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ORIGINAL PAPER

Elena S. Garnova · Tatjana N. Zhilina
Tatjana P. Tourova · Nadezhda A. Kostrikina
Georgy A. Zavarzin

Anaerobic, alkaliphilic, saccharolytic bacterium *Alkalibacter saccharofermentans* gen. nov., sp. nov. from a soda lake in the Transbaikal region of Russia

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Abstract Three strains of new obligately anaerobic alkaliphilic bacteria have been isolated as a saccharolytic component from the cellulolytic community of alkaline Lake Nizhnee Beloe (Transbaikal region, Russia), a lake with low salt concentration. DNA analysis of these strains showed an interspecies level of DNA similarity of 96–100%. Strain Z-79820 was selected for further investigations. Cells were Gram-positive, asporogenous, nonmotile short rods with pointed ends. The strain was a true alkaliphile: growth occurred from pH 7.2 to 10.2 with the optimum at pH 9.0. Strain Z-79820 was halotolerant and could grow in medium with up to 10% (w/v) NaCl, with the optimum between 0 and 4% NaCl. The new isolate obligately depended on Na^+ ions in the form of carbonates or chlorides. Total Na^+ content needed for optimal growth was 0.46 M Na^+, with a wide range from 0.023–0.9 M Na^+ at which growth also occurred. The isolate was a mesophile and grew at temperatures from 6 to 50°C (slow growth at 6 and 15°C) with an optimum at 35°C. The organotrophic organism fermented ribose, xylose, glucose, mannose, fructose, sucrose, mannitol, and peptone. The products of glucose fermentation were acetate, ethanol, formate, H_2, and CO_2. Yeast extract was required for some anabolic needs. The DNA G+C content of the type strain Z-79820 was 42.1 mol%. The new bacterium fell into the 16S rRNA gene cluster XV of the Gram-positive bacteria with low G+C content, where it formed an individual branch. Based on its growth characteristics and genotype traits, we propose the new genus and species named *Alkalibacter saccharofermentans* with the type strain Z-79820 (=DSM14828), Uniqem-218 (Institute Microbiology, RAS; http://inmi.da.ru).

Keywords 16S rRNA · Alkaliphile · Anaerobe · Saccharolytic bacterium · Soda lakes

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E. S. Garnova (✉) · T. N. Zhilina · T. P. Tourova
N. A. Kostrikina · G. A. Zavarzin
Laboratory of Relict Microbial Communities,
Institute of Microbiology,
Russian Academy of Science (RAS),
Prospect 60-let Oktyabrya 7/2,
117312 Moscow, Russia
Tel.: +1-908-358-80-93
E-mail: egarnova@yahoo.com

Introduction

During the study of cellulose decomposition in the soda lakes of central Asia with low salt concentration (Kevbrin et al. 1999), some strains of saccharolytic, anaerobic, alkaliphilic bacteria have been isolated. According to a preliminary phylogenetic analysis, the bacteria belong to the "clostridia group with low G+C content" (Tourova et al. 1999). Two strains were new members of the 16S rRNA gene cluster XI "clostridia" (Collins et al. 1994), in which they represented the first saccharolytic, aerotolerant, alkaliphilic anaerobe among other anaerobic representatives isolated from soda lakes (Zhilina et al. 1998; Kevbrin et al. 1998; Jones et al. 1998). These new strains were described as a new genus *Anoxynatronum sibiricum* (Garnova et al. 2003). The other strains belonged to the 16S rRNA gene cluster XV "clostridia," which is composed of neutrophiles of the genera *Acetobacterium*, *Eubacterium*, and *Pseudoramibacter* (Willems and Collins 1996). Here, we present the first alkaliphile from the 16S rRNA gene cluster XV: *Alkalibacter saccharofermentans* gen. nov., sp. nov.

Materials and methods

Bacterial strains and their source

Strains Z-7980, Z-79820, and Z-7983 have been isolated together with *Anoxynatronum sibiricum*(Garnova et al. 2003) from an anaerobic microbial community decomposing 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 (southeastern Transbaikal region, Russia) with a pH of 9.6, salt concentration of 4.57 g/l, and temperature of 27°C.

Methods of isolation and cultivation of pure cultures

The mineral medium, which was used for the culture enrichment, had a total salt concentration 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 glucose as a substrate. Incubation was carried out anaerobically under N_2 gas flow at pH 9.5 and 36°C. Pure cultures were obtained under strictly anaerobic conditions by a series of dilutions in liquid media with subsequent single-colony isolation on the surface of solidified media, prepared using 3% agar (w/v). Uniformity of the colonies on agar media and microscopy of the cells confirmed the purity of the cultures.

