Extremophiles (2000) 4:53–60

ORIGINAL PAPER

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Halomonas magadii sp. nov., a new member of the genus *Halomonas*, isolated from a soda lake of the East African Rift Valley

Received: July 20, 1999 / Accepted: October 29, 1999

Abstract A number of novel alkaliphilic organotrophic bacteria have been isolated from several saline and alkaline East African soda lakes. The new isolates grow at pH values between 7.0 and 11.0, with pH optima for growth between 9.0 and 10.0. Growth occurs at total salts concentration between 0% and 20% (w/v) with optimum at 0%–7% (w/v). Phylogenetic analyses based on 16S rDNA sequence comparison indicate that these isolates are related (>96% similarity) to members of the *Halomonadaceae* within the γ-3 subdivision of the *Proteobacteria*. These analyses indicate that existing species within the *Halomonadaceae* fell within three main groups, one group comprising the type species of *Halomonas*, *Halomonas elongata*, and a number of other known species including one soda lake isolate. A second group constituting most of the remaining known species of *Halomonas* and related *Chromohalobacter* spp. includes 3 soda lake isolates with high DNA–DNA homologies. The third group included *Halomonas halodenitrificans*, *Halomonas desiderata*, *Halomonas cupida*, and 13 soda lake isolates. Phenotypic comparisons indicated that the majority of soda lake strains shared similar morphological, phenotypic, and chemotaxonomic properties to known strains of *Halomonas* but grew under alkaline conditions. The 3 soda lake isolates with high DNA–DNA homologies were, however, significantly different in antibiotic sensitivity pattern and in the utilization of several substrates, were unable to reduce nitrite, and showed low DNA–DNA homologies with known halomonads in the same group. We propose that these isolates comprise a new species of the genus *Halomonas* that we name *Halomonas magadii* sp. nov. The type strain is strain 21 MI (NCIMB 13595).

Key words *Halomonas magadii* sp. nov. · Alkaliphile · Soda lake · 16S rDNA · Phylogeny · Halomonad taxonomy

Communicated by K. Horikoshi

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Introduction

The family *Halomonadaceae*, which includes several moderately halophilic and halotolerant bacteria, until recently consisted of four genera: *Halomonas*, *Halovibrio*, *Deleya*, and *Chromohalobacter*. Members of these genera have been isolated from a range of terrestrial, marine, and hypersaline environments (Franzmann et al. 1988). Recently, the phylogeny of this family has been reexamined with the genera *Halomonas*, *Halovibrio*, and *Deleya* being united to form the single genus *Halomonas* (Dobson and Franzmann 1996). The family was also expanded to include the genus *Zymobacter*. There is clear evidence that the genus *Chromohalobacter* also belongs to the family *Halomonadaceae* (Mellado et al. 1995). The organisms cannot be distinguished on the basis of polar lipid profiles or fatty acid signatures, and all contain ubiquinone 9 (Franzmann and Tindall 1990). There are also limited 16S rRNA signature sequences available for the group (Franzmann et al. 1988; Mellado et al. 1995; Dobson and Franzmann 1996).

The Great Rift Valley running through Eastern Africa is an arid tropical zone where evaporation exceeds the rate of inflow of water. In the Kenyan-Tanzanian section, the Gregory Rift Valley, in many areas dissolved minerals concentrate into alkaline brines with carbonate as the major anion, forming shallow soda lakes (Jones et al. 1994). The salinities of these lakes vary from around 5% (w/v) total salts to saturation. The lakes are extremely productive as a consequence of dense blooms of cyanobacteria, notably *Spirulina* spp. (Grant and Tindall 1986; Jones et al. 1994). The lakes also harbor dense stable populations of organotrophic bacteria, viable counts (colony-forming units, cfu) being

between 10^5 and 10^6 cfu/ml^{-1}. Recent studies have identified many of the trophic groups responsible for the recycling of carbon and sulfur in the lakes, and there are obvious parallels with other aquatic systems (Jones et al. 1998).

