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Dietzia natronolimnaios sp. nov., a new member of the genus *Dietzia* isolated from an East African soda lake

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Abstract Two novel alkaliphilic aerobic organotrophic bacteria have been isolated from a moderately saline and alkaline East African soda lake. The new isolates grow at pH values between 6 and 10, with a pH optimum for growth of 9.0, and at a salt concentration between 0% and 10% (w/v). Phylogenetic analysis based on 16S rDNA sequence shows that these isolates are very closely related (99.6% similarity) and are members of the monospecific genus *Dietzia* (98.8% and 98.7% similarity). DNA/DNA hybridization revealed a relatedness of 83% between the two isolates, but only 8% between them and the type strain *Dietzia maris*. The G + C content as measured by thermal denaturation is 66.1 mol%. Phenotypic comparisons between *D. maris* and one isolate showed that they share very similar morphological and chemotaxonomic properties, but differ significantly in carbon source utilization profiles and halotolerance in alkaline medium. We propose a second species of this genus which we name *Dietzia natronolimnaios* (type strain 15LN1 = CBS 107.95).

Key words *Dietzia natronolimnaios* sp. nov. · Alkaliphile · Soda lake · 16S rDNA · Phylogeny · Chemotaxonomy

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Introduction

The East African soda lakes are found within the Kenyan-Tanzanian Rift Valley in areas of alkaline trachyte lavas, low rainfall, and high rates of evaporative concentration (Jones et al. 1994; Grant 1992). The salinities of these alkaline (pH 8–12) lakes vary from around 5% (w/v) total salts to almost saturation. The lakes are alkaline because of high concentrations of sodium carbonate (usually as $Na_2CO_3 \cdot 10H_2O$ or $Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$) and have very low concentrations of Ca^{2+} and Mg^{2+}, which are insoluble as carbonate minerals under alkaline conditions. Little Lake Naivasha (also known as Lake Oloidien) at 0°49′S and 36°13′E is a moderately saline and alkaline example of a soda lake with a pH of 8.5 and conductivity values of around 1–3 mS cm^{-1} [compared with more normal pH values of 10–11 and conductivities of 10– > 100 mS cm^{-1} exhibited by other soda lakes in the Rift Valley (Duckworth et al. 1996; Jones et al. 1994)], representing an interesting water chemistry intermediate between an adjacent neutral-pH fresh water lake, Lake Naivasha (0°46′S and 36°20′E) (conductivity 0.4 mS cm^{-1}) and the soda lake Lake Sonachi (0°46′S and 36°16′E) (conductivity 10 mS cm^{-1}).

Soda lakes are probably the most productive naturally occurring aquatic environments on earth, with gross primary productivities an order of magnitude greater than the average productivity for streams and lakes (Talling et al. 1973), largely as a consequence of dense blooms of cyanobacteria, notably *Spirulina* spp. The lakes also harbor dense, stable populations of aerobic organotrophic bacteria, viable counts (colony-forming units; cfu) of such being between 10^5 and 10^6 cfu ml^{-1}, with direct counts two orders of magnitude greater than this (Jones et al. 1994; Grant 1992; Grant et al. 1990). Several hundred of strains of aerobic, nonphototrophic organotrophs have been isolated from samples of soil, water, and sediment in and around soda lakes (Duckworth et al. 1996; Jones et al. 1994) on a variety of media. The majority of isolates have an obligate requirement for high pH and all are alkaliphilic. 16S rRNA genes from around thirty of these isolates have been sequenced

and subjected to phylogenetic analysis (Duckworth et al. 1996). Gram-negative alkaliphiles were found to be confined to the γ3 subdivision of the Proteobacteria, whereas Gram-positive isolates were found in both high mol% G + C and low mol% G + C divisions of the Gram-positive lineage. Isolates 14LN1 and 15LN1 from L. Oloidien were shown to be most closely related to the organism *Dietzia maris* within the high mol% G+C division of the Gram-positive lineage. The genus *Dietzia* was first proposed by Rainey et al. (1995a) to accommodate *Rhodococcus maris* (Nesterenko et al. 1982) which, after phylogenetic analysis (Rainey et al. 1995b), proved not to be a member of the genus *Rhodococcus sensu strictu*. To date, *Dietzia maris*, originally isolated from halibut by Harrison (1929) and later from soil and the intestinal tract of carp by Nesterenko et al. (1982) remains the only species assigned to this genus. In this study we report in detail on the properties of the soda lake isolates and propose that these represent a new species of the genus *Dietzia*.

