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ORIGINAL PAPER

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Anoxynatronum sibiricum gen.nov., sp.nov. alkaliphilic saccharolytic anaerobe from cellulolytic community of Nizhnee Beloe (Transbaikal region)

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Abstract New alkaliphilic anaerobic fermentative bacteria, strains Z-7981 and Z-7981′, with Gram-positive cell walls, were isolated from the cellulolytic community from the soda lake Nizhnee Beloye, south-east of Baikal. Cells were motile rods, which differed in dimensions but, according to 98% DNA/DNA homology, belonged to the same species. Strain Z-7981 was chosen as the type and studied in detail. It did not produce spores and its cells were non-thermoresistant. It was a true alkaliphile with a growth range from pH 7.1 to pH 10.1 and optimal pH for growth at pH 9.1. It was obligately dependent on Na^+ and carbonate ions but not on Cl^-. Growth occurred in media with total sodium content from 0.076 M to 1.27 M Na^+ with a broad optimum from 0.25 to 0.86 M Na^+. Growth showed an optimum at 35°C, with absence of growth above 46°C. The organism was aerotolerant and was capable of fermentation in non-reducing medium at less than 4.75% O_2 in the gas phase. Strain Z-7981 fermented mono- and disaccharides, sugar alcohols, but only glutamate and cysteine among the amino acids, and the proteinaceous substrates, chitin and dried *Spirulina* biomass. Fermentation products were acetate and ethanol. Fe^{3+} was reduced in a process that yielded no energy. Phylogenetically the new organism belonged to cluster XI of the Gram-positive bacteria with low G+C content and its closest neighboring taxon was *Tindallia magadiensis*. However, according to its phenotypic and genotypic characters it did not belong to any known genus from this group. We suggest a new genus and species with the name *Anoxynatronum sibiricum* and strain Z-7981 as its type (=DSM15060).

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Introduction

Saccharolytic anaerobic alkaliphilic microorganisms from soda lakes from different geographic locations include phylogenetically different bacteria: spirochetes (Zhilina et al. 1996), haloanaerobes (Zhilina et al. 2001a), and bacilli (Zhilina et al. 2001b), but also strains belonging to "Clostridia with low G+C" (Jones et al. 1998; Tourova et al. 1999). Within this group most clostridia strains from African soda lakes (Jones et al. 1998) belong to "cluster XI", which also includes newly described alkaliphilic bacteria from soda lakes, such as *Tindallia magadiensis* (Kevbrin et al. 1998), *Natronincola histidinovorans* (Zhilina et al. 1998), and *Alkaliphilus transvaalensis* (Takai et al. 2001). The new isolate Z-7981, which is described here, belongs to the same group according to the data from partial sequence of 16S rDNA (Tourova et al. 1999).

Strain Z-7981 was isolated from the soda Lake Nozhnee Beloye, south-east Baikal region as a component of cellulolytic community. Alkaline lakes in central Asia represent an extreme type of prairie lake with a considerable input of cellulosic material. High numbers of cellulolytic bacteria are characteristic of these habitats (Kulyrova 1999). We studied the anaerobic decomposition of cellulose (Kevbrin et al. 1999) and these enrichments were the source from which the new strains were isolated.

Materials and methods

Bacterial strains and their source

Strains Z-7981 and Z-7981′ were isolated from an anaerobic community degrading cellulose. The source for this community was a sample representing a mixture of mud and surface cyanobacterial

mat taken from a lagoon of Lake Nizhnee Beloe (south-eastern Transbaikal region, Russia). At the time of sampling (July 1995), the water of the lagoon had a pH of 9.6, mineralization of 4.57 g/l, and a temperature of 27°C.

Methods of isolation and cultivation of pure cultures

Pure cultures were obtained by serial dilutions in liquid medium under strictly anaerobic conditions using anaerobic techniques. Isolation was carried out in mineral medium which mimicked the mineral composition of the water in the lake, having pH 9.5 and total mineralization of 13.4 g/l (NaCl 3.4 g/l, Na_2CO_3 4.45 g/l and $NaHCO_3$ 5.5 g/l) and 5 g/l of glucose as a substrate. Subsequent isolation was made from colonies grown in Hungate roll tubes in a N_2 atmosphere with the medium mentioned above solidified using 3% (w/v) of agar. Uniformity of colonies on agar media and microscopy of the cells confirmed the purity of the culture.