Recently, a large number of aerobic organotrophic strains were isolated from in and around soda lakes (Duckworth et al. 1996; Jones et al. 1994) on a variety of media. The majority of the isolates had an obligate requirement for high pH, and all were alkaliphilic. Phylogenetic analysis of about 30 of the isolates based on 16S rDNA sequence comparisons indicated that many of the isolates belonged to the γ-3 division of the *Proteobacteria*, and notably the majority (17) of the isolates clustered within the halomonad lineage (Duckworth et al. 1996), although all the isolates were distinct from known halomonads. There have since been reports of other alkaliphilic halomonads (Romano et al. 1996; Berendes et al. 1996) derived from less obviously alkaline environments. In this study we report in detail on the properties of the soda lake isolates and propose that one group of these isolates represents a new species of the genus *Halomonas*.

Materials and methods

Source of organisms

Littoral zone sediment samples were collected from Lake Bogoria (Kenya) (0°20′ N and 36°15′ E), Lake Elmenteita (Kenya) (0°25′ S and 36°15′ E), Lake Magadi (Kenya) (1°43′–2°00′ S and 36°13′–36°18′ E), Lake Nakuru (Kenya) (0°22′ S and 36°05′ E), and Lake Sonachi (Kenya) (0°46′ S and 36°16′ E) and transported back to the UK or the Netherlands as previously described (Duckworth et al. 1996). Details of the sample collection and water chemistry are given in Jones et al. (1994) and Duckworth et al. (1996). Isolation procedures are described in Duckworth et al. (1996). The isolates used in this study and sample locations are listed in Table 1 of Duckworth et al. (1996).

Halomonas meridiana DSM 5425^T, *Halomonas halmophila* ATCC 19717^T, and *Halomonas salina* ATCC 49509^T were used for comparisons as strains able to grow under alkaline conditions on the medium described below. *Halomonas aquamarina* NCIMB 557^T and *Halomonas halophila* CCM 3622^T were also used for DNA–DNA hybridization comparisons.

Media and cultivation

The strains were grown in alkaline media and incubated at 37°C aerobically. The alkaline agar medium contained the following (in gl^{-1} of deionized H_2O): glucose, 10.0; peptone, 5.0 (Difco); KH_2PO_4, 5.0; $MgSO_4 \cdot 7H_2O$, 1.0; NaCl, 40.0; Na_2CO_3, 10.0; and agar, 20.0. The NaCl and Na_2CO_3 were autoclaved separately and added to the organic components at 60°C before pouring the agar medium (Duckworth et al. 1996). All strains were maintained on this medium.

Lipid analyses

Polar lipid analyses were performed following the polar lipid extraction procedure of Ross et al. (1985). Samples were analyzed by two-dimensional thin-layer chromatography (TLC), the plate being sprayed with 0.1% (w/v) $Ce(SO_4)_2$ in 1 N H_2SO_4 followed by heating at 150°C to visualize total polar lipids, ninhydrin for aminolipids, and 0.2% α-naphthol in *n*-butanol followed by heating at 120°C to visualize glycolipids. The identity of polar lipids was confirmed by cochromatography. Standards for phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine were purchased from Sigma (St. Louis, MO, USA).

Isoprenoid quinone analyses were performed by the modified procedure of Collins (1985). Dry, stationary-phase cells were extracted in 1:1 (v/v) $CHCl_3$:CH_3OH at 50°C for 16 h and examined by reverse-phase TLC on Merck HPTLC RP18 F254 plates (Merck, Darmstadt, Germany).

Antibiotics

Antibiotic sensitivity was tested by spreading a light suspension of bacteria in alkaline broth on the surface of alkaline agar and applying commercially available antibiotic sensitivity test disks (Oxoid). The plates were cultivated at 37°C for 48 h.

