Extremophiles (2004) 8:193–200
DOI 10.1007/s00792-004-0377-4

ORIGINAL PAPER

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Alkalimonas amylolytica gen. nov., sp. nov., and *Alkalimonas delamerensis* gen. nov., sp. nov., novel alkaliphilic bacteria from soda lakes in China and East Africa

Received: 8 August 2003 / Accepted: 25 December 2003 / Published online: 21 February 2004

Abstract Two related novel alkaliphilic and slightly halophilic bacteria are described. They are strain N10 from Lake Chahannor in China and strain 1E1 from Lake Elmenteita in East Africa. Both strains are strictly aerobic, heterotrophic, alkaliphilic, mesophilic, and require NaCl for growth. The optimal conditions for growth were at pH 10–10.5 and 2–3% (w/v) NaCl. Cells of both strains were Gram-negative, rod-shaped, non-spore-forming, and motile with a single polar flagellum. Cellular fatty acids in both strains were predominantly saturated and mono-unsaturated straight-chain fatty acids (16:0, 16:1ω7c and 18:1ω7c). The major isoprenoid quinone of both strains was Q8. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglycerol phosphate and phosphatidylethanolamine. The guanine plus cytosine (G + C) content of the DNA was 52.5 mol% and 55.4 mol%, respectively. Phylogenetic analysis revealed that the two strains formed a distinct lineage within the gamma-3 subclass of the *Proteobacteria*. The strains shared a 16S rDNA sequence similarity of 96.1% and showed less than 93.7% of sequence similarity to any other known species. Based on polyphasic data, the two strains were differentiated from currently recognized genera and represent a new genus, *Alkalimonas* gen. nov., with two species, *Alkalimonas amylolytica* sp. nov. (type strain is N10^T = AS 1.3430) and *Alkalimonas delamerensis* sp. nov. (type strain is 1E1$^{P,\ T}$ = CBS 391.94). The GenBank accession numbers for the 16S rRNA gene sequence of strains N10 and 1E1 are AF250323 and X92130, respectively.

Keywords 16S rDNA · *Alkalimonas* gen. nov. · Alkaliphile · Chemotaxonomy · Moderate halophile · Phylogeny · Soda lake

Communicated by K. Horikoshi

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Introduction

Alkaliphiles are defined as organisms that grow optimally at alkaline pH, with pH optima for growth being in excess of pH 8 (usually between 9 and 10), with some being capable of cultivation at pH values >11 (Grant and Jones 2000; Horikoshi 1999). Although they were once considered to be curiosities, awareness of alkaliphiles has blossomed in recent years due to an interest in their physiological adaptations to high pH (Krulwich and Guffanti 1989) and their potential uses in biotechnological applications (Horikoshi 1996, 1999; Grant et al. 1990). This has led to more systematic studies and a rapid expansion in the numbers and types of alkaliphiles isolated from a variety of environments (Zhang et al. 2001; Sorokin et al. 2001; Jones et al. 1994, 1998; Duckworth et al. 1996). While alkaliphiles can be isolated from many different environments, even from those not considered to be particularly alkaline, it is now very clear that soda lakes appear to be the most bountiful environment when it comes to isolating new varieties. Soda lakes are naturally occurring, stable high-alkaline environments often with pH values between 9 and 10, and sometimes >11, that are characterized by large concentrations of sodium carbonate minerals and NaCl. They form in arid geographical zones in closed

drainage basins under conditions of low Ca^{2+} and Mg^{2+} content and where high rates of evaporative concentration exceed the rates of water inflow, allowing Na^+, Cl^- and CO_3^{2-} ions to accumulate in molar concentrations (Grant and Jones 2000). Undoubtedly the best-studied soda lakes are those of the Kenyan–Tanzanian section of the Great Rift Valley in equatorial East Africa. Here, continental rifting and volcanic activity have created shallow depressions with a high marginal relief containing more or less permanent standing bodies of water within a geology depleted in Ca^{2+} and Mg^{2+}. Although more numerous and often ephemeral, the soda lakes of Inner Mongolia are, by contrast, less well documented. Situated in a cryoarid zone of the Gobi Desert in the continental interior of Asia, this area experiences little rainfall and has less vegetation cover than in the East African Rift Valley. The chemical composition of the waters is broadly similar in the soda lakes of both regions, with Na^+, Cl^- and CO_3^{2-} forming the major ions in solution, although absolute amounts vary considerably between the different lakes. However, local geological conditions provide for a unique total ion composition in individual lakes.