After optimization, the growth medium contained (g/l): KH_2PO_4 0.2, $MgCl_2$ 0.1, NH_4Cl 0.5, KCl 0.2, Na_2CO_3 9.3, $NaHCO_3$ 44.1, Na_2Sx9H_2O 0.7, yeast extract 0.2, glucose 5.0, trace element solution 1 ml/l (Kevbrin and Zavarzin 1992), vitamin solution 10 ml/l (Wolin et al. 1963), and resazurin 0.001; pH 9.05. Gas phase was N_2.

To establish the spectrum of substrates utilized, mono- and disaccharides, sugar alcohols, mono- and dicarboxylic organic acids, monohydroxylic alcohols, amino acids and nitrogen compounds were added at a concentration of 3 g/l. Xylan (Sigma), microcrystalline cellulose (Sigma), carboxymethylcellulose (Fluka, Germany), dried *Spirulina* biomass (Sigma), starch (Sigma), dextrin (Fluka), gum arabic (Sigma) and avicel (Fluka) were added at concentrations of 2 g/l. Hydrolysis of agar (Difco) and gelatin (Sigma) was tested by adding them to the growth media at concentrations of 3% and 15% (w/v) respectively. All substrates were tested in the optimal growth medium. Sterile aqueous solutions of sugars were added to the alkaline medium immediately before inoculation. Polymers were added to the growth medium before sterilization. To avoid polymer caramelization in the alkaline media during sterilization, the tenfold-concentrated solution of sodium carbonate and bicarbonate was sterilized separately. The sodium mineralization was maintained with an equimolar amount of NaCl.

Determination of physiological characteristics

Electron acceptors were introduced into a sterile medium in the following concentrations (mM): $Na_2S_2O_4$ 1; Na_2SO_3 2 or 10; $NaNO_2$, $NaNO_3$, Na_2SO_4 10; $Na_2S_2O_3{\times}5H_2O$ 10 or 20; $Fe(OH)_3$ 30; S 2% (w/v). The utilization of sulfur compounds as electron acceptors was determined from hydrogen sulfide formation, and sodium thioglycollate was used as the reducing agent. Ammonium compounds were omitted from the growth medium when $NaNO_3$ or $NaNO_2$ were added as electron acceptors. Since $Fe(OH)_3$ reduces readily, whenever it was added to the growth medium, we did not use any reducing agents and used peptone as a substrate [because glucose would also reduce $Fe(OH)_3$]. Other electron acceptors were tested at the optimal growth media with glucose as a substrate. The capacity for dinitrogen assimilation was determined in a nitrogen-free medium except for N_2 in gas phase. In the experiments about growth dependence on pH, the carbonate concentration in the medium was first reduced tenfold, so the only carbonate species added was sodium bicarbonate, and optimal sodium concentration was maintained with sodium chloride. Then 10% HCl or 10% NaOH solutions were used to adjust the pH of the medium. To determine whether sodium carbonates are required for bacterial growth, they were replaced with an equimolar amount of NaCl and pH 9.0 was maintained with 20 mM Tris-base buffer ($pK_a = 9.0$). The need for chloride ion was tested in a medium where NaCl was replaced with an equimolar amount of sodium carbonate and sodium bicarbonate and all other chlorides were replaced with sulfates. The dependence of the bacterial growth on temperature was studied in the range from 6° to 60°C at optimal pH and mineralization.

Analytical methods

Growth was determined by measuring optical density of the culture in Hungate tubes on a Specol-10 (Jena) spectrophotometer at 600 nm. Glucose was analyzed in a reaction with phenol (Hanson and Phillips 1984). The amounts of hydrogen and nitrogen were quantitatively determined on a LKhM-80 gas chromatograph equipped with a katharometer. Volatile fatty acids were analyzed on a model 3700 gas chromatograph equipped with a flame ionization detector. Dissolved hydrogen sulfide was determined colorimetrically from the formation of methylene blue (Trüper and Schlegel 1964). Ammonium concentration was determined using Nessler's reagent after isothermal microdistillation of free ammonia. The presence of nitrite was determined with Griss's reagent. Fe (II) was determined with 2,2′-dipyridil after dissolution of precipitate in HCl (Novikov et al. 1990). The presence of catalase was judged from the foaming of a drop of 3% hydrogen peroxide when added to the biomass washed three times in 10% NaCl to remove carbonates.