DNA extraction and sequencing of 16S rDNA genes

DNA was extracted from colonies using the Pitcher et al. (1989) extraction procedure. The PCR protocol of Embley (1991) was used. The reaction mix and subsequent direct cycle sequencing of the amplified PCR fragments was as previously described (Duckworth et al. 1996). A similarity and distance matrix was calculated using the Jukes and Cantor equation (1969). The distance data were then used to construct an unrooted tree using the Fitch and Margoliash method (1967).

Bootstrapping was employed to determine the accuracy of the tree constructed. The following Genbank/EMBL sequences were used during the phylogenetic analysis: *Chromohalobacter marismortui* (strain ATCC 17056^T) X87219, *C. marismortui* (strain A-492) X87222, *C. marismortui* (strain A-65) X87220, *C. marismortui* (strain A-100) X87221, *Halomonas aquamarina* M93352, *H. cupida* L42615, *H. elongata* M93355, *H. eurihalina* L42620, *H. halodurans* L42619, *H. halmophila* M59153, *H. halodenitrificans* L04942, *H. halophila* M93353, *H. marina* M93354, *H. meridiana* M93356, *H. pacifica* L42616, *H. salina* L42617, *H. subglaciescola* (strain ACAM 12^T) M933548, *H. subglaciescola* (strain ACAM 21) L42614, *H. variabilis* M93357, *H. venusta* L42618, *H. pantelleriense* X93493, *H. desiderata* X92417, *Zymobacter palmae* D141555, Lake Bogoria isolate 8B1 X92138, Lake Bogoria isolate 24B1 X92151, Lake Bogoria isolate 25B1 X92139, Lake Bogoria isolate 65B4 X92142, Lake Bogoria isolate WB2 X92141, Lake Bogoria isolate WB4 X92145, Lake Bogoria isolate

WB5 X92136, Crater Lake isolate 12C1 X92135, Crater Lake isolate 29C1 X92148, Crater Lake isolate 75C4 X92146, Lake Elmenteita isolate 35E2 X92147, Lake Elmenteita isolate 44E3 X92143, Lake Elmenteita isolate WE5 X92140, Lake Magadi isolate 21M1 X92150, Lake Magadi isolate 27M1 X92137, Lake Nakuru isolate 19N1 X92149, and Lake Nakuru isolate 28N1 X92144.

DNA base composition

Exponential-phase cells of strains were ruptured, and DNAs were purified using the method of Marmur (1961). The guanine-plus-cytosine (G + C) content of each DNA was determined from midpoint of the thermal denaturation profile (T_m) (Marmur and Doty 1962) obtained with a model UV-Vis 551S spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) at 260 nm; this instrument was programmed for temperature increases of 1.0°C/min. The T_m was determined by the graphic method described by Ferragut and Leclerc (1976), and the G + C content was calculated from this temperature by using 0.1 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The T_m of reference DNA from *Escherichia coli* NCTC 9001 was 74.6°C in 0.1 × SSC (Owen and Pitcher 1985). The mol% G + C calculated was the mean of three determinations.

Phenotypic characterization

The Gram's stain reaction was carried out using the Dussault (1955) modification with safranin as counterstain. The oxidase reaction was performed on filter paper moistened with a 1% (w/v) aqueous solution of *N, N, N′, N′*-tetramethyl-*p*-phenylenediamine. The aminopeptidase test (Cerny 1976) was performed on test strips (bactident Aminopeptidase; Merck). The KOH sensitivity tests was performed by the method of Halebian et al. (1981). Gelatin hydrolysis was tested on charcoal-gelatin discs (BioMérieux, Charbonières-les-Bains, France) incubated in alkaline broth at 37°C. The pH range for growth was determined on pH gradient plates as previously described (Jones et al. 1991). Otherwise, the methodology that we used has been described previously (Garcia et al. 1987; Quesada et al. 1984; Ventosa et al. 1982). Unless otherwise indicated the tests were carried out in the alkaline medium at 4% (w/v) NaCl, and incubated at 37°C in sealed containers. A total of 98 characteristics, including morphological, cultural, physiological, biochemical, and nutritional tests, were determined for each strain (Table 1).

