During a study of microbial communities in athalassic bodies of water, three new species within the genus *Spirochaeta* were described. These are alkalophilic *Spirochaeta alkalica* sp. nov. *Z-7491* (DSM 8900) and haloophilic *S. africana* sp. nov. *Z-7692* (DSM 8902) from the soda-depositing Lake Magadi in Central Africa and haloalkaliphilic *S. asiatica* sp. nov. *Z-7591* (DSM 8901) from Lake Khutyn, Central Asia. These mesophilic spirochetes develop at pHs of >9 as anaerobic saccharolytic dissipotrophs. The DNA base compositions (moles percent G+C) of the strains were as follows: *S. alkalica* Z-7491, 57.1; *S. africana* Z-7692, 56.1; and *S. asiatica* Z-7591, 49.2. The optimum growth parameters (temperature, pH, and NaCl concentration [percent, wt/vol], respectively) were as follows: for *S. alkalica* Z-7491, 35°C, 9.2, and 5 to 7%; for *S. africana* Z-7692, 35°C, 9.3, and 5 to 7%; and for *S. asiatica* Z-7591, 35°C, 8.9, and 3 to 6%. The products of glucose fermentation were acetate, hydrogen, ethanol, and lactate, in different proportions, for *S. alkalica* and *S. africana*; for *S. asiatica*, they were acetate, ethanol, and lactate. The spirochetes are strictly anaerobic, while *S. alkalica* and *S. africana* are rather aero-tolerant. All three species group within the radiation of the majority of the species of the genus *Spirochaeta*. The studies of the genes encoding 16S rRNA indicate a possible fanning out of the phylogenetic tree of spirochetes.

The genus *Spirochaeta* represents a group of dissipotrophic (i.e., utilizing low-molecular-weight compounds dissipating from the sites of their origin [29]) saccharolytic bacteria associated with decomposition of (poly) carbohydrates in aquatic habitats. The spirochetes form a fairly coherent group (3) belonging to a separate phylogenetic lineage which correlates with their distinctive morphology (18, 21). Members of the genus *Spirochaeta* exist in diverse environments; however, they still exhibit marked similarities in growth substrate preferences. All species of *Spirochaeta* utilize carbohydrates and exhibit restricted hydrolytic activity. They utilize soluble hexoses, disaccharides, and pentoses released by polysaccharide-degrading microorganisms (13). Recently, quite a number of these organisms were isolated from various habitats. Species of *Spirochaeta* are quite common in anaerobic methanogenic communities in sewage sludge treatment systems. They are easily enriched from cultures of decomposing cellulose material (3). Being common in mesophilic environments, *Spirochaeta* spp. have also recently been isolated from moderately extreme habitats. The first report of the isolation of spirochetes from an extreme environment was that of *Pate1* in Utah was the site of enrichment of methanogenic communities in sewage sludge treatment systems. They are easily enriched from cultures of decomposing cellulose material (3). Being common in mesophilic environments, *Spirochaeta* spp. have also recently been isolated from moderately extreme habitats. The first report of the isolation of spirochetes from an extreme environment was that of *Pate1* in Utah was the site of enrichment of methanogenic communities in sewage sludge treatment systems. They are easily enriched from cultures of decomposing cellulose material (3). 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glucose, as no decomposition in the alkaline medium was observed after autoclaving at 110°C for 30 min. Cultures were incubated at 37°C in an N₂ atmosphere.

Routine transfers were performed with Bellco glass tubes and rubber-stoppered screw-cap flasks by a standard strictly anaerobic technique. Growth was recorded by optical density measurements at 600 nm in Bellco glass tubes using a photometer (Spekol, Jena, Germany) with the tube adapter ER.

Analytical procedures. Volatile products of glucose fermentation were quantified by using a Chrom-5 gas chromatograph equipped with a flame ionization detector, using argon as a carrier gas and a Chromosorb 101 column (0.9 m by 3 mm) at 160°C. Lactate was identified by using a lactate dehydrogenase test kit (Boehringer Mannheim). Gaseous products were determined in an LHM-80 gas chromatograph with a catarometer using argon as a carrier gas and a molecular-sieve 5A column (0.75 m by 3 mm) at ambient temperature. Glucose was quantified by phenol-H₂SO₄ reaction (11).