Dense populations of aerobic and anaerobic organotrophic prokaryotes have been demonstrated to inhabit soda lakes (Jones et al. 1998; Zhilina and Zavarzin 1994). Phylogenetic surveys of these prokaryotes indicate that soda lakes harbor considerable taxonomically diverse bacterial populations (Rees et al. 2003; Duckworth et al. 1996; Jones et al. 1994). Most of the isolates have an obligate requirement for high pH and all are alkaliphiles. Some of the organisms seem to be unique to these lakes and some appear to represent distinct phylogenetic lineages (Rees et al. 2003; Zhang et al. 2001; Duckworth et al. 1996). In this paper we describe two new alkaliphilic, aerobic, Gram-negative bacteria, designated strains N10 and 1E1, which could not be assigned to any existing genus. Strain 1E1 was originally isolated in 1987 from the East African Rift Valley lake, Lake Elmenteita. Subsequent 16S rDNA sequencing revealed that this isolate belonged to the gamma-3 subdivision of the *Proteobacteria* but without any close affinity to a known taxon (Duckworth et al. 1996). More recently, strain N10 was isolated from Lake Chahannor in Inner Mongolia; its 16S rDNA sequence indicated that strain 1E1 is its only close relative. Based on the data for the 16S rDNA sequences, as well as phenotypic and chemotaxonomic properties of these strains, we propose the new genus *Alkalimonas* within the gamma subdivision of the *Proteobacteria* to accommodate two new species, *Alkalimonas amylolytica* sp. nov., and *Alkalimonas delamerensis* sp. nov.

Materials and methods

Bacterial strains and culture conditions

Strain N10 was isolated from Lake Chahannor (39°14′ N and 108°04′ E), located in Inner Mongolia Autonomous Region, China. Strain 1E1 was isolated from Lake Elmenteita (0°25′ S and 36°15′ E), located in Kenya, East Africa, as described previously (Duckworth et al. 1996). Both lakes have a pH value of 9.5. The chemical composition of the lakes is given in Table 1. Isolation and cultivation were performed according to Horikoshi (1971). The strains were incubated aerobically at 37°C in alkaline medium containing (g l^{-1}): glucose 10, peptone 5, yeast extract 5, K_2HPO_4 1, $MgSO_4 \cdot 7H_2O$ 0.2, NaCl 40, and Na_2CO_3 10. Solid medium was supplemented with 20 g agar l^{-1}.

Morphological and phenotypic characteristics

The Gram-type was determined by staining using the Dussault (1955) modification and confirmed by the KOH lysis method (Gregersen 1978, Halebian et al. 1981) and aminopeptidase reaction (Cerny 1976, 1978). Cell morphology and motility were examined by phase-contrast microscopy and electron microscopy. For platinum shadowing, cells were applied to a carbon-coated grid, washed once with 4% (w/v) NaCl and shadowed with Pt-C at an angle of 18°. Photomicrographs were taken with a Hitachi transmission electron microscope.

The presence of oxidase, catalase, urease, and poly-β-hydroxybutyrate, the production of endospores, indole, and hydrogen sulfide, and nitrate reduction were determined as previously described (Duckworth et al. 1998; Smibert and Krieg 1994). Substrate utilization and antibiotic sensitivity tests were performed as previously described (Duckworth et al. 1998). Hydrolysis of polymers was performed as described by Smibert and Krieg (1994) using the alkaline medium without glucose as the basal medium. The temperature range for growth was tested in the alkaline medium and incubated at 4°, 10°, 20°, 25° 30°, 37°, 42°, and 50°C. The pH range for growth was determined in the medium, the pH being adjusted to 5.5, 6.0, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, and 12.0 with HCl or NaOH. Na^+ requirement and tolerance of various NaCl concentrations were determined in NaCl-free alkaline medium. The growth was monitored by turbidity at OD_{600}.