Morphological studies

Light-microscopic examination of morphology was performed using a Zetopan (Austria) phase-contrast microscope. Agarose slides were used when taking photographs (Pfennig and Wagener 1986). Ultrathin sections were obtained as described earlier (Zhilina et al. 2001a). The sections and whole cells stained with 1% phosphotungstic acid to reveal flagella were examined under JEM-100C (Japan) electron microscope.

DNA analysis

DNA was isolated and purified by Marmur method (Marmur 1961). The G + C content of DNA was determined from the thermal denaturation curves obtained using a Pye-Unicam SP 1800 spectrophotometer. DNA homology was determined using the method of De Ley (De Ley et al. 1970).

Determination of nucleotide sequences of 16S rRNA genes

The amplification of the 16S rRNA genes was performed using 11F-1492R primers (Lane 1991). The PCR mixtures (20 μl) had the following content: 1× PCR buffer (17 mM $(NH_4)_2SO_4$, 6 mM Tris-HCl, 2 mM $MgCl_2$, pH 8.8), 1 nM of each dNTP, 10–50 ng of DNA, 100 pM of each primer and 2 units of BioTaq DNA polymerase (Dialat, Russia). Samples were amplified through 30 three-step cycles of DNA denaturation at 94°C for 30 s, primer annealing at 40°C for 1 min, and elongation at 72°C for 2.5 min, with a final extension step of 72°C for 7 min. PCR products were analyzed by electrophoresis in 1% agarose gel stained with EtBr. PCR fragments were purified on low-melting-point agarose using "Wizard PCR Preps" kit (Promega, USA).

Sequencing of original 16S rDNA PCR fragments was performed by the Sanger method (Sanger et al. 1977) using "Silver Sequencing" kit (Promega) in accordance with the recommendations of the manufacture with minor modifications. Electrophoresis was run on SQ3 sequencer (Hoefer, USA) in 0.19-mm thick polyacrylamide gel. Universal prokaryotic primers (Lane 1991) and Sequenase (Biochemicals, Cleveland, OH, USA) were used for sequencing in both directions.

Analysis of the nucleotide sequences of the 16S rRNA genes

The sequence was pre-aligned and analyzed using the database and software package from the Ribosomal Databases Project. Then it was aligned with a representative set of 16S rDNA sequences obtained from the Ribosomal Database Project and from recent GenBank releases using a BIOEDIT sequences editor (http://jwbrown.mbio.ncsu.edu/bioedit/bioedit.html). Positions of sequence with alignment uncertainties were omitted, and in total 1,250 positions of alignment were used in the analysis. Phylogenetic trees were constructed using various algorithms implemented in the TREECON (Van de Peer and De Wachter 1994) and PHYLIP (Felsenstein 1990) software packages.

The 16S rRNA sequence of strain Z-7981 was submitted to GenBank under accession number AF522323.

Results and discussion

Morphology

Strains Z-7981 and Z-7981′ are thin, slightly curved rods with pointed ends (Fig. 1a), 0.7 μm in diameter for Z-7981 and 0.5 μm in diameter for Z-7981′, 3.8–5 μm in length for both strains. They occur usually singly or in pairs, rarely forming very long chains. The new isolates have 98% DNA–DNA homology between strains and G + C content 48.4 mol% for Z-7981 and 48.1 mol% for Z-7981′. These two strains differ only in their cell diameter (Tourova et al. 1999), so strain Z-7981 was chosen for future investigations.

Cells of strain Z-7981 multiply by binary division (Fig. 1a), which is not quite symmetrical in long chains. They are actively motile and the flagellation is peritrichous (Fig. 1b). No sporulation was observed. Thermoresistance was studied by heating cell suspension for 10, 20, and 50 min at 70° and 80°C, with subsequent incubation for 14 days in glucose medium at 36°C. Growth was observed in the samples that were heated for 10 and 20 min at 70°C and no growth was observed after pasteurization at 70°C for 50 min. The addition of 5 mM Ca^{2+} [which is the real concentration in the place of habitat (Gorlenko et al. 1999)] to the culture, and heating for 10 and 20 min at 70°C did not result in sporulation.

Judging by the type of its cell wall on ultrathin section (Fig. 2), strain Z-7981 is a Gram-positive bacterium.