**Microscopy.** The morphology of the living cultures was observed with an anaptral Reichardt Zetopan microscope.

**Electron microscopy.** Negative staining by phosphotungstate was performed after fixation with glutaraldehyde added to the culture at a final concentration of 2.5% (vol/vol). Cells were centrifuged, resuspended in tap water, and stained with 1% (wt/vol) phosphotungstate (pH 7). For ultrathin sectioning, cells were prefixed with glutaraldehyde in culture medium for 30 min at ambient temperature. The cells were centrifuged, washed once with 0.15 M K-phosphate buffer (pH 7.2) with 3% (wt/vol) NaCl, and centrifuged, and the pellet was fixed with 1% (vol/vol) OsO₄ in acetate-Veronal buffer (pH 7.2) with 3% (wt/vol) NaCl for 18 h at 4°C. Dehydration and embedding in Epon 812 were done by standard methods. Microscopy was done with a JEM-100C microscope.

**Genome characterization.** DNA was isolated and purified from lysosome- and sodium dodecyl sulfate-treated cells by the method of Marmur (16). The G+C content was determined by a thermal denaturation method (17). Escherichia coli K-12 DNA was used as a standard. DNA-DNA hybridization was carried out by optical reassociation as described by De Ley et al. (4). The genome size was determined according to an equation in reference 9.

**16S rRNA sequencing.** Genomic DNAs were extracted from the three strains and the genes coding for 16S rRNA (16s rDNAs) were amplified as described previously (21). PCR products were sequenced directly by using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. The purified sequence reaction products were electrophero-

**RESULTS**

**Enrichment and isolation.** Positive enrichments of spirochetes from the lake sediments were obtained in the alkaline medium with starch as the selective substrate. Glucose or other monosaccharides were nonselective, and rod-shaped organisms proliferate when these substrates are used. Spirochetes were enriched by filtration of the enrichment cultures through 0.45-pm-pore-size Millipore filters and subsequent serial dilutions in glucose-containing medium. Dilutions were repeated in appropriate stages of growth until cultures of uniform morphology were obtained. Softening of agar at high pHs limited the usefulness of solidified media in culture purification. Purity of the cultures was checked by microscopic examination of the growth in nonselective glucose- and peptone-containing medium. Purity was checked further during physiological studies of growth on a variety of substrates as well as during molecular studies. A single strain was chosen from each site for further studies: strain Z-7692 from a lagoon in Lake Magadi, strain Z-7491 from a warm spring in Lake Magadi, and strain Z-7591 from Lake Natron.

**Morphology.** All three strains are highly motile, with wavy movement and/or rotation. They have typical spirochetal structure (Fig. 1) and periplasmic flagella attached to the ends of a screw-shaped protoplasmic cylinder (Fig. 2). Strain Z-7692 has thin cells 0.25 to 0.3 µm in diameter in the exponential phase and mainly 15 to 30 (occasionally 7 to 40) µm in length. Strain Z-7491 has cells mostly 0.4 to 0.5 µm in diameter and 9 to 18 µm in length, with a range of 6 to 35 µm. Cells of strain Z-7591 are thin, delicate, and transparent, 0.2 to 0.25 µm in diameter, and mostly 15 to 20 µm in length; however, short cells (7.5 µm) were observed. In the late exponential phase, spherical bodies which dominate in the stationary phase or during dilution were observed (Fig. 1A). The type of spherical-body formation dif-
FIG. 2. Negatively stained cells of alkaliphilic spirochetes. Bars, 0.5 μm. Typical structures with flagella in the periplasmic space are shown for *S. alkalica* Z-7491 (A), *S. africana* Z-7692 (B), and *S. asiatica* Z-7591 (C).