Lipid analysis

Polar lipid analysis was performed following the polar lipid extraction procedure of Ross et al. (1985) as described previously (Duckworth et al. 1998).

Isoprenoid quinones were extracted and purified from freeze-dried cells using the method of Collins (1985), determined by HPLC and as described previously (Duckworth et al. 1998).

Fatty acid analyses were performed as previously described (Duckworth et al. 1998) and also by the Deutsche Sammlung von Mikroorganismen und Zelkulturen (Braunschweig, Germany).

Table 1 Chemical composition (mM) of Lake Chahannor and Lake Elmenteita

Lake	Na^+	K^+	Ca^{2+}	Mg^{2+}	SiO_2	PO_4^{3-}	Cl^-	CO_3^{2-}	SO_4^{2-}	B_2O_3	Reference
Chahannor	5,826	48.7	0	2.9	NT	NT	2,535	950	573	18.6	Zheng et al. 1992
Elmenteita	195.7	3.6	0.07	< 0.004	2.9	0.03	65.1	68.0	2.0	NT	Jones et al. 1994

NT, not tested

Genotypic and phylogenetic analysis

Genomic DNA of the strains was prepared by the methods of Marmur (1961) or Pitcher et al. (1989), and the purity was checked spectrophotometrically. The G+C content of DNA was determined by the thermal denaturation method according to Marmur and Doty (1962) using *Escherichia coli* DNA (51 mol% G + C)) as standard. DNA–DNA reassociation values were determined by the spectrophotometric renaturation rate (Huss et al. 1983).

PCR and sequencing of 16S rDNA were carried out as described previously by Duckworth et al. (1996). The 16S rDNA sequence obtained was compared to sequences available in GenBank databases by using the Basic BLAST 2.0 option (Altschul et al. 1997). Close relatives were retrieved and manually aligned with each other using ClustalW version 1.8 (Thompson et al. 1994). Positions of sequence and alignment uncertainty were omitted from the analysis. The similarity values were calculated by pairwise comparisons of the sequences within the alignment. A phylogenetic tree was reconstructed by the neighbor-joining method with Kimura two-parameter calculation model in TreeconW v.1.2 (Van de Peer and De Wachter 1994). The accession numbers of the reference strains used in the sequence comparison are shown in the fIgures. The GenBank accession numbers for the 16S rRNA gene sequence of strains N10 and 1E1 are AF250323 and X92130, respectively.

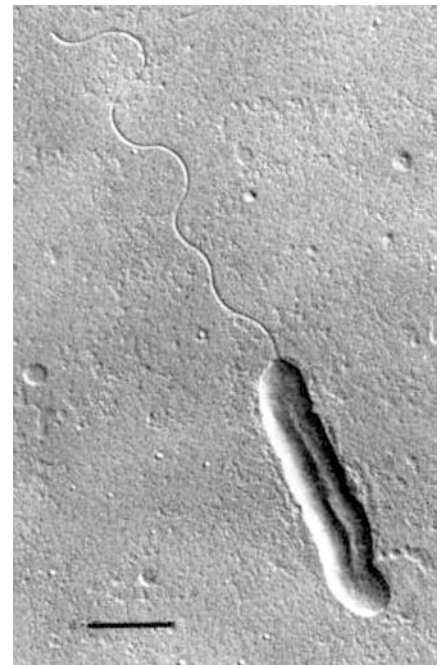

Fig. 1 Electron micrograph of strain N10. Platinum shadowed cell, showing single polar flagellum. *Bar* 1.0 μm

Results

Phenotypic characteristics

Both strains formed smooth, circular and convex colonies, with entire margins, 2–4 mm in diameter after 5 days incubation on alkaline-agar. The colonies of both strains were initially translucent, but strain 1E1 became opaque and strain N10 became pale brown after a few days. In alkaline-broth, growth is flocculent, with the formation of a sediment and surface pellicle. Cells of both strains were Gram-negative, strictly aerobic, motile, slightly curved to straight rod-shaped, 0.5–0.7 μm in width and 2.0–4.0 μm long, with a single polar flagellum (Fig. 1). Endospores and poly-β-hydroxybutyrate were not detected. Cells of both strains became leaky but did not lyse completely in distilled water and the cell shape appeared flatter. Leakage could be prevented by the addition of NaCl, but not by higher pH. Both strains were positive for catalase, oxidase, nitrate reduction, and formation of H_2S, whereas they were negative for indole production, Voges-Proskauer test, and urease.