Results

Morphology of isolates

All 17 isolates produced cream-beige, circular, convex, entire opaque colonies 2–3 mm in diameter at 37°C after 24 h. Microscopic examination showed that all isolates were non-spore-forming motile rods, 4–6 × 0.6–0.8 μm that were determined as gram negative on the basis of the Dussault modification of the Gram stain, the KOH test, and the amino peptidase reaction.

Phylogenetic analysis

PCR amplification and subsequent DNA sequencing allowed the determination of approximately 96% of the entire 16S rRNA gene in the region between positions 27 and 1512 (*E. coli* numbering).

Phylogenetic analysis revealed a relatively close relationship between the soda lake isolates and members of the genera *Halomonas* and *Chromohalobacter* with 16S rDNA sequence similarities between 94.6% and 99.0% to known strains. Tree construction (Fig. 1) indicated that the soda lake isolates, together with known *Halomonas* and *Chromohalobacter* species, formed three clusters with only *H. marina* and *Z. palmae* outside these main clusters. Group 1 comprises six *Halomonas* species and the soda lake isolate 65 B4 originally isolated from Lake Bogoria. Group 2 comprises *Chromohalobacter marismortui*, eight *Halomonas* species, and the soda lake organisms 19 N1, 21 M1, and 24 B1. Group 3 contains the majority of soda lake isolates, *Halomonas halodenitrificans*, *Halomonas desiderata*, and *Halomonas cupida*.

All strains were oxidase- and catalase positive, capable of growth at salt concentrations between 0% and 20% (w/v) with salt optima at 0%–7% (w/v). All soda lake isolates were alkaliphilic with pH optima for growth between 9 and 10, whereas the three *Halomonas* spp. able to grow on alkaline media had pH optima around 7.5. Of the 98 tests listed in Table 1, the capacity to grow anaerobically with or without nitrate, the inability to reduce nitrite, to utilize ethionine and creatine, and the production of H_2S distinguished strains 19 N1, 21 M1, and 24 B1 from all the other strains, including the known *Halomonas* spp. Strain 65 B4 was also distinct by also being able to grow anaerobically, incapable of reducing nitrate and utilizing creatine, but differed from 19 N1, 21 M1, and 24 B1 by being unable to grow on betaine, arabinose, D-galactose, lactose, D-mannose, D-melibiose, α-amino valerate, aconitate, DL-glycerate, dulcitol, D-mannose, *N*-acetylglucosamine, and L-serine and being unable to produce H_2S. Group 3 organisms were not homogeneous in test results and did not appear to have any clear discriminatory features.

The soda lake isolates exhibited similar antibiotic sensitivity patterns, the majority being insensitive to gentamycin (10 mg), kanamycin (30 mg), bacitracin (10 IU), novobiocin (5 mg), neomycin (30 mg), and vancomycin (30 mg) but sensitive to erythromycin (5 mg), streptomycin (10 mg), polymyxin (300 IU), nalidixic acid (10 mg), chloramphenicol (25 mg), and oleandomycin (5 mg). Strains 19 N1, 21 M1, and 24 B1 could be distinguished by insensitivity to rifampicin (2 mg) and sensitivity to sulfafurazole (100 mg). Strain 64 B4 was also distinct by virtue of its sensitivity to fusidic acid (10 mg), tetracycline (25 mg), and