The isolated strains are strictly saccharolytic and do not utilize amino acids. Each strain utilized pentoses, hexoses, and disaccharides with marked preferences (Table 1). In addition to these conventional substrates, alkaliphilic spirochetes could grow on some polysaccharides. All of them utilized starch; strain Z-7491 is agarolytic, strain Z-7692 utilized glycogen, and strain Z-7591 could grow slowly on xylan and pectin in addition to starch and glycogen. Used substrates arranged in the order of preference are given in diagnoses for each strain.

The strains were found to differ in ion requirements. Only strain Z-7491 grows in medium when the Cl⁻ anion is omitted and the medium is supplemented by equimolar Na₂CO₃ + NaHCO₃ instead of NaCl. For strains Z-7692 and Z-7591, NaCl is an essential component. When Na₂CO₃ + NaHCO₃ is substituted by NaCl and the pH is supported by 50 mM serine buffer (pKₐ 9.2), only strain Z-7692 grows. On the basis of these characteristics, strain Z-7491 could be considered an alkaliphile, strain Z-7692 could be considered a halophile, and strain Z-7591 could be considered a haloalkaliphile. None of these strains had a requirement for the divalent cations Mg²⁺ and Ca²⁺.

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The three strains differ from one another in their metabolic products of glucose fermentation. *S. alkalica* Z-7491 forms acetate and hydrogen as the main products (1.5 and 1.7 mol/
mol of glucose, respectively), while lactate and ethanol are formed as minor components in the stationary phase (6.8 and 2 mM, respectively). *S. africana* Z-7692 excretes acetate and ethanol as the main products (1.1 and 0.7 mol/mol of glucose, respectively) and hydrogen and lactate as minor components (2.8 and 5 mM in the stationary phase, respectively). *S. asiatica* Z-7591 decomposes glucose without formation of minor components, while the main products are acetate, ethanol, and lactate (0.7, 0.75, and 0.4 mol/mol of glucose, respectively).

The relationship of these organisms to oxygen also differs between strains. Strain Z-7591 is an obligate anaerobe. Strains Z-7692 and Z-7491 from Lake Magadi survived two or three transfers in glucose-containing medium under microaerobic conditions (5 ml of medium in a tube under a cotton plug). Growth is weak, however; when the strain is inoculated back into anaerobic medium, normal growth is observed after a prolonged lag phase. Thus, the H₂-producing spirochetes from Lake Magadi are aerotolerant, while the spirochete from Lake Khatyn is quite sensitive to oxygen.

**Phylogenetic analysis.** DNA-DNA homology studies indicated that there are significant differences between the strains (Table 2). The results indicate that all three strains represent distinct species.

The phylogenetic dendrogram (Fig. 7) shows the relationship of the alkaliphilic spirochetes to other spirochetes for which sequence data are available. All three strains group within the radiation of the majority of the species of the genus *Spirillum*.
Three new species are proposed, with names derived from Spirochaeta. Medium combination with the DNA-DNA homology data, show them lakes are members of the genus to represent three new species within the genus rochete strains isolated from extremely alkaline continental liter, and the final pH was brought up to 9 with concentrated HCl. The reductant described marine differential traits for the alkaliphilic isolates and previously de-

![Image](image-url)

**FIG. 5.** Effect of pH on the specific growth rate of alkaliphilic spirochetes. Medium II without Na₂CO₃ was used, and the pHs shown were obtained by addition of 5 M NaOH in an anaerobic flask equipped with a pH electrode. Incubation was carried out at 35°C.

**Spirochaeta.** The highest levels of 16S rDNA similarity for the three strains are to *S. halophila* (89.2 to 94.5%) (Table 3). Two strains, Z-7591 and Z-7692, group together at the level of 96.4% 16S DNA sequence similarity, while the third strain, Z-7491, clusters with *S.* halophila, showing 94.5% sequence similarity.

**Taxonomic considerations.** A comparison of the main differential traits for the alkaliphilic isolates and previously described marine *Spirochaeta* species is shown in Table 4. Both phenotypic and phylogenetic data indicate that the three spirochete strains isolated from extremely alkaline continental lakes are members of the genus *Spirochaeta.* These data, in combination with the DNA-DNA homology data, show them to represent three new species within the genus *Spirochaeta.* Three new species are proposed, with names derived from their ecophysiology and their place of origin: *S.* alkalica sp. nov., *S.* africana sp. nov., and *S.* asiatica sp. nov.