The temperature range for growth was 10°–42°C with an optimum of around 37°C. No growth occurred at 4° or 50°C. Both strains required Na^+ for growth, and no growth occurred without added NaCl in the medium when Na_2CO_3 was substituted by K_2CO_3. Both strains grew in medium containing 0–7% (w/v) NaCl with no growth at 8% (w/v) NaCl. The pH range for growth of strain N10 was from 7.5 to 11.0, with an optimum of 10. The pH range for growth of strain 1E1 was from 8.0–11.0, with an optimum of 10–10.5. Both strains are obligate alkaliphilic and slightly halophilic.

Both strains are chemoorganotrophs and possess a strictly respiratory type of metabolism. Both strains grew on complex substrates such as yeast extract and peptone. The following compounds could be used by both strains: glucose, mannose, maltose, cellobiose, trehalose, pyruvate, serine, proline, asparagine, arginine, alanine, lysine, and glutamine. Only strain N10 could utilize rhamnose and sucrose; however, only strain 1E1 used fructose and valine. Both strains were unable to utilize lactose, galactose, ribose, xylose, arabinose, sorbose, raffinose, sorbitol, mannitol, inositol, salicin, inulin, succinate, fumarate, acetate, lactate, citrate, propionate, methionine, phenylalanine, and glycine. Both strains hydrolyzed starch, casein, gelatin, and Tween 80. Pectin and cellulose were not hydrolyzed, and only strain N10 hydrolyzed esculin.

Strain N10 was susceptible to streptomycin, erythromycin, lincomycin, kanamycin, and chloramphenicol, but was resistant to penicillin, vancomycin, bacitracin, anisomycin, and lysozyme. Strain 1E1 was susceptible to streptomycin, erythromycin, penicillin, chloramphenicol, oleandomycin, and rifampicin, but was resistant to vancomycin, bacitracin, and kanamycin.

Chemotaxonomic and genotypic characteristics

The fatty acid profiles of strain N10 and 1E1 are shown in Table 2. Both strains possess very similar whole-cell fatty acid profiles. Independent analyses confirmed the composition of the major fatty acids, although the absolute values varied slightly. Unsaturated fatty acids accounted for 52%–59% of the total fatty acids with C18:1ω7c, and C16:1ω7c predominating. Saturated fatty acids accounted for 26%–32% of the total fatty acids, with C16:0 predominating. Both strains also

Table 2 Cellular fatty acid profiles of *Alkalimonas* species

Fatty acid	Fatty acid composition (%)	
	A. amylolytica N10	*A. delamerensis* 1E1
Unknown	0.34	0.49
C12:0	0.32	0.32
C11:0 3OH	0.27	ND
C13:0	0.28	ND
C12:0 3OH	1.55	3.01
C14:0	1.27	0.65
Sum 1	1.65	0.49
C15:0 a	0.31	ND
C15:1ω 8c	1.47	0.25
C15:1ω 6c	0.17	ND
C15:0	4.29	0.28
C14:0 i 3OH	0.45	ND
Sum 2	4.07	4.85
C16:0 I	1.84	0.39
Sum 3	14.31	8.21
C16:0	18.91	18.08
C15:0 2OH	0.29	ND
C17:1 Iω 9c	0.27	ND
C17:0 i	0.80	0.45
C17:0 a	1.75	1.26
C17:1ω 8c	6.94	1.98
C17:1ω 6c	0.89	0.33
C17:0	6.99	1.94
C18:0 i	0.69	0.56
C18:1ω 9c	0.28	0.47
C18:1ω 7c	27.87	47.72
C18:0	0.77	4.81
C18:1ω 7c 11m	0.51	0.91
C20:1ω 7c	0.30	1.92

Sum 1, C13:0 3OH /C15:1 i
Sum 2, C14:0 3OH /C16:1 i
Sum 3, C15:0 i 2OH/C16:1ω 7c
ND, not detected

contained small amounts of hydroxy- and iso-branched fatty acids. Ubiquinone Q8 was the major lipoquinone in strain N10 and strain 1E1. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglycerol phosphate, and phosphatidylethanolamine.