Table 1. Phenotypic characteristics of original isolates and reference strains

Characteristics	19N1	21M1	24B1	65B4	29C1	35E2	75C4	28N1	WB4	44E3	WB5	WB2	8B1	25B1	WE5	12C1	27M1	CR1[a]	CR2[a]	CR3[a]
Anaerobic growth without nitrate	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Anaerobic growth with nitrate	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Growth at salts (%)	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0–20	0.5–20	0–20	0–20	1–20	0–20	0–20
Optimal growth at salts (%)	0–7	0–7	0–7	0–7	0–7	0–7	0–7	0–7	0–7	0–7	0–7	0–7	0–7	0–7	0.5–7	0–7	0.5–7	1–3	ND[b]	7.5
Growth at temperature (°C)	20–50	20–50	20–50	20–55	20–50	20–55	20–55	20–55	20–55	20–55	20–55	20–55	20–55	20–50	20–50	20–55	20–50	20–55	20–45	20–45
Optimal growth at temperature (°C)	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	73	37	37	37	37
pH range for growth	7–11	7–11	7–11	7–11	8–11	8–11	8–11	8–11	8–11	8–11	8–11	8–11	7–11	7–11	7–11	8–11	8–11	6–9	ND	5–10
Optimal pH for growth	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	9–10	7.6	7.5	7.2
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
Nitrite reduction	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−
H_2S production	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth on:																				
Amygdalin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Arabinose	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
D-Galactose	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Lactose	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
D-Mannose	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
D-Melibiose	+	+	+	−	+	+	−	+	+	−	+	+	+	+	+	+	+	+	−	−
D-Ribose	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	−	+	−	−
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	−	−
D-Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−	−
Adonitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
D-Gluconolactone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
D-Glucosamine	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−
Dulcitol	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Erythritol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
D-Mannitol	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−

m-Inositol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Propanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
D-Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
N-Acetylglucosamine	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
α-Aminovalerate	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Aconitate	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DL-α-Aminobutyrate	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	+	+
α-Ketoglutarate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Benzoate	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
DL-Glycerate	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
D-Glucuronate	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Glutamate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
p-Hydroxybenzoate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Hippurate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
DL-Malate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Oxalate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Pyruvate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Allantoin	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
DL-Arginine	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−	−
L-Asparagine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
DL-Aspartic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Betaine	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−	−
Ethionine	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Creatine	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
DL-Phenylalanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
L-Glutamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
L-Isoleucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
L-Leucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
DL-Lysine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
L-Methionine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
L-Proline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Sarcosine	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
L-Serine	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	+
L-Threonine	+	+	+	+	−	+	+	+	+	−	+	+	−	+	+	−	−	+	−	−
L-Tryptophan	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
L-Valine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+

[a] CR1, CR2, CR3: *Halomonas meridiana* DSM 5425, *H. halmophila* ATCC 19717, and *H. salina* ATCC 49509, respectively

[b] ND; not determined

All positive: cream pigmentation, motility, cytochrome oxidase, catalase, growth on D-cellobiose, D-fucose, L-raffinose, sucrose, D-xylose, acetate, butyrate, caprylate, fumarate, D-gluconate, malonate, propionate, quinate, D-saccharate, salicylate, suberate, succinate, tartrate, L-alanine, L-histidine, and L-ornithine

All negative: Gram stain, spore production, indole production, hydrolysis of casein, gelatin, starch, and Tween 80, phosphatase, phenylalanine deaminase and growth on inulin and L-rhamnose