![Image](image-url)

**FIG. 6.** Effect of NaCl on specific growth rate of alkaliphilic spirochetes. Medium II was used. Na₂CO₃-NaHCO₃ was substituted by 5 g of K₂CO₃ per liter, and the final pH was brought up to 9 with concentrated HCl. The reductant was 0.5 g of K₂S per liter. Incubation was carried out at 35°C.

**TABLE 1.** Substrate utilization by alkaliphilic spirochetes

<table>
<thead>
<tr>
<th>Substrate</th>
<th><em>S. alkalica</em> (Z-7491)</th>
<th><em>S. africana</em> (Z-7692)</th>
<th><em>S. asiatica</em> (Z-7591)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mannose</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ribose</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Glycojen</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pectin</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylan</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of agar</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* ++, final optical density at 600 nm (OD₆₀₀) of 0.6 to 0.4 at stationary phase; +, final OD₆₀₀ of 0.4 to 0.1; −, final OD₆₀₀ of 0.1 to 0.05. The following substrates were not utilized by any of the alkaliphilic strains: raffinose, lactose, melibiose, erythritol, sorbose, tagatose, sorbitol, rhhamnose, dulcitol, adonitol, inositol, acetate, pyruvate, Casamino Acids, lactate, glycerol, humic acids, Whatman paper no. 1, carboxymethyl cellulose, microcrystalline cellulose, and pepsitone.

**Description of *Spirochaeta alkalica* sp. nov.** *Spirochaeta al-
kalica* (al.ka.li.ca, L. fem. adj. alkalica, of alkali, developing in the alkaline medium). Motile, helical cells, 0.4 to 0.5 by 9 to 18 μm; shorter (6-μm) and longer (up to 35-μm) cells occur in culture. The outermost structure is an outer membrane enclosing the periplasmic flagella and the protoplasmic cylinder. The cells have regular, stable primary coils. Cell mass is orange.

Alkaliphilic; growth in sodium carbonate medium optimally at pH 8.7 to 9.6. No growth at pH 8.3 or 10.8. Dependent on sodium; no growth below 3% (w/vol) or above 10% (w/vol) NaCl. Growth is possible when NaCl is substituted by equimolar Na₂CO₃ + NaHCO₃. Requires carbonate anion. Optimum temperature for growth, 33 to 37°C; limits, 15 to 44°C; slow growth at 6°C after a long lag phase.

Anaerobic, aerotolerant, fermentative; utilizes carbohydrates, mainly mono- and disaccharides, as carbon and energy sources. Preferred substrates are as follows: sucrose > trehalose > cellobiose > glucose = maltose > xylose > starch; poor growth with fructose, galactose, ribose, or N-acetylglucosamine; no growth with mannose or glycogen.

Cellobiolytic and agarolytic. Amino acids do not serve as fermentable substrates. Aerotolerant; growth develops under a cotton plug in liquid medium.

The main products of glucose fermentation are acetate, H₂, 

![Image](image-url)

**TABLE 2.** Genome characteristics of alkaliphilic *Spirochaeta* spp.

<table>
<thead>
<tr>
<th>Strain</th>
<th>G+C mol%</th>
<th>Genome size (10⁶)</th>
<th>DNA-DNA homology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. alkalica</em> (Z-7491)</td>
<td>57.1</td>
<td>2.7</td>
<td>100</td>
</tr>
<tr>
<td><em>S. africana</em> (Z-7692)</td>
<td>56.1</td>
<td>2.5</td>
<td>22</td>
</tr>
<tr>
<td><em>S. asiatica</em> (Z-7591)</td>
<td>49.2</td>
<td>2.1</td>
<td>14</td>
</tr>
</tbody>
</table>

**TABLE 3.** Genome characteristics of alkaliphilic *Spirochaeta* spp.
Spirochaeta africana strain 2-7491

Spirochaeta alkalica strain 2-7491

Spirochaeta thermophila

Spirochaeta aurantia

strain DSM 8211

Spirochaeta stenostrepata

"Spirochaeta caldaria"

"Treponema phagedenis"

Borrelia burgdorferi

Brevinema anderssonii

Leptonema illini

Leptospira interogens

FIG. 7. Phylogenetic dendrogram indicating the positions of the three alkaliphilic Spirochaeta species within the radiation of the spirochetes. Scale bar, 10 substitutions per 100 nucleotides. The root was determined by the inclusion of E. coli as an out-group organism.

and CO₂. Minor products in stationary phase are ethanol and lactate.