The G+C content of the DNA of strain N10 determined by the thermal denaturation temperature was 52.5 mol%. Strain 1E1 possessed a value of about 55.4 mol%. The strains shared 40% DNA–DNA hybridization.

Phylogenetic analysis

About 1,500 bp sequences of 16S rDNA from strain N10 and 1E1 were determined and aligned with close relatives, namely representatives of the gamma subdivision of the *Proteobacteria*. The phylogenetic comparison indicated that the two strains cluster within the gamma-3 subclass of the *Proteobacteria*, forming a robust monophyletic lineage with a 100% bootstrap value. For computing evolutionary distance, 1,376 unambiguous nucleotides were aligned with 18 of the most closely related sequences. The phylogenetic analysis showed that the two strains were more closely related to each other (96.1% nucleotide sequence similarity) than to any other bacteria whose sequences were available. The closest phylogenetic neighbors of all the validly described taxa were *Rheinheimera baltica* (92.5% and 90.3% sequence similarity for strains N10 and 1E1, respectively), and *Alishewanella fetalis* (92.0% and 90.0% sequence similarity for strains N10 and 1E1, respectively). The next closest phylogenetic neighbors of all the validly described taxa were *Erwinia quercina* (90.8% and 88.1% sequence similarities for strains N10 and 1E1, respectively) and *Vibrio fluvialis* (90.0% and 87.1% sequence similarities for strains N10 and 1E1, respectively). Much lower sequence similarity values (<87%) were found to other members of the gamma-3 subclass of *Proteobacteria*. This relationship is also shown in the phylogenetic tree (Fig. 2), whereby the two strains formed a stable monophyletic unit with 100% bootstrap value, together with the genera *Rheinheimera* and *Alishewanella*.

Discussion

Two new alkaliphilic and slightly halophilic bacteria, designated strains N10 and 1E1, have been isolated from soda lakes in China and East Africa, respectively. The conditions necessary for the formation of a soda lake are fairly similar to those for the generation of a salt lake, with the major exception being that evaporative concentration leads to carbonate (or carbonate complexes) becoming the major anion in solution. Sodium and chloride ions, being ubiquitous in the environment, also co-concentrate, producing a highly alkaline brine. However, these lakes often have a unique chemical signature depending upon local geological conditions. This can give rise to circumstances where some ions such as K^+, Li^+, SO_4^{2-}, F^-, Br^-, PO_4^{3-} and borate complexes, which are normally only present in trace amounts, achieve significant proportions, and this may be reflected in the microbiology of individual lakes. In general, soda lakes harbor a rich diversity of prokaryotes (Rees et al. 2003; Zhang et al. 2001; Duckworth et al. 1996) and many isolates have been the subject of taxonomic and phylogenetic analysis. Not surprisingly, most isolates are alkaliphilic and some at least are obligate alkaliphiles, but phylogenetic analysis has often been unable to place them within existing taxa (Duckworth et al. 1996).

Phylogenetic analysis based on 16S rDNA sequence similarity unambiguously places these two new strains within the gamma-3 subdivision of the *Proteobacteria*. This confirms earlier data (Duckworth et al. 1996) that suggested that strain 1E1 from East Africa was somewhat loosely related to the *Enterobacteria–Aeromonas–Vibrio* part of the gamma-3 subdivision, but without any clear affinity to any of these genera. The new isolate N10 from Inner Mongolia is clearly most closely related to the East African strain (96.1% sequence similarity), forming a monophyletic lineage with 100% bootstrap