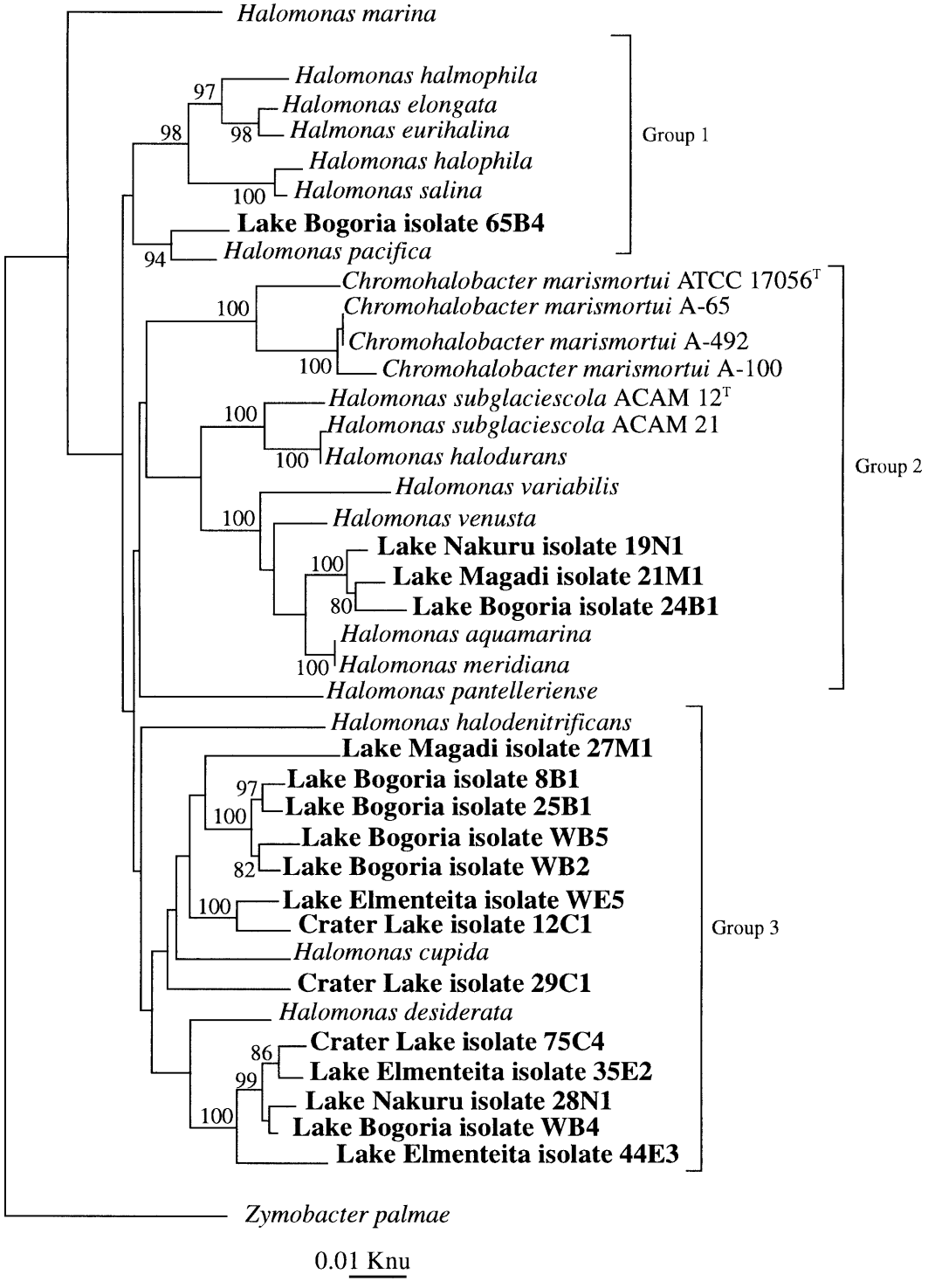

Fig. 1. Unrooted phylogenetic tree of halophilic and alkaliphilic *Halomonas* isolates. The least squares algorithm of Fitch and Margoliash (1967) was used to construct the tree using an evolutionary distance matrix (Jukes and Cantor 1969). Values at nodes indicate >50% percentage of occurrence in 100 bootstrapped trees

vancomycin (30 mg). Otherwise, there were no particular group-specific patterns.

All strains possessed phosphatidylglycercol (PG), diphosphatidylglycerol (DPG), phosphatidylglycerol phosphate (PGP), and phosphatidylethanolamine (PE), plus ubiquinone 6 and 9 (data not shown). A number of strains, notably 19 Nl, 21 Ml, and 24 Bl, produced small amounts of an unknown glycolipid.