Materials and methods

Source of organisms

Littoral zone sediment and water samples were collected in October 1988 from Little Lake Naivasha (L. Oloidien) (0°49′S and 36°13′E), Kenya and transported back to the UK for enrichment and cultivation. Details of the sample collection and water chemistry are given in Jones et al. (1994) and Duckworth et al. (1996). *Dietzia maris* strains DSM 43672^T and DSM 41602 were used for comparison.

Media and cultivation

Dilutions of sediment samples were made in Alkaline Broth and then plated out on alkaline agar media for incubation at 37°C. The Alkaline Broth contained the following (in g l^{-1} of deionized H_2O): glucose (10.0), peptone (5.0; Difco, Detroit, MI, USA), yeast extract (5.0; Difco), KH_2PO_4 (1.0), $MgSO_4 \cdot 7H_2O$ (0.2), NaCl (40.0), Na_2CO_3 (10.0). NaCl and Na_2CO_3 were autoclaved separately and added to the organic components (including 1.5% w/v agar) for agar media. *Dietzia maris* strains were maintained on Brain Heart Infusion Broth (Oxoid, Basingstoke England). This medium was also used to carry out some phenotypic comparisons, since growth of *D. maris* on the alkaline medium was much less vigorous than that of the soda lake isolates.

Lipid analysis

Mycolic acid and cell wall composition analyses were performed by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany).

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) using chloroform-methanol-glacial acetic acid-water (80:12:15:4 v/v/v/v), the plate being sprayed with 0.1% (w/v) $Ce(SO_4)_2$ in 1N 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 phosphatidyl glycerol, diphosphatidyl glycerol, 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).

For fatty acid analyses, freeze dried stationary phase cells (200–300 mg) were extracted for 16 h at 75°C in toluene:methanol:concentrated H_2SO_4 (2.5 ml:2.5 ml:0.2 ml). After cooling, the lipid esters were partitioned into hexane (2 × 1.0 ml) and residual acid removed with NH_4HCO_3.

Lipid extracts were concentrated under O_2-free N_2, dissolved in 300 µl hexane, and applied to preparative silica gel plates (Merck F254, type T). The plates were developed in hexane:diethyl ether 85:15 (v/v) and the fatty acid methyl esters scraped off, extracted in hexane, and concentrated under a stream of O_2-free N_2. The fatty acid methyl esters were analyzed by gas chromatography using a Packard model 439 gas chromatograph equipped with flame ionization detectors. The samples were divided over a sample splitter and analyzed simultaneously over two columns: CP-SIL-88 (Chrompack, Nederland BV) (length 50 m, internal diameter 0.20 mm) and Ultra-2 (Hewlett Packard, Avondale, PA, USA) (length 50 m, internal diameter 0.20 mm). The carrier gas was N_2; the injection temperature 120°C; temperature gradient 2.5°C per min to 240°C and isothermal at 240°C for 30 min. Fatty acid methyl esters were assigned by reference to known standard mixtures. The identity of some peaks was confirmed by gas chromatography-mass spectroscopy (GC-MS) using a Carlo Erba HRGC 5160 Mega series gas chromatograph equipped with a CP-SIL-88 column (length 50 m, internal diameter 0.22 mm) with helium as carrier gas, and direct injection into the source of a AMD 403 mass spectrometer.

Physiological tests

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 was performed on test strips (Bactident Aminopeptidase; E. Merck, Darmstadt, Germany). The KOH sensitivity test 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.