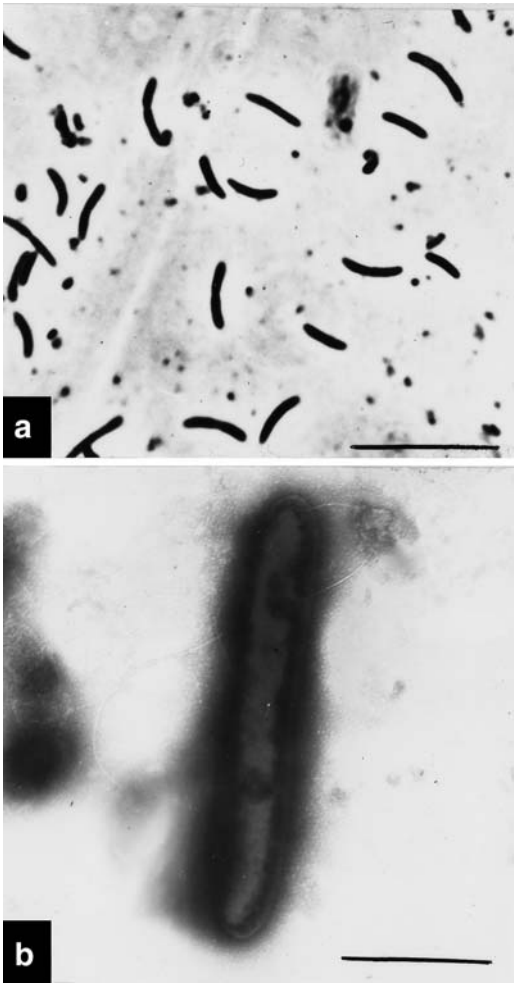

Fig. 1 Morphology of strain Z-7981: **a** cells as viewed under phase contrast microscope, *bar* 10 μm; **b** negatively stained cell with peritrichous flagella, *bar* 1 μm

Growth characteristics

Strain Z-7981 is a modest alkaliphile since it grows at alkaline pH values of 7.55–9.5 (Fig. 3a), with very slow growth at pH 7.1 and 9.8–10.1 and a sharp peak at optimum pH 9.1. There is no growth at pH 7.0 and 10.5. The

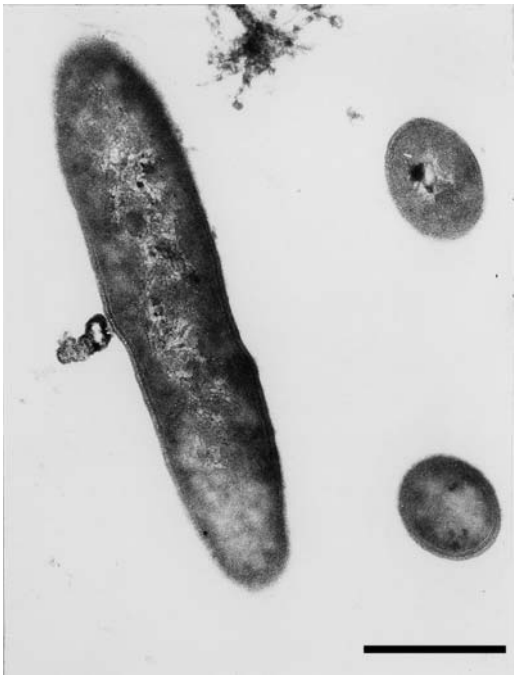

Fig. 2 Ultrastructure of Z-7981 (*bar* 0.5 μm). Longitudinal and cross sections demonstration Gram-positive type of cell wall structure