The optimized growth medium contained (g/l): KH_2PO_4 (0.2), $MgCl_2$ (0.1), NH_4Cl (0.5), KCl (0.2), NaCl (6.0), Na_2CO_3 (15.5), $NaHCO_3$ (3.5), 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, with a pH of 9.0. The gas phase was N_2.

Physiological characteristics

To establish the spectrum of substrates utilized by the isolate, we added mono- and disaccharides, sugar alcohols, mono- and dicarboxylic organic acids, monohydroxylic alcohols, amino acids, nitrogen compounds, and polymers to the optimized growth medium at concentrations of 3 g/l against the background of 0.2 g/l of yeast extract, as it was described previously (Garnova et al. 2003). Growth in H_2/CO_2 was tested under an atmosphere of 80% H_2/20% CO_2.

Electron acceptors supplemented an optimized growth medium with glucose as a substrate in the following concentrations (mM): $Na_2S_2O_4$(1); Na_2SO_3 (2 or 10); $NaNO_2$, $NaNO_3$, Na_2SO_4 (10); $Na_2S_2O_3$(10 or 20); S° 2% (w/v). The ability to fix N_2 was tested by using the acetylene-reduction assay in a N_2-free medium except for N_2 in the gas phase. For this purpose, bacterial cultures were grown in 16-ml tubes, where 5 ml medium was added; after 24 h of inoculation, 1/20 of tube air volume of acetylene was added (Burris 1972). The presence of ethylene was detected 10 h later.

In the study of bacterial growth dependence on the pH of the medium, the carbonate was replaced by bicarbonate. The total concentration of bicarbonate in the medium was set at one tenth concentration of the carbonate, and the optimal sodium concentration was maintained with NaCl. Solutions (10%) of HCl or NaOH were used to adjust the pH of the medium. In order to establish NaCl dependence (for the range of concentrations from 0 to 12%), an appropriate amount of NaCl was added directly to Hungate tubes prior to dispensing growth medium. The need for Cl^- ions was tested in a medium where NaCl was replaced with an equimolar amount of Na_2CO_3 and $NaHCO_3$, and all other chlorides were replaced with sulfates. To determine if $Na_2CO_3$is required for growth, they were replaced with an equimolar amount of NaCl, while pH 9.0 was maintained with 20-mM Tris-base buffer ($pK_a = 9.0$). The dependence of the bacterial growth on the temperature was studied in the temperature range from 6 to 60°C, using optimized growth medium.

Analytical methods

Growth was determined by measuring the optical density of the culture in Hungate tubes using a Specol-10 spectrophotometer (Carl Zeiss, Jena, Germany) at 600 nm. Glucose concentration was analyzed by a reaction with phenol (Hansson and Phillips 1981). The amounts of H_2 and N_2 were determined on a LKhM-80 gas chromatograph (Krohm-Service, Moscow, Russia) equipped with a katharometer (heat-conductance sensor). Volatile fatty acids and ethylene were analyzed on a model 3700 gas chromatograph equipped with a flame-ionization detector. Dissolved H_2S was determined colorimetrically from the formation of methylene blue (Trüper and Schlegel 1964). Ammonium concentration was determined using Nessler's reagent. The presence of $NO_2$was determined with Griess's reagent. The presence of catalase was judged from the foaming of a drop of 3% H_2O_2solution, when added to the biomass washed three times in 10% NaCl solution in order to remove carbonates.

Morphological studies

Light-microscope examination of morphology was performed using a Zetopan phase-contrast microscope (Reichert, Vienna, Austria). Agarose slides were used when taking photographs (Pfennig and Wagener 1986). Whole cells were stained with 1% phosphotungstic acid to reveal flagella. To obtain ultra thin sections, cells were fixed with 5% glutaraldehyde for 2 h at 4°C, then treated with 1% OsO_4 for 4 h at 4°C. Cells were embedded in Epon-812, and thin sections were stained by uranyl acetate and lead citrate. Whole cells and thin sections were examined with a JEM-100C electron microscope (JEOL, Tokyo, Japan).

Binary cultures of saccharolytic and sulfate-reducing bacteria

The type strains of *Desulfonatronovibrio hydrogenovorans*, *Desulfonatronum lacustre*, *Halonatronum saccharophilum*, and *Amphibacillus fermentum* were taken from

the culture collection maintained in the Laboratory of Relict Microbial Community (Institute of Microbiology, RAS, Russia). Because saccharolytic bacteria grow in the medium with broader range of Na^+ salt concentration than sulfate reducers can grow, experiments were carried out in the mineral medium optimal for *D. hydrogenovorans* (Zhilina et al. 1997) and *D. lacustre* (Pikuta et al. 1998). Due to the significant difference in the growth rate between fermentative bacteria and sulfate reducers, *D. hydrogenovorans* and *D. lacustre* were grown first with formate as substrate, and when the consumption of formate and H_2S production stopped, the inoculates of *Halonatronum saccharophilum*, *Amphibacillus fermentum*, and *Alkalibacter saccharofermentans*, together with glucose as a substrate, were added. This enabled the observation of the interaction of sulfate reducers with saccharolytic bacteria, because now formate and H_2 were immediately removed from the medium by the sulfate reducers.