Profiles for utilization of substrates as sole carbon and energy source were performed by two methods. Method (1) used a basal mineral agar medium containing ($g l^{-1}$): $Na_2HPO_4 \cdot 7H_2O$ (28.5), KH_2PO_4 (3.0), NaCl (8.0), $NaNO_3$ (1.0) pH 7.6. Solutions of substrates were made up in deionized water (20% w/v), filter sterilized, and added at a final concentration of 1% (w/v). Cells were grown in Brain Heart Infusion Broth, spun down after 48 h, resuspended in the same volume of 0.85% (w/v) NaCl, and 10-µl amounts of cell suspension were spotted on to the surface of agar plates containing the test substrates. Plates were read after 7 days at 30°C, comparing substrate-containing plates with control plates without any added carbon source. Method (2) was used for a comparison of soda lake isolates by employing a medium composed ($g l^{-1}$) of yeast extract (1.0), KNO_3 (10.0), K_2HPO_4 (1.0), $MgSO_4 \cdot 7H_2O$ (0.2), NaCl (40.0), Na_2CO_3 (10.0), and agar (20.0), supplemented with 2.0 $g l^{-1}$ of the carbon source under test. Bacteria were inoculated as described from a bacterial suspension cultivated for 48 h in Alkaline Broth. The plates were incubated at 37°C for 48 h and the results recorded by comparing bacterial growth on plates containing the carbohydrate supplement with control plates without the added substrate.

Amino acids as sole carbon and nitrogen source for growth were tested using method (2) with KNO_3 omitted from the medium.

Use was also made of the commercially available test strip ATB 32N (API-BioMérieux, La Balme des Grottes, France). The strips were used according to the manufacturer's instructions, cell suspensions for the inoculation of the strips being prepared from cultures on Brain Heart Infusion Agar. Strips were incubated at 30°C and 37°C for 48 h and read automatically with the ATB32 strip reader.

Enzymatic activities of the soda lake isolates were compared using the commercially available test strip APIZYM (API-BioMérieux) which was used according to the manufacturer's instructions, except that bacterial cells were suspended in Alkaline Broth. The strips were incubated at 37°C for 24 h.

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 discs (Oxoid). The plates were cultivated at 37°C for 48 h.

The optimum temperature for growth was determined by growing the cells in Brain Heart Infusion Broth at temperatures of 20°C, 25°C, 28°C, 30°C, 37°C, 42°C, 45°C, and 50°C. The optimal pH for growth of soda lake isolates was determined by growing the cells in Brain Heart Infusion Broth, the pH being adjusted with either HCl or NaOH. The optimum NaCl concentration for growth was determined by growing cells in NaCl-free minimal medium containing fructose, and NaCl-free Alkaline Broth to which was added varying amounts of NaCl between 0% and 16% (w/v). Optical densities at 600 nm were monitored over 1–6 days until the highest optical density was achieved.

DNA–DNA hybridization

The DNA–DNA relatedness of the two soda lake strains to the type strain of *D. maris* was determined using a modification of the procedure described by Nielsen (1994). The DNA concentration of each sample was determined spectrophotometrically. Probe DNA (300 ng) was prepared by random priming of total chromosomal DNA isolated by the Pitcher et al. (1989) method labeled with [^{35}S] dCTP using the NEBLOT kit (New England Biolabs, Beverly, MA, USA). Target DNA (300 ng per slot) was immobilized on nitrocellulose membranes (BA85, Schleichorand Schuell, London UK) by heating at 80°C for 90 min.

Hybridizations were carried out overnight at 61°C in the presence of 35% (v/v) formamide and the membranes were then washed at the hybridization temperature in changes of 2 × sodium saline citrate (SSC) containing 0.1% (w/v) sodium dodecyl sulfate (SDS) for 5 min each, followed by two changes of 0.1 × SSC containing 0.1% (w/v) SDS for 20 min each. Pieces of membrane were subjected to scintillation counting and percentage reassociation was calculated as described by Seldin and Dubnau (1985).

DNA base composition

The mol% G + C content (mean of five determinations) of DNA isolated by the method already described was determined by thermal denaturation analysis (Owen and Pitcher 1984) using a spectrophotometer equipped with a programmable heated sample carriage (Philips PU8700). *Eschericheria coli* B DNA (Sigma) was used as internal standard to check the accuracy of the system.

DNA extraction, sequencing, and analysis of 16S rDNA genes

DNA was extracted from colonies using the Pitcher et al. (1989) extraction procedure. The polymerase chain reaction (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 reconstruct a 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: *Actinomyces hyovaginalis* X69616, *Arthrobacter globiformis* X80736, *Clavibacter xylii* M60935, *Corynebacterium variabilis* X53185, *Corynebacterium xerosis* M59058, *Dietzia maris* (DSM 46102) X79291, *Dietzia maris* (DSM 43672^T) X79290, *Gordona bronchialis* X79287, *Gordona terrae* X53202, *Micrococcus luteus* M38242, *Mycobacterium paratuberculosis* X52934, *Mycobacterium tuberculosis* X52917, *Nesterenkonia halobia* X80747, *Nocardia asteroides* X57949, *Nocardioides fastidiosa* X76862, *Propionibacterium jensenii* X53219, *Rhodococcus rhodochrous* X79288, *Rhodococcus*

erythieus X79289, *Rhodococcus* sp. X77780, *Rothia dentocariosa* M59055, and *Tsukamurella paurometabolum* X53207.