G + C content and DNA–DNA relatedness

On the basis of the phenotypic characterization, strains 19 N1, 21 M1, and 24 B1 were selected for further genotypic characterization. DNA–DNA hybridization experiments carried out between strain 21 M1 and others in group 2 indicated high relatedness between strains 21 M1, 19 N1, and 24 B1 and low relatedness with *H. aquamarina* and

Table 2. DNA–DNA homologies of soda lake strains

Strain	% DNA relatedness with	
	Lake Magadi isolate 21M1	Lake Nakuru isolate 28N1
Lake Magadi isolate 21M1	100	28
Lake Nakuru isolate 19N1	86	28
Lake Bogoria isolate 24B1	86	24
Lake Nakuru isolate 28N1	11	100
Lake Bogoria isolate 25B1	20	33
Halomonas halophila CCM 3622^T	19	34
Halomonas meridiana DSM 5425^T	40	44
Halomonas aquamarina NCIMB 557^T	41	44

H. meridiana (Table 2), the closest relatives in the phylogenetic tree shown in Fig. 1 (98.8% and 98.9% 16S rDNA sequence similarities, respectively). Isolates 25 B1 and 28 N1 as representatives of group 3 had low DNA–DNA relatedness with strain 21 M1, again reflecting low 16S rRNA sequence similarity (96.0% and 95.9%, respectively). On the basis of the high DNA–DNA relatedness values between strains 21 MI, 19 N1, and 24 B1, we consider these to be different isolates of the same species as having DNA–DNA homologies higher than 70% (Wayne et al. 1987).

The DNA of strain 21 M1 had a G + C content of 62.2 mol% as determined by thermal denaturation, comparable to values quoted for members of the genus *Halomonas* (Dobson et al. 1993).

Discussion

The conditions necessary for the formation of a soda lake have much in common with those for the generation of an athalassohaline salt lake, but with the major difference that in a soda lake carbonates (or carbonate complexes) become the major anions in solution, producing highly alkaline brines (pH 10–12). Very high primary productivities are associated with these lakes, presumably because of the unlimited supply of CO_2 combined with high light intensities and high ambient temperatures. The Rift Valley soda lakes maintain dense, stable populations of aerobic organotrophic bacteria, many isolates of which have been subject to phenotypic, chemotaxonomic, and phylogenetic analysis (Duckworth et al. 1996; Jones et al. 1994). The majority of isolates are, as expected, alkaliphilic, and some at least are obligate alkaliphiles. In the recent phylogenetic analysis of more than 30 gram-positive and gram-negative isolates obtained from different lakes carried out by Duckworth et al. (1996), a few isolates were associated with *Pseudomonas* spp. and others had little affinity to known taxa within the enteric/vibrio lineages, but surprisingly the majority were related to but not identical with members of the *Halomonadaceae*.

Aerobic gram-negative organotrophic bacteria are abundant in brines of medium salinity. Originally classified in several genera such as *Halomonas*, *Pseudomonas*, *Deleya*, *Paracoccus*, *Halovibrio*, and *Volcaniella* (Ventosa 1994), these have now been reclassified into a single generic grouping as *Halomonas* spp. on the basis of 16S rDNA sequence analysis (Dobson and Franzmann 1996). 16S rDNA sequence comparison studies have also shown that members of the genus *Chromohalobacter* belong to this phylogenetic branch (Mellado et al. 1995) (see Fig. 1).

It is becoming increasingly clear that halomonads often constitute the major group in moderately saline environments, and this study extends that view to include the saline and alkaline environment. The soda lake isolates are clearly halomonads in terms of phylogenetic placement, quinone, polar lipid composition (Franzman and Tindall 1990), and general phenotypic properties. However, the soda lake halomonads differ from the majority of isolates from other natural environments in being alkaliphilic with growth optima at pH 9.5–10.0. They are also distinct and phylogenetically distant from the other alkaliphilic halomonads recently described, *H. pantelleriense* and *H. desiderata* (Fig. 1).

Isolates 21MI, 19N1, and 24B1 are clearly closely related in terms of phenotypic characters, DNA–DNA homology, and 16S rDNA sequence analysis. We believe that these isolates differ from the other soda lake isolates sufficiently to merit classification as a new species, which we have named *Halomonas magadii* sp. nov. It is worth noting that isolate 65B4 may also represent a single example of another new species in view of its phenotypic features and 16S rDNA sequence analysis, but a detailed comparison with *Halomonas pacifica* would be necessary to confirm this. The soda lake halomonads in group 3 may also represent one or more new species, but again detailed comparisons with *Halomonas cupida*, *Halomonas halodenitrificans*, and *Halomonas desiderata* are required.