A supplement of vitamins and yeast extract is required.

The guanine-plus-cytosine content of the DNA is 57.1 mol% (thermal denaturation method).

Habitat: isolated from the cyanobacterial mat in the warm spring from under the horst in the equatorial alkaline Lake Magadi.

Type strain: Z-7491. Deposited with the Deutsche Sammlung von Mikroorganismen, DSM 8900T.

Description of Spirochaeta africana sp. nov. Spirochaeta africana (af.ri.ca.na, L. fem. adj. africana, of African continent, found in African alkaline Lake Magadi). Motile, helical cells, 0.25 to 0.3 by 15 to 30 μm; shorter (7.5-μm) and longer (up to 40-μm) cells occur in culture. The outermost structure is an outer membrane enclosing the periplasmic flagella and the protoplasmic cylinder. The cells have regular, stable primary coils. Cell mass is orange.

Halophilic, growing in sodium carbonate medium but not requiring it. Depends on sodium; no growth below 3% (wt/vol) or above 10% (wt/vol) NaCl. Optimal growth at pH 8.8 to 9.75. No growth at pH 8.0 or 10.8. Optimum temperature for growth, 30 to 37°C; range, 15 to 47°C; slow growth at 6°C after

### TABLE 3. 16S rDNA similarities between alkaliphilic Spirochaeta species and related taxa

<table>
<thead>
<tr>
<th>Strain</th>
<th>S. africana</th>
<th>S. alkalica</th>
<th>S. thermophila</th>
<th>S. aurantia</th>
<th>S. stenostrepata</th>
<th>S. caldaria</th>
<th>T. phagedenis</th>
<th>B. burgdorferi</th>
<th>B. burgdorferi, strain DSM 8211</th>
<th>T. phagedenis, strain 2-7491</th>
<th>B. burgdorferi, strain DSM 8211</th>
<th>B. burgdorferi, strain 2-7491</th>
<th>S. illini</th>
<th>L. interogens</th>
<th>L. interogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. africana Z-7491</td>
<td>100</td>
<td>96.4</td>
<td>96.4</td>
<td>96.4</td>
<td>96.4</td>
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<tr>
<td>S. alkalica Z-7491</td>
<td>96.4</td>
<td>100</td>
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<tr>
<td>S. thermophila</td>
<td>96.4</td>
<td>96.4</td>
<td>100</td>
<td>96.4</td>
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% Similarity
a long lag phase. Aerotolerant; develops under a cotton plug in liquid medium.

Anaerobic, aerotolerant, fermentative; utilizes carbohydrates, mainly mono- and disaccharides, as carbon and energy sources. The order of preference is as follows: fructose > maltose = trehalose > saccharose > cellobiose > glucose > glycogen > starch; poor growth with mannose or xylose; no growth with galactose, N-acetylglucosamine, or ribose. Amino acids do not serve as fermentable substrates.

The main products of glucose fermentation are acetate, ethanol, and H₂. The minor product in stationary phase is lactate. A supplement of vitamins is required; however, yeast extract cannot be omitted for strain Z-7692.

The guanine-plus-cytosine content of the DNA is 56.1 mol%. Growth is Na dependent; no growth below 2% (wt/vol) NaCl or above 8% (wt/vol) NaCl; optimum, 3 to 6% (wt/vol) NaCl. Requires carbonate anion. Broad thermal adaptation with prolonged lag phase; optimum at 33 to 37°C, limits at 20 to 43°C.