Results

Isolation of soda lake strains

Dilutions of several littoral sediment samples plated on Alkaline Agar incubated at 30°C produced colonies that were initially orange in color but gradually became coral red as the culture aged. A dimethylsulfoxide (DMSO) extract of the cells gave a peak at 468 nm with shoulders at 388 nm and 445 nm in the UV spectrum. Two isolates, 14LN1 and 15LN1, were selected for further study. The colonies were smooth, glistening, circular, convex, entire, opaque, and 1–2 mm in diameter after 4–5 days at 30°C.

Cultures grown in Alkaline Broth for 24 h showed nonmotile, short, straight rods (0.9–1.1 μm by 1.2–2.3 μm) exhibiting snapping division and producing V forms. Cells adhered together in microcolonies in liquid media even when shaken at 180 rpm (Fig. 1). The isolates also grew on Brain Heart Infusion Agar.

Physiological and chemotaxonomic characteristics

The two soda lake isolates exhibited similar antibiotic sensitivity patterns, being sensitive to chloramphenicol (25 mg), erythromycin (5 mg), fusidic acid (10 mg), methicillin (10 mg), streptomycin (10 mg), tetracycline (25 mg), sulphafurazole (100 mg), oleanodomycin (5 mg), rifampicin (2 mg), neomycin (30 mg), vancomycin (30 mg), and bacitracin (10 IU), but insensitive to penicillin G (1 IU), novobiocin (5 mg), polymixin (300 IU), and kanamycin (30 mg). In addition, strain 15LN1 is sensitive to gentamicin (10 mg) and ampicillin (25 mg). There were, however, some differences between the two isolates in enzymatic activity (Table 1) and utilization of substrates (Table 2). Both had identical profiles with regard to Gram stain, polar lipids, lipoquinones, and major fatty acids (Table 3). Strain 15LNI was selected for further study and comparison with *Dietzia maris*.

Both *Dietzia maris* and strain 15LN1 were oxidase negative and catalase positive. Strain 15LN1 differs from *D. maris* in utilizing D-glucose, D-fumarate, succinate (and derivatives), mannitol, propionate, citrate, L-proline, suberate, glycogen, and hydroxybutyric acids under neutral conditions (Table 4). Our results differ from those reported for *D. maris* in that we repeatedly failed to get this strain to utilize D-glucose although the original report indicates acid production from glucose rather than utilization (Nesterenko et al. 1982).

The polar lipid patterns of *D. maris* and 15LN1 were identical (not shown) with spots corresponding to phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Table 5 summarizes chemotaxonomic and other data for *D. maris* and strain 15LN1. It was not possible to discriminate between the two strains on the basis of these tests. The fatty acid pattern is diagnostic for all members of the genus *Rhodococcus* and their relatives *Mycobacterium*, *Nocardia*, *Dietzia*, *Tsukamurella*, and