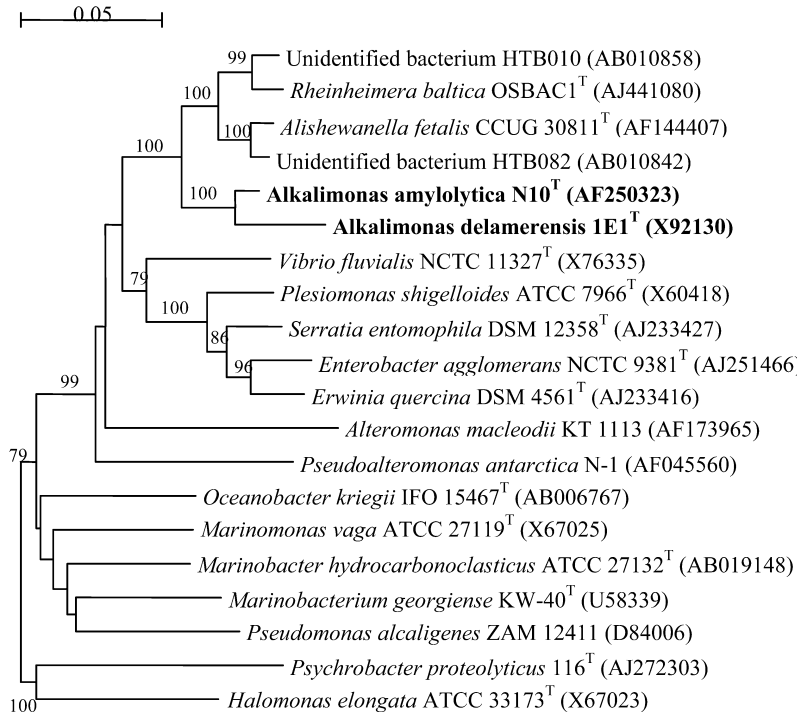

Fig. 2 Phylogenetic position of strains N10 and 1E1 within the gamma-*Proteobacteria*. Evolutionary distances were calculated by the neighbor-joining method and Kimura's two-parameter calculation model. Numbers at the nodes represent the confidence level from 100 replicate bootstrap samplings. *Bar* 0.05 substitutions per nucleotide

value (Fig. 2) separate from any members of the family *Alteromonadaceae* or the *Oceanospirillum* group.

In terms of phenotypic characteristics, the new strains exhibited a superficial resemblance to representatives of the genera *Pseudoalteromonas* (Gauthier et al. 1995), *Marinobacter* (Gauthier et al. 1992), and *Marinobacterium* (González et al. 1997) of the family *Alteromonadaceae*, and the genus *Marinomonas* (Gauthier and Breittmayer 1992) of the *Oceanospirillum* group (Table 3). The new strains showed the general taxonomic features typical of these genera, being Gram-negative, strictly aerobic, chemoorganotrophic with a non-fermentative metabolism, non-spore forming, and rod-shaped cells, motile with a single polar flagellum.

Table 3 Phenotypic characteristics differentiating *Alkalimonas* spp. from related genera. All strains are Gram-negative, chemoorganotrophic with a non-fermentative metabolism, do not form endospores, are rod-shaped, do not accumulate poly-β -hydroxybutyrate as an intracellular reserve product, oxidase-positive, mesophilic (except *Rheinheimera*), and require NaCl for growth. Data from previous studies (Holt et al. 1994; Brettar et al. 2002; Fonnesbech Vogel et al. 2000; Gauthier and Breittmayer 1992; Gauthier et al. 1992, 1995; González et al. 1997; Akagawa-Matsushita et al. 1992)

Character	1	2	3	4	5	6	7	8
Strictly aerobic	+	+	–	+	+	+	+	+
Motile by a polar flagellum	+	+	–	+	+	+	+	+
Na^+ required for growth	+	+	+	–	+	+	+	+
Growth in 20% (w/v) NaCl	–	–	+	–	–	–	+	+
Growth at 4°C	–	–	–	+	–	d	–	+
Growth at 37°C	+	+	+	–	+	+	+	+
Optimal temperature for growth (°C)	37	37	37	20–25	20–25	25–30	32	37
Optimal pH for growth	10	10–10.5		6.0			7–7.5	7.5
Utilization of glucose	+	+	–	+	+	+	–	+
Acid from glucose	–	–	–	–	+	+	–	–
Gelatin hydrolysis	+	+	+	+	–	+	–	–
Starch hydrolysis	+	+	ND	+	–	d	–	–
Nitrate reduction	+	+	+	–	–	–	+	–
Quinone	Q8	Q8	ND	ND	Q8	Q8	ND	ND
Major fatty acids	18:1ω 7c 16:0 16:1ω 7c	18:1ω 7c 16:0 16:1ω 7c	16:1ω 7c 17:0 17:1ω 8c	16:1ω 7c 16:0 18:1ω 7c	ND	16:0 16:1 17:1	16:0 16:1ω 9c 18:1ω 9c	16:0
G+C mol %	52.5	55.4	50.6	47–49	44–48	37–50	52.7	54.9