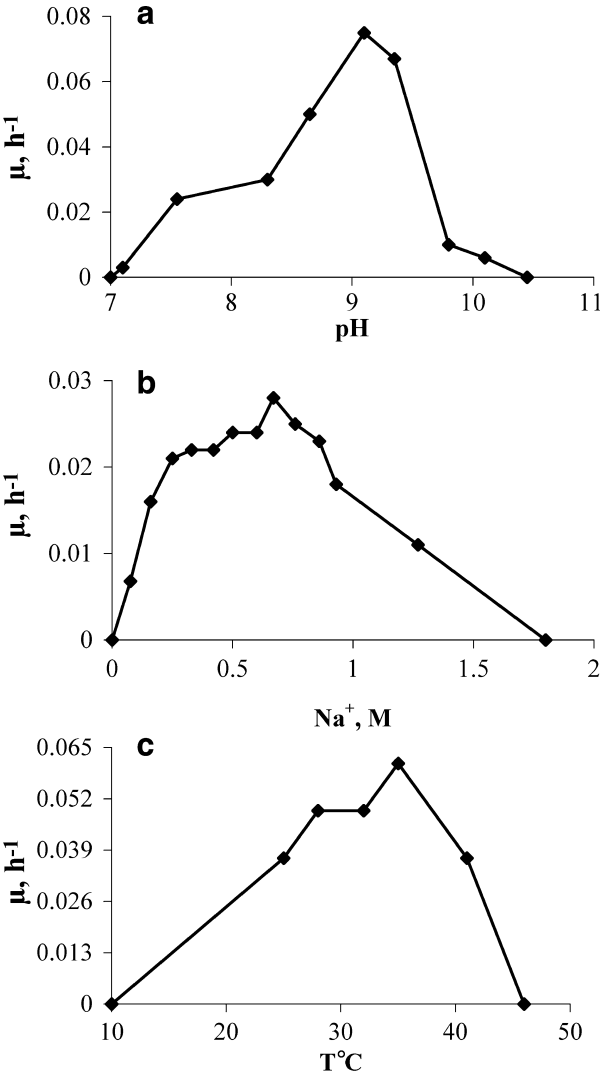

Fig. 3 Dependence of the specific growth rate of strain Z-7981 on **a** pH of the medium, **b** mineralization of the medium and **c** temperature

new isolate is a typical athalassophilic organism: it does not need Cl^- ions and has an obligatory requirement for sodium carbonates. The strain Z-7981 obligately required Na^+ and no growth was observed after replacing sodium ions with potassium ions. The bacterium grows at mineralization of the medium in the range of 0.076–1.27 M Na^+ and there is no growth at 0 or 1.8 M Na^+. The dependence of the specific growth rate on mineralization has a weakly pronounced maximum at 0.67 M Na^+ with a rather broad plateau in the region of 0.25–0.86 M Na^+ (Fig. 3b), which allows this organism to adapt to the seasonal changes in mineralization.

The strain Z-7981 is a mesophile: under the optimal value of mineralization and pH the optimal temperature for growth is 35°C. The shape of its growth–temperature curve is asymmetrical (Fig. 3c), with a gradual rise from zero growth at 10°C to a maximum at 35°C and an abrupt drop to zero at 46°C. Due to the continental climate of the region, the temperature of sediments rarely exceeds 40°C and this might explain the rapid decline of growth rate above 41°C and its limit at 46°C. These physiological characters of the strain fit well with climatic conditions of the region.

Strain Z-7981 is a catalase-positive anaerobe but does not grow aerobically under cotton stoppers. However, under microaerobic conditions the presence of 0.9%, 2.7%, or 4.5% of O_2 in nitrogen gas phase does not inhibit growth. There is no improvement of growth in the presence of microamounts of O_2. It can grow without Na_2S as a reducing agent on glucose or peptone as substrates. The optical density and growth rate without reducing agents or with sodium sulfide or sodium ascorbate are the same. The growth rate was lower with thioglycollate as a reducing agent. Thus Na_2S is not required as the source of anabolic sulfur. The strain is incapable of nitrogen assimilation. The source of nitrogen can equally well be NH_4Cl or $NaNO_3$. Anabolic needs include yeast extract as an obligatory component of the growth medium which can serve as a possible source of sulfur and nitrogen but cannot be substituted by casamino acids. The growth yield of strain Z-7981 is proportional to the concentration of yeast extract within the range 50–750 mg/l. Vitamin solution (Wolin et al. 1963) considerably improves growth in the medium with yeast extract, but does not influence the growth with casamino acids. Unidentified factors from yeast extract might be needed.

Z-7981 possesses true fermentative metabolism. It is incapable of dissimilatory reduction of nitrite, nitrate, or sulfur compounds (S, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$). The bacterium reduces Fe^{3+} to Fe^{2+} with a formation of 3.4 mmol Fe^{2+} from 30 mmol Fe^{3+}. However, this process, which was also reported for *Tindallia magadiensis* (Kevbrin et al. 1998), is not coupled to energy generation because the growth yield in the presence of Fe^{3+} is the same as without it with peptone as a substrate. None of the tested electron acceptors inhibits or stimulates growth except for $NaNO_2$, which prolongs lag phase and reduces the growth rate of the strain Z-7981.

Strain Z-7981 physiologically belongs to the primary anaerobes, which closely interact with hydrolytic microorganisms. It utilizes a wide variety of the substrates of catabolism: mono- and disaccharides, sugar alcohols, a few amino acids, and proteinaceous materials (Table 1). It is able to use glycerol, this ability indicating the possibility of utilizing osmoprotectant from *Dunaliella* or cyanobacteria (Reed et al. 1986). Quite remarkable is its capability for slow growth on dried *Spirulina* biomass. This ability indicates another possible source of nutrition in addition to the products of cellulose decomposition.