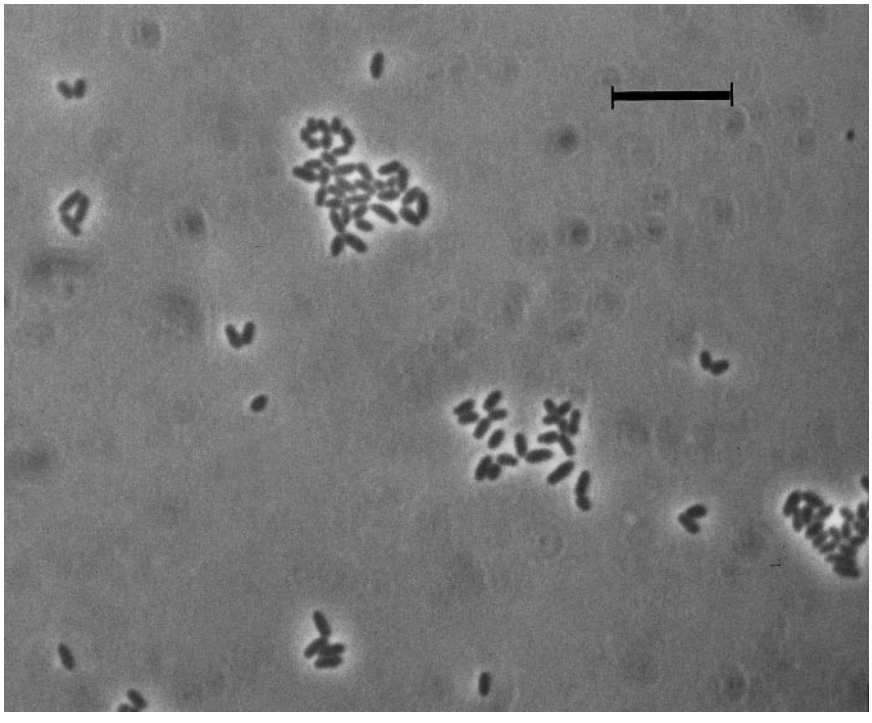

Fig. 1. Phase contrast micrograph of strain 15LN1 grown in Alkaline Broth for 48 h at 28°C. *Bar* represents 10 μm

Table 1. Enzymatic activity (APIZYM)

Enzyme	Strain 14LN1	Strain 15LN1
Alkaline phosphatase	+	+
Esterase (C4)	+	+
Esterase/lipase (C8)	+	+
Lipase (C14)	−	−
Leucine arylamidase	+	+
Valine arylamidase	+	−
Cystine arylamidase	+	+
Trypsin	+	−
Chymotrypsin	+	−
Acid phosphatase	+	+
Naphthol-AS-BI-phosphohydrolase	+	+
α-Galactosidase	−	−
β-Galactosidase	−	−
β-Glucuronidase	−	−
α-Glucosidase	+	+
β-Glucosidase	−	−
N-Acetyl-β-glucosaminidase	−	−
α-Mannosidase	−	−
α-Fucosidase	−	−

Table 2. Utilization of substrates under alkaline conditions

Substrate	Strain 14LN1	Strain 15LN1
L-Serine	+	+
L-Proline	+	+
L-Asparagine	+	+
L-Arginine	+	+
L-Alanine	+	−
L-Lysine	+	−
L-Methionine	+	+
L-Phenylalanine	+	+
Glycine	+	+
L-Valine	+	+
Fumarate	−	−
D-Fructose	−	w
Succinate	+	+
Formate	−	−
D-Lactose	−	−
D-Galactose	−	−
D-Xylose	−	−
Pyruvate	−	−
Starch	+	+

nd, not determined; w, weak growth.

Table 3. Characteristics of soda lake strains

	Strain 14LN1	Strain 15LN1
Cell morphology	Short rods	Short rods
Colony pigmentation	Red	Red
Gram reaction	Positive	Positive
KOH test	Negative	Negative
Aminopeptidase reaction	Negative	Negative
Oxidase test	Negative	Negative
Catalase text	Positive	Positive
Hydrolysis of gelatin	Negative	Negative
Major polar lipids	Phosphatidylglycerol Diphosphatidylglycerol Phosphatidylethanolamine	Phosphatidylglycerol Diphosphatidylglycerol Phosphatidylethanolamine
Isoprenoid quinones	MK8(H_2)	MK8(H_2)
Major fatty acids [1–10% (+); 10–30% (++)]	C14:0 (+) C16:0 (++) C16:1 (+) C17:0 (+) C18:0 (++) C18:1 (++) C18:2 (+) C18:Me (+) C20:0 (+) C22:0 (+)	C14:0 (+) C16:0 (++) C16:1 (+) C17:0 (+) C18:0 (++) C18:1 (+) C18:2 (+) C18:Me (+) C20:0 (++) C22:0 (+)

MK8(H_2), menaquinone with 8 isoprene units.

some *Corynebacterium* spp., but here again identification to the species level is not possible (Rainey et al. 1995b).