1 *A.amylolytica* N10, 2 *A. delamerensis* 1E1, 3 *Alishewanella*, 4 *Rheinheimera*, 5*Marinomonas*, 6 *Pseudoalteromonas*, 7 *Marinobacter*, 8 *Marinobacterium*.

+ positive, – negative, d differs among species, ND not determined

The new strains do not accumulate poly-β-hydroxybutyrate as intracellular reserve product, and are oxidase-positive, mesophilic, require NaCl for growth, and have ubiquinone Q8 as the major lipoquinone. However, it is clear that the substrate utilization patterns and chemotaxonomic data, in particular the fatty acid profiles, of the new strains do not closely resemble those of established taxa (Table 3). Moreover, the new strains are obligate alkaliphiles and the high pH requirement for growth also distinguishes them from the genera mentioned above. It is concluded that the soda lake isolates can be differentiated from other aerobic and halophilic bacteria on the basis of phenotypic and chemotaxonomic analyses that we have carried out and that the new isolates should therefore be assigned to novel taxa.

This conclusion is supported by the phylogenetic analysis. The 16S rRNA of the new strains exhibited less than 87% sequence similarity with the 16S rRNA of all described members of the family *Alterononadaceae* and *Oceanospirillum* group, confirming that the new strains have no close affinity with the described members of the family *Alterononadaceae* and *Oceanospirillum* group. The new strains are phylogenetically most closely related to the genera *Rheinheimera* (Brettar et al. 2002) and *Alishewanella* (Fonnesbech Vogel et al. 2000). However, it is clear that the soda lake strains form an independent unit that can be recognized as a novel taxon within the gamma-3 subclass of the *Proteobacteria* (Fig. 2). The 100% bootstrap replication and the low values of the 16S rDNA sequence similarity to the closest relatives (90.0%–92.5%) reflect the fact that the new strains are not closely related to members of *Rheinheimera* and *Alishewanella*. Furthermore, differences in physiological and chemotaxonomic traits can also exclude the new strains from these closest phylogenetic relatives (Table 3). The new strains require Na^+ for growth and have different temperature, pH requirement, and salt tolerance for growth, which probably reflects the particular conditions of the soda lake environment that they inhabit. The new strains are also able to reduce nitrate to nitrite. A combination of the phenotypic traits can distinguish the new stains from the genus *Rheinheimera*. Likewise, the characteristics that distinguish the new strains from the genus *Alishewanella* were motility, aerobic cells, and high pH requirement for growth, different fatty acid profiles and utilization of a different spectrum of substrates.

The polyphasic analysis described above clearly demonstrates that both strains N10 and 1E1 are novel members of the gamma-3 subclass of the *Proteobacteria*. The 16S rDNA dissimilarity between strain N10 and strain 1E1, the low level of DNA–DNA relatedness, and phenotypic differences between the two strains indicate two different species. The new strains can be readily distinguished from close phylogenetic relatives by being alkaliphilic and halophilic, probably reflecting the fact that the new strains are well suited for survival in soda lake conditions. Therefore, we proposed a new genus, *Alkalimonas* gen. nov., containing two new species: *Alkalimonas amylolytica* gen. nov., sp. nov., and *Alkalimonas delamerensis* gen. nov., sp. nov. Strain N10 has been deposited in the Chinese Collection of Microorganisms as strain AS 1.3430. Strain 1E1 has also been deposited at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands as CBS 391.94 under the Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedures. The GenBank accession numbers for the 16S rRNA gene sequence of strains N10 and 1E1 are AF250323 and X92130, respectively.

Description of *Alkalimonas* gen. nov.