The products of glucose fermentation are acetate and ethanol. Strain Z-7981 forms 8.05 mM acetate and 1.55 mM ethanol from 3.22 mM glucose:

$$2C_6H_{12}O_6 \rightarrow 5CH_3COOH + C_2H_5OH \quad (1)$$

with carbon balance 99.3%, hydrogen balance 110%, and oxygen balance 91.3%. The main product of fermentation for Z-7981 is acetate, and thus it should be

Table 1 Substrate utilization by strain Z-7981

Substrates	Maximum optical density, $\lambda = 600$ nm
Monosaccharides	
D-Ribose	0.05
D-Glucose	0.1
D-Mannose	0.1
D-Fructose	0.11
Disaccharides	
Sucrose	0.09
Sugar alcohols	
Glycerol	0.11
L-Inositol	0.06
Monocarboxylic organic acids	
Pyruvate	0.09
Amino acids	
Glutamate	0.1
Cysteine	0.07
Nitrogen compounds	
Peptone	0.1
Tryptone	0.04
Meat extract	0.1
Yeast extract	0.1
Spirulina biomass	+
Chitin	0.04

The following substrates are not utilized: D-xylose, L-arabinose, galactose, sorbose, L-sorbitol, mannitol, dulcitol, D-fucose, erythritol, D-maltose, D-lactose, trehalose, D-cellobiose, methanol, ethanol, formate, acetate, propionate, butyrate, glycolate, lactate, malonate, succinate, betaine, N-acetyl-D-glucosamine, trimethylamine, choline chloride, β-alanine, arginine, asparagine, aspartate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, β-phenyl-α-alanine, serine, threonine, tryptophan, tyrosine, valine, casamino acids, casein, carboxymethylcellulose, microcrystalline cellulose, gum arabic, avicel, and pectin. Starch, glycogen, xylan, and dextrin were not hydrolyzed. Gelatin and agar were not liquefied

considered as an acetogen. In the trophic system of the anaerobic community in soda lakes it could be regarded as a copiotrophic component of the saccharolytic pathway of biomass degradation.

Under optimal growth conditions, the generation time is 9 h.

DNA and phylogenetic analyses

The G + C content of the DNA of strain Z-7981 was determined from T_m to be 48.4%. The G + C content of the most closely related (according to the data of 16S rDNA sequencing) *Tindallia magadiensis* is 37.6 mol%.

Almost the complete sequence of the 16S rRNA gene of strain Z-7981 was determined: 1,410 nucleotides between *E. coli* position from 43 to 1,461. Comparative analysis of this sequence with the complete database of the 16S rDNA sequences within the GenBank BLAST program confirmed our previous conclusion based on partial sequencing of strain Z-7981 (Tourova et al. 1999) that this isolate is a member of the low G + C subphylum of Gram-positive bacteria. It is in the subgroup of *Clostridium felsineum* cluster XI (Collins et al. 1994) in which alkaliphilic genera *Tindallia* (Kevbrin et al. 1998), *Natronincola* (Zhilina et al. 1998), and *Alkaliphilus* (Takai et al. 2001) have been described. Comparison of the 16S rDNA sequence of Z-7981 with the validated alkaliphilic members of subgroup *Cl. felsineum* reveals that the closest relative is the anaerobic alkaliphilic ammonifier *Tindallia* isolated from the extremely saline Lake Magadi, Kenya (Kevbrin et al. 1998) with similarity in sequence of 16S rDNA as high as 94.2%, while similarity with *Alkaliphilus* (Takai et al. 2001) and *Natronincola* (Zhilina et al. 1998) is 91.7% and 89.5%, respectively. However Z-7981 differs from *Tindallia* by 10% in G + C content and has a low rate of DNA homology with it (11%). The new isolate differs from *Tindallia magadiensis* and related alkaliphiles by its ability to ferment saccharides, the substrates utilized, and products of metabolism (Table 2). Thus strain Z-7981 obviously does not belong to *Tindallia*.