The pH range for growth of 15LN1 was found to be between 6 and 10 with a pH optimum around 9 at 28°C (Fig. 2). The pH optimum for *D. maris* has not apparently been determined and, surprisingly, proved to be virtually identical to that of 15LN1 (data not shown) in Brain Heart Infusion Broth at 28°C. Both *D. maris* and 15LN1 were capable of growth at between 20°C and 42°C with a temperature optimum around 30°C in Brain Heart Infusion Broth. Although both 15LN1 and *D. maris* grew best in the absence of NaCl, strain 15LN1 was markedly more tolerant of NaCl in Alkaline Broth, showing growth at between 0% and 10% (w/v) NaCl after 7 days at 28°C. *D. maris* was incapable of growth at salt concentrations above 4% (w/v) under these conditions. In neutral pH medium such as minimal medium containing fructose, 15LN1 and *D. maris* showed identical NaCl profiles, growing at between 0% and 8% (w/v) NaCl, again with optimum growth in the absence of NaCl.

G + C content, 16S rDNA analysis, and DNA/DNA homology

PCR amplification and subsequent DNA sequencing allowed the determination of approximately 96% of the entire 16S rRNA gene of both strain 14LN1 and 15LN1 in the region between positions 27–1512 (*Escherichia coli* numbering). These sequences have been deposited at the EMBL sequence data library under accession numbers X92156 and X92157. Phylogenetic analysis revealed a close relationship between the 16S rDNA sequences of both *D. maris* strains, which were identical, and strain 15LN1 (98.8% similarity) and the other soda lake isolate 14LN1 (98.7% similarity). The degree of sequence similarity between 15LN1 and 14LN1 was found to be at the level of 99.6%. The tree (Fig. 3) supported this close association, with high bootstrapping values separating the strains of *D. maris* from the two soda lake isolates. However, DNA hybridization revealed only a 8% relatedness between 15LN1 and *D. maris*, with 15LN1 being 83% related to 14LN1.

The G + C content of isolate 15LN1 was 66.1% ± 0.4 mol% ($n = 5$) by the thermal denaturation method.

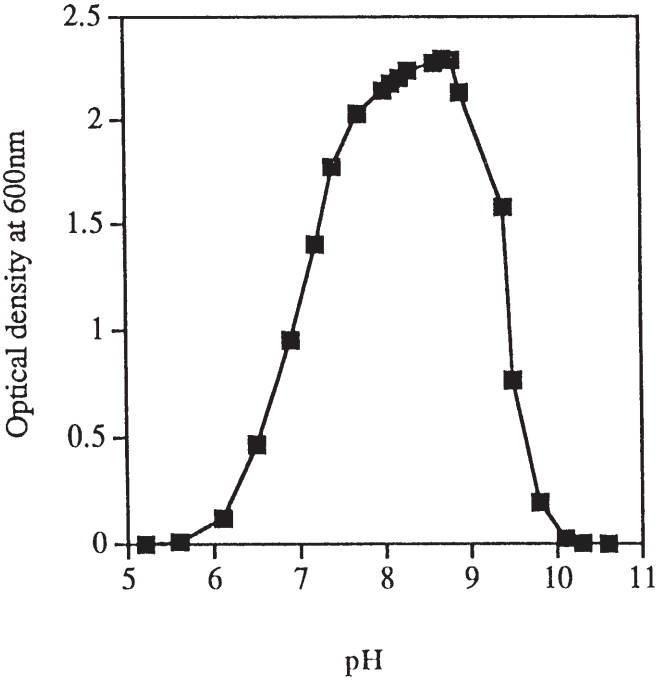

Fig. 2. Growth of 15LN1 in liquid Brain Heart Infusion media over a range of pH values

Discussion

It is clear that, on the basis of phylogenetic analysis; both 15LN1 and 14LN1 are members of the genus *Dietzia* (Fig. 3). Chemotaxonomic analyses confirms the placement of 15LN1, especially the presence of 10-methyloctadecanoic acid (tuberculostearic acid) (Table 5). However, 15LN1 has no significant DNA/DNA homology with the type species of *Dietzia maris*, whereas 15LN1 and 14LN1 are related at the level of 83% and are therefore members of the same species (Stackebrandt and Goebel 1994; Wayne et al. 1989). It is not possible to differentiate 15LN1 from *D. maris* on the basis of the chemotaxonomic analyses that we have