Alkalimonas. Al.ka.li.mo′ nas. N.L. n. alkali (from Arabic *al-qaliy* ashes of salt wort), Gr. fem. n. monas a unit, monad, N.L. fem. n. *Alkalimonas*, alkaline monad.

Gram-negative, strictly aerobic, chemoorganotrophic rods. Motile with a single polar flagellum. No endospores formed. Mesophilic, alkaliphilic and slightly halophilic. Na^+ is required for growth. Growth occurs at salinity of 0%–7% (w/v) NaCl and temperatures of 10°–42°C, with an optimum of around 37°C. Does not accumulate poly-β-hydroxybutyrate as an intracellular reserve product. Oxidase, catalase and H_2S production are positive. Urease, indole, and Voges-Proskauer tests are negative. Nitrate is reduced to nitrite. Strictly respiratory type of metabolism. Wide range of substrates is utilized. Hydrolyses casein, gelatin, starch, and Tween 80. Does not hydrolyze pectin or cellulose. Utilizes glucose, mannose, maltose, trehalose, cellobiose, pyruvate, serine, proline, asparagine, arginine, alanine, lysine, and glutamine. Acid is not produced from substrates. Lactose, galactose, ribose, xylose, arabinose, sorbose, raffinose, sorbitol, mannitol, inositol, salicin, inulin, succinate, fumarate, acetate, lactate, citrate, propionate, methionine, phenylalanine, and glycine are not utilized. Sensitive to streptomycin, erythromycin, and chloramphenicol, but insensitive to vancomycin and bacitracin. Major quinone is Q8. The predominant cellular fatty acids are 16:0, 16:1ω7c, and 18:1ω7c. Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglycerol phosphate, and phosphatidylethanolamine. DNA G+C content is 52−56 mol%. Habitat: soda lake. The type species is *Alkalimonas amylolytica*.

Description of *Alkalimonas amylolytica* sp. nov.

Alkalimonas amylolytica (a.my.lo.ly′ ti.ca. Gr. n. amylon starch, Gr. adj. lytikos dissolving, N.L. fem. adj. *amylolytica* starch dissolving).

Same as genus description plus the following. Cells are rods (2.0–4.0×0.5–0.7 μm). NaCl optimum is 2%–3% (w/v). The pH range for growth is 7.5–11.0 with an optimum of 10. Esculin hydrolyzed. Utilizes sucrose and rhamnose but not fructose and valine. Sensitive to lincomycin and kanamycin but insensitive to penicillin, anisomycin, and lysozyme. The G+C content of the

DNA is 52.5 mol% (determined by thermal denaturation). Isolated from Lake Chahannor, a soda lake in Inner Mongolia, China. The type strain is strain $N10^T$, which has been deposited as AS 1.3430 in the China General Microbiological Culture Collection Center.

Description of *Alkalimonas delamerensis* sp. nov.

Alkalimonas delamerensis (de.la.me.ren′ sis. N.L. fem./masc. adj. *delamerensis* pertaining to the Delamere estates, which include Lake Elmenteita, Kenya).

Same as genus description plus the following. Cells are rods (1.7–3.3×0.5–0.7 μm). NaCl optimum is 3% (w/v). The pH range for growth is 8.0–11.0 with an optimum of 10–10.5. Does not hydrolyze esculin. Utilizes fructose and valine but not rhamnose and sucrose. Sensitive to penicillin, oleandomycin, and rifampicin but insensitive to kanamycin. The G+C content of the DNA is 55.4 mol% (determined by thermal denaturation). Isolated from Lake Elmenteita, a soda lake in Kenya. The type strain is strain $1E1^{P, T}$, which has been deposited as CBS 391.94 at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Acknowledgments Part of this study was supported by grants from the Knowledge Innovation Program of the Chinese Academy of Sciences, Chinese National Programs for High Technology Research and Development (863 programs), the European Commission (“Biotechnology of Extremophiles”, contract no. BIO2-CT93-0274 and “Multigenome Access Technology for Industrial Catalysts”, contract no. QLK3-CT-2002-01972). We are grateful to Professor Hans Trüper for his assistance with the correct names and etymologies of the strains.

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