3.1833° S, 35.5333° E), a soda lake in the Arusha region, Tanzania. The DNA G+C content is 48.9 mol%. The 16S rRNA gene and genome sequence were deposited at DDBJ/

EMBL/GenBank under accession numbers MG189964 and VCRO01000000, respectively.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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