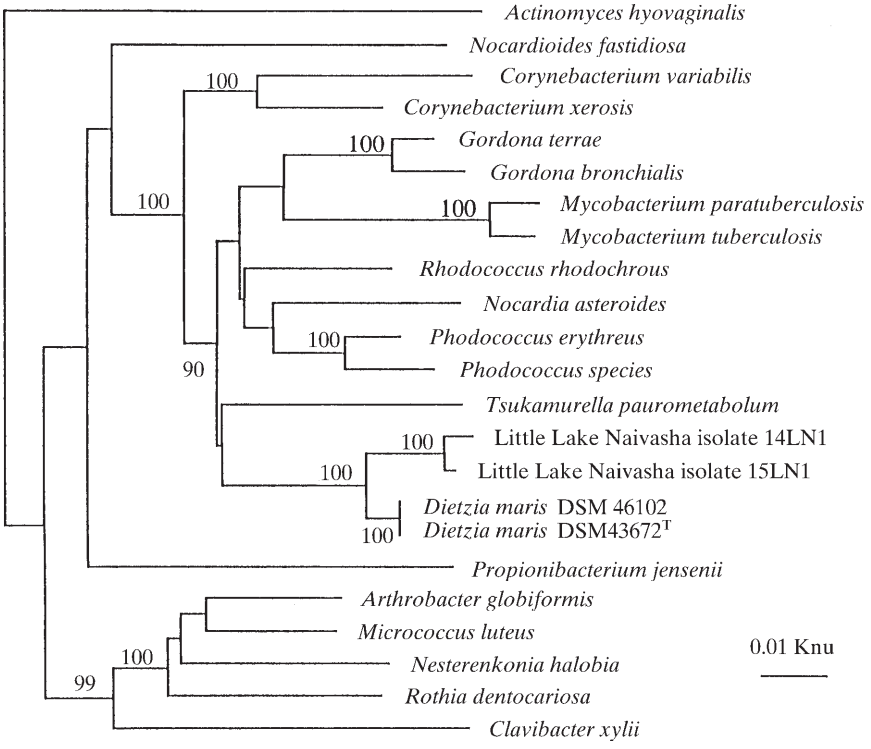

Fig. 3. Unrooted phylogenetic tree showing the relationship between both soda lake isolates and representatives of closely related genera. Bootstrap values for 100 estimations are shown at *nodes*. The least squares algorithm of Fitch and Margoliash (1967) was used to construct this tree using a Jukes and Cantor (1969) evolutionary distance matrix.

carried out, but these tests are not particularly discriminatory for the mycolic acid-containing bacteria (Rainey et al. 1995b). However, the G + C mol% value for 15LN1 is within the range reported for the mycolic acid group, although some 6% different from that reported for *D. maris* (Rainey et al. 1995a). Even so, most of the genera of mycolic acid-containing bacteria exhibit ranges of G + C mol% of more than 6%, so the difference may reflect the small number of isolates available for study. Strain 15LN1 can be readily distinguished from *D. maris* on the basis of a number of phenotypic characters, notably utilization of a range of carbon substrates (Table 4) and its relative halotolerance in alkaline media, probably reflecting the fluctuating salinities in Lake Oloidien as a consequence of climatic conditions. A steady increase in salinity and alkalinity in L. Oloidien has been observed over the years (Mbogo 1997) and these strains are thus well suited to survive as conditions approach those of "native" soda lakes in the Rift Valley. The observation that *D. maris* is also somewhat alkaliphilic is not surprising, since the original site of isolation, namely fish intestine, might be expected to be alkaline due to ammonification, and the generic description should reflect this.

We conclude that isolate 15LN1 is sufficiently different from the only known species of *Dietzia*, *D. maris*, to merit description as a new species.