Phylogenetic analysis of sequences from Genbank of invalidated strains from cluster XI demonstrates that the strains of saccharolytic alkaliphilic anaerobes included under the names *Clostridium elementeitii* E2SE1-B from Lake Elementeita and *Cl. aminovorans* B7FT-A, *Cl. bogorii* B8NS1, and *Cl. alkaliphilum* B8NS1-A isolated from Lake Bogoria (Kenyan section of the East African Rift Valley) (Jones et al. 1998) and *Tindallia* sp. from Mono Lake (D. Marsic et al., unpublished) definitely belong to the same cluster as *Tindallia magadiensis* (bootstrap analysis 100%) (Fig. 4) with high similarity of 16S rDNA at 97.4–99.8%. All the Kenyan strains grow at high alkalinity with upper limit at pH 10.5. They ferment sugars and amino acids, producing acetate, propionate, and ammonia. Phylogenetically all of them should be included in the genus *Tindallia*. Phenotypic features do not contradict this conclusion, though the ability of these strains to use carbohydrate expands the description of the genus *Tindallia* which was originally described on the grounds of the monotypic species *T. magadiensis*, which uses only amino acids.

The resulting phylogenetic tree (Fig. 4) shows that, within the subgroup of *Clostridium felsineum*, strain Z-7981 forms (bootstrap 100%) a separate cluster with the alkaliphilic strain *Cl. alcalibutyricum* E2SE1 from Lake Elmenteita, Kenya (Jones et al. 1998) related to the *Tindallia* cluster at 93.4–94.4% sequence similarity. *Cl. alcalibutyricum* produces, besides acetate, butyrate, which was not detected among fermentation products of Z-7981. It is not yet a validated species, since it needs to be studied in detail in order to define its taxonomic status.

All the data presented here indicate that Z-7981 and the related strain Z-7981′ belong to a new genus and species, for which we suggest the name *Anoxynatronum sibiricum* gen.nov., sp.nov. with type strain Z-7981.

Description of *Anoxynatronum* gen.nov.

Anoxynatronum, Garnova, Zhilina, Tourova (an.oxy. natron.um. N.L. *an* – without; *oxy* – oxygen; *natron*

Table 2 Comparison of properties for the closest alkaliphilic representatives of cluster XI and Z-7981

Characteristics	*Tindallia magadiensis* Z-7934^T (Kevbrin et al. 1998)	*Natronincola histidinovorans* Z-7940^T (Zhilina et al. 1998)	*Alkaliphilus transvaalensis* SAGM1^T (Takai et al. 2001)	Z-7981^T
Morphology	Slightly curved rods with pointed ends	Rods, terminal round mini-cells were detected	Straight or slightly curved rods, vesicle-like structures were detected	Thin slightly curved rods with pointed ends
Motility	Nonmotile	Peritrichous flagellation	Multiple flagella	Peritrichous flagellation
Spore shape and location	Absent	Lacking in type strain	Sphericalterminal	Absent
Gram-positive wall structure	+	+	+	+
Na^+ mineralization, M (optimum)	0.17–1.7 (0.7–1.4)	0.7–2.7 (1.4–1.7)	0.77–1.3 (0.84)	0.08–1.3 (0.25–0.86)
Obligate dependence on carbonates	+	+	ND	+
pH (optimum)	7.5–10.5 (8.5)	8.0–10.5 (9.4)	8.5–12.5 (10.0)	7.1–10.1 (9.1)
Temperaturerange, °C (optimum)	19–47 (37)	ND (37–40)	20–50 (40)	25–41 (35)
Obligate dependence on Na^+	+	+	ND	+
Electron acceptors				
Nitrate	–	ND	–	–
Thiosulfate	–	ND	+	–
Elementary sulfur	ND	ND	+	–
Dimethilsulfoxide	+	ND	–	ND
Fe^{3+}	+	ND	ND	+
Fumarate	ND	ND	+	ND
Substrates utilized				
Carbohydrates	–	–	–	+
Sugar alcohols	–	–	–	+
Pyruvate	+	–	–	+
Arginine	+	–	–	–
Ornithine	+	–	–	–
Glutamine	±	–	–	–
Glutamate	±	+	–	+
Cysteine	–	–	–	+
Histidine	±	+	–	–
Spirulina biomass	ND	ND	ND	+
Chitin	ND	ND	ND	+
Proteinaceous substrates	±	+	+	+
Products of fermentation	From amino acids Acetate, NH_3, propionate, H_2	Acetate, NH_3, formate	ND	From carbohydrates Acetate, ethanol
G+C, mol.%	37.6	31.9	36.4	48.4
Isolation source	Lake Magadi, Kenya	Lake Magadi, Kenya	Mine water containment dam at 3.2 km below land surface in an ultra-deep gold mine near Carletonville, South Africa	Lake Nizhnee Beloe (South-Eastern Transbaikal region, Russia)