Strictly anaerobic, fermentative, utilizes simple and complex carbohydrates. The order of preference is as follows: glucose > maltose > glycerogen > mannose > trehalose > cellobiose > saccharose > starch > galactose > pectin > xylene; poor growth with xylose or arabinose; no growth with fructose, ribose, lactose, agar, or N-acetylglucosamine. Amino acids are not fermented.

Fermentation products from glucose include acetate, ethanol, and lactate; H₂ not produced. A supplement of vitamins is required, and yeast extract enhances growth.

The guanine-plus-cytosine content of the DNA is 49.2 mol% (thermal denaturation method). Habitat: isolated from the mud of alkaline Lake Khatyn in Tuva, Central Asia. Type strain: Z-7591. Deposited with the Deutsche Sammlung von Mikroorganismen, DSM 8901T.

### DISCUSSION

Soda lakes are formed by evaporation of continental carbonate-containing waters produced by the leaching of rocks by CO₂-containing meteoric or thermal water. Depending on the rock composition, Na- or Ca-dominated waters are produced by the hydrolysis of silicates. Athalassic habitats essentially differ from thalassic ones and presumably might contain organisms from different origins.

Continental bodies of alkaline water harbor quite diverse microbial communities capable of completely decomposing organic matter. The usual prime producers in these habitats are cyanobacteria; however, green algae also proliferate in soda lakes. This differentiates continental bodies of water from marine habitats, in which cyanobacteria and green algae are in the minority. Green algae produce cellulose material. Decomposition of cellulose occurs in alkaline lakes, and extremely alkaliphilic anaerobic cellulose-decomposing bacteria have been reported (30).

**Spirochaeta** spp. are typical representatives of the functional group of dissipotrophs. It has for a long time...
been recognized that Spirochaeta strains are active in the fermentation of sugars formed by the enzymatic activity of polysaccharide-degrading microorganisms (13). Only rarely can spirochetes from the original bacterial bloom in the soda lakes be observed by direct microscopy; however, they are easily enriched by using starch-containing media. Trophically linked phylogenetically unrelated groups (27). The genus Spirochaeta, which represents a distinct phylogenetic line, provides a good example of this type of relationship, being a trophical intermediate between hydrolytic bacteria and secondary anaerobes. These organisms produce compounds which are normally consumed by sulfate-reducing bacteria. However, we have as yet been able to isolate only an H₂-consuming sulfate reducer and not lactate- or acetate-utilizing species from the sulfide sediments in Lake Magadi.

Thermophilic spirochetes differ from each other in their salt requirements: S. thermophila, from a marine origin, needs 0.5 to 4.5% NaCl (1, 2), while "S. caldaria," from the continental hot spring in Utah, does not tolerate 0.4% NaCl (20). Also, the continental strains are distinct from each other (Table 4). Alkaliphilic spirochetes from continental bodies of water belong to the same phylogenetic cluster as marine forms, and thus it is not possible to discriminate between thalassic and athalassic groups. The spirochetes of marine origin fall within one phylogenetic cluster, while S. stenostrepta, S. zuelzeriae, and "S. caldaria," spirochetes of freshwater origin, group with the Trepoden lineage. The freshwater spirochete S. aurantia is an exception to this observation in that it groups with the marine spirochetes, albeit deeply within the phylogenetic cluster. The previously described Spirochaeta species have relatively low levels of 16S rDNA sequence similarity to each other (81.6 to 93.1%) (Table 3). The high degree of similarity at the 16S rDNA sequence level (96.4%) between S. asiatica and S. africana, complemented by a low level of DNA-DNA homology (10%), indicates the possible fanning out of the phylogenetic tree as more branches are added when new Spirochaeta species are discovered and investigated.

The description of three new, extremely alkaliphilic spirochetes broadens understanding of both the diversity of the genus Spirochaeta and the role of this peculiar group in extreme environments.

ACKNOWLEDGMENTS

G. A. Zavarzin acknowledges full-hearted help from the members of the Russian Embassy to Kenya. The hospitality of the International Ussu-Nur Biospheric Center is appreciated.

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REFERENCES