Table 4. Substrate utilization under neutral conditions

	Dietzia maris	Strain 15 LN1
(a) Substrate (minimal agar)		
acetate	+	+
D-fructose	+	+
fumarate	−	+
D-galactose	−	−
D-glucose	−	+
glutamate	+	+
lactose	−	−
succinate	−	+
sucrose	−	−
(b) Substrate (ATB 32N)		
mannitol	−	+
D-glucose	−	+
salicin	−	−
D-melibiose	−	−
L-fucose	−	−
D-sorbitol	−	−
L-arabinose	−	−
propionate	−	+
caprate	−	−
valerate	+	+
citrate	−	+
histidine	−	−
2-ketogluconate	−	−
3-hydroxybutyrate	+	+
4-hydroxybenzoate	−	−
L-proline	−	+
rhamnose	+	−
N-Acetylglucosamine	−	−
D-ribose	−	−
inositol	−	−
sucrose	−	−
maltose	−	−
itaconate	−	−
suberate	−	+
malonate	−	−
acetate	+	+
DL-lactate	−	−
L-alanine	−	−
5-ketogluconate	−	−
glycogen	−	+
3-hydroxybenzoate	−	−
L-serine	+	+

Description of *Dietzia natronolimnaios* sp. nov.

Dietzia natronolimnaios Duckworth, Grant, Grant, Jones, and Meijer (na. tro. no. lim.na.ios. Gr. n. *natrum* soda, salt; Gr. adj. *limnaios* of a lake; Gr. gen. *natronolimnaios* of the soda lake). Gram-positive nonmotile nonspore-forming rods, 0.9–1.1 μm by 1.2–2.3 μm. Catalase positive, oxidase negative. Short, rod-shaped cells exhibit snapping division and produce V forms. Cells in liquid culture form microcolonies of up to 50–60 cells. Growth occurs on Brain Heart Infusion Agar and alkaline agar media. Circular, convex, glistening colonies with entire margins are formed on agar. Colonies initially orange in color, becoming coral red with age. Alkaliphilic; growth occurs at between pH 6 and 10 with an optimum around pH 9. The diagnostic amino acid of the peptidoglycan is meso-diaminopimelic acid. Grows well at between 20°C and 40°C with a temperature optimum around 30°C. Grows slowly at 10°C and 45°C; no growth at 50°C. Short-chain mycolic acids are present (34–38 carbon atoms). The long-chain fatty acids are predominantly saturated and monounsaturated fatty acids (C16:0, C18:0, C18:1, C20:0).

Tuberculostearic acid (10-methyloctadecanoic acid) is present. The major polar lipids are phosphatidylethanola-

Table 5. Chemotaxonomic characteristics of *Dietzia maris* an strain 15LN1

	Colony color pigmentation	Morphology	Fatty acids[a]						Mycolic acids chain length	Cell wall chemotype	Quinone	Polar lipids
			br	16:0	16:1	18:0	18:1	18:Me				
D. maris	Orange	R-V	−	++	+	+	++	++	L34–38	Meso DAP	$MK8(H_2)$	PE, PG, DPG
15LN1	Coral red[b]	R-V	−	++	+	+	++	++	L34–38	Meso DAP	$MK8(H_2)$	PE, PG, DPG

R-V, Short rods that show snapping division and V forms; Meso DAP, meso diaminopimelic acid; $MK8(H_2)$, menaquinone with 8 isoprene units; PG, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol.

[a] Additional data provided by Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany): fatty acids 1%–5% (+); 15%–30% (++); br, branched fatty acids; 18:Me, tuberculostearic acid.

[b] Particularly pronounced on fructose, acetate, glutamate.

mine, phosphatidylglycerol, and diphosphatidylglycerol. Menaquinone MK8(H_2) is the major isoprenoid.

Under neutral conditions, acetate, fructose, fumarate, glutamate, succinate, mannitol, D-glucose, propionate, suberate, valerate, 3-hydroxybutyrate, citrate, glycogen, L-serine, L-proline, L-asparagine, L-arginine, methionine, phenylalanine, L-glycine, and L-valine are utilized for growth, but not D-galactose, lactose, D-sucrose, rhamnose, *N*-acetylglucosamine, D-ribose, inositol, itaconate, malonate, DL-lactate, salicin, D-melibiose, L-fucose, D-sorbitol, L-arabinose, caprate, 5-ketogluconate, 2-ketogluconate, 4-hydroxybenzoate, 3-hydroxybenzoate, L-alanine, L-histidine, or L-lysine.

Isolated from littoral sediments of Lake Oloidien (Little Lake Naivasha), Kenya. The guanine plus cytosine content of the DNA is 66.1% ± 0.4 mol% (as determined by the thermal denaturation method). The type strain is 15LN1, deposited as CBS 107.95 at the Central Bureaux Schimmel Cultures, Baarn, The Netherlands.

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