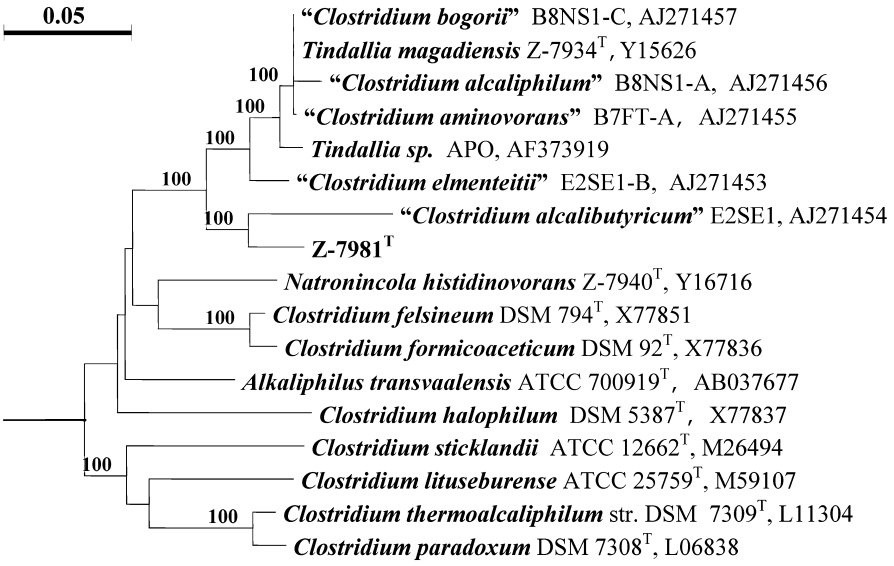

Fig. 4 Phylogenetic tree of *Cl. felsineum* group of *Clostridium* cluster XI constructed on the comparison of nucleotide sequence of the 16S rRNA genes. *Clostridium butyricum* was taken as outgroup. *Bar* corresponds to 5 nucleotide substitutions per 100 nucleotides. Bootstrap values (expressed as percentage of 100 replications) are shown at branch points; values >95 were considered significant

M.L.n. – soda, organism which inhabits anaerobic and sodic environment).

Anaerobic, alkaliphilic, fermentative rods with Gram-positive cell wall structure. Organotroph: able to utilize mono- and disaccharides and proteinaceous substrates. Belongs to cluster XI of subgroup *Clostridium felsineum* of the low G+C Gram-positive bacteria. Monotypic, type strain is Z-7981.

Description of *Anoxynatronum sibiricum* sp.nov.

Anoxynatronum sibiricum Garnova, Zhilina (si.bi.ri.cum M.L. – Sibir′ Russian region).

Thin slightly curved rods with pointed ends, 0.7×3.8–5 μm in size. Usually occurring singly or in pairs, rarely forming very long filaments. Motile by peritrichous flagella. Multiplication by fission. Cell wall of Gram-positive structure. Asporogenous. Thermosensitive. Growth was not observed after heating for 50 min at 70°C.

Aerotolerant. Catalase-positive. Growth occurs without any reductants.

Moderately alkaliphilic. The optimum pH for growth is 9.1 with the range of pH 7.1 to 10.1. Obligately depends on sodium and bicarbonate ions, but does not require chloride ions. The optimal mineralization for growth is 0.25–0.85 M Na^+.

Mesophile. Growth occurs at temperatures from 25° to 41°C with optimum at 35°C. Under optimal growth conditions, the generation time is 9 h.

Fermentative. Chemoorganoheterotrophic. Ferments mono- and disaccharides, sugar alcohols, pyruvate, proteinaceous substrates, and slowly dried *Spirulina* biomass and chitin. The products of glucose fermentation are acetate and ethanol. Reduces Fe^{3+} to Fe^{2+}, but this process is not coupled to energy generation. Amino acids are used and cannot be replaced by casamino acids. Does not require vitamin solution, but its addition improves the growth rate. Does not need sulfide as a sulfur source.

The G+C content of genomic DNA is 48.4 mol% (T_m method).

Habitat: brackish-carbonate lake. Type strain has been isolated as a member of the anaerobic cellulolytic community from a mixture of mud and surface cyanobacterial mat taken from a lagoon of Lake Nizhnee Beloe (south-eastern Transbaikal region, Russia).

The type strain is Z-7981^T (=DSM15060).

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