Figure 1 indicates that the inclusion of *Halomonas marina* and particularly *Zymobacter palmae* as members of the family may need to be reexamined.

Description of *Halomonas magadii* sp. nov.

Halomonas magadii, Duckworth, Grant, Jones, Márquez and Ventosa (ma.ga'di.i. M.L. gen. n. *magadi* of Magadi, named for lake Magadi, a saline soda lake in Kenya). Gram-negative, non-spore-forming motile rods 4.0–6.0 × 0.6–0.8 μm. Catalase positive, oxidase positive, circular, low, convex, opaque cream-colored colonies are formed on agar. Alkaliphilic; growth occurs between pH 7.0 and 11.0 with an optimum around 9.5. Halotolerant: able to grow at 0% to 20% salts. Grows well between 25°C and 40°C with an optimum at 37°C. Phosphatase and phenylalanine deaminase negative. Does not hydrolyze starch, gelatin, or casein. Indole not produced. No anaerobic growth in the presence or absence of nitrate. Nitrate reduced as far as nitrite. H_2S produced from cysteine. Under alkaline conditions, amygdalin, D-cellobiose, esculin D-fructose, D-fucose, D-galactose, D-glucose, lactose, D-mannose, D-melibiose, L-raffinose, D-ribose, salicin, sucrose, starch, D-trehalose, D-xylose, adonitol, D-glucuronolactone, D-glucosamine,

dulcitol, erythritol, ethanol, glycerol, D-mannitol, *m*-inositol, propanol, D-sorbitol, acetate, *N*-acetylglucosamine, propionide, quinate, D-saccharate, salicylate, suberate, succinate, tartrate, L-alanine, DL-arginine, L-asparagine, DL-aspartic acid, DL-phenylalanine, L-glutamine, L-histidine, α-aminovalerate, aconitate, α-ketoglutarate, butyrate, caprylate, citrate, fumarate, DL-glycerate, D-gluconate, D-glucuronate, glutamate, *p*-hydroxybenzoate, hippurate, DL-malate, malonate, oxalate, L-ornithine, L-isoleucine, L-leucine, DL-lysine, L-methionine, L-proline, L-serine, L-threonine, L-tryptophan, L-valine, and pyruvate are metabolized, but not sarcosine, ethionine, creatine, DL-aminobutyrate, Tween 80, inulin, or L-rhamnose. Sensitive to erythromycin, streptomycin, polymyxin, sulphafurazole, chloramphenicol, and oleandomycin, but insensitive to gentamycin, sulphamethoxazole, kanamycin, nitrofurantoin, trimethoprim, fusidic acid, tetracyclin, rifampicin, bacitracin, ampicillin, penicillin G, methicillin, neomycin, novobiocin, and vancomycin. The major polar lipids are phosphatidyl glycerol, phosphatidyl glycerol phosphate, diphosphatidyl glycerol, and phosphatidyl ethanolamine with a small amount of an unidentified glycolipid. Cells contain ubiquinones 6 and 9.

Isolated from the littoral sediments of Lake Magadi, Lake Nakuru, and Lake Bogoria, Kenya. The guanine plus cytosine content of the DNA is 62 mol% (as determined by the thermal denaturation method). The type strain is 21M1, deposited at the National Collection of Industrial and Marine Bacteria Ltd., 23 St Machar Drive, Aberdeen, AB2 1RY as NCIMB 13595.

Acknowledgments This study was supported by grants from the European Commission (Generic Project "Biotechnology of Extremophiles" BIO2-CT93-0274 and Extremophiles as Cell Factories BI04-CT96-0488), from the Spanish Ministerio de Educacióu y Cultura and the Junta de Andalucia.

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