DNA analysis

The G + C content of the DNA and DNA homology were determined as described earlier (Tourova et al. 1999).

Determining 16S rRNA gene sequence and phylogenetic analysis

Amplification, sequencing of 16S rRNA genes, and analysis of the nucleotide sequences of the 16S rRNA genes were carried out as described earlier (Garnova et al. 2003). The 16S rRNA sequence of strain Z-79820 was submitted to GenBank under accession number AY312403.

Results and discussion

Strains Z-7980, Z-79820, and Z-7983 had morphologically uniform cells in liquid medium but produced colonies of two types in solid medium. The colonies were different by their shape, diameter, consistency, and edge. All three strains and their morphotypes had similar G + C content (40.8–42.1%) and high levels of DNA–DNA hybridization (96–100%) (Tourova et al. 1999). Based on these results, the morphotypes of the strains Z-7980, Z-79820, and Z-7983 belonged to the same species, and the morphology of the colonies was not a differentiating characteristic. Strain Z-79820 was chosen for more detailed investigations.

Morphology

Cells of the strain Z-79820 taken from the exponential phase (which lasted 12 h on average) usually occurred singly or in pairs (Fig. 1a); sometimes cells were at an angle to each other, or rarely, in short chains consisting of three to five cells. They were 0.5 μm in width and 1.5–2.5 μm in length. Cells were nonmotile short rods with pointed ends (Fig. 1b). The cellular morphology varied somewhat with the age of the culture. At the late stationary phase (after 20 h of growth) most of the cells were swollen, had irregular shape, and were predominantly arranged in chains. The cell wall of the new isolate had a Gram-positive structure, and cells were divided by a septum (Fig. 1c). Fission in the old culture was sometimes not quite symmetrical. Sporulation has never been observed. The addition of 5 mM Ca^{2+} or treatment of the cell suspension for 10, 20, and 50 min at 70 and 80°C did not induce spore formation. Bacteria grew after pasteurization at 70°C for 50 min, but not at 80°C for 10 min.

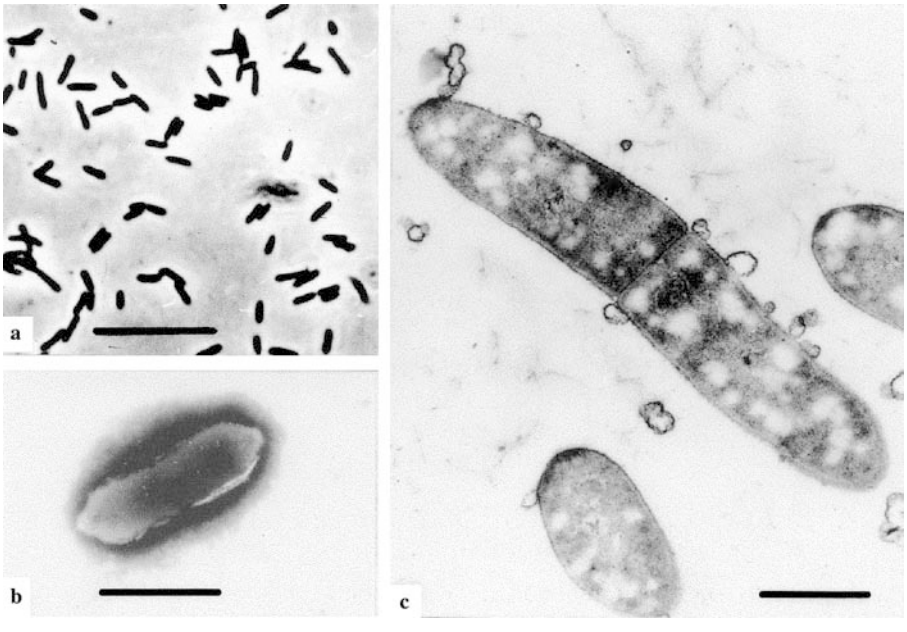

Fig. 1a–c Morphology of strain Z-79820. **a** Cells as viewed under phase-contrast microscope. *Bar* = 10 μm. **b** Negatively stained cell without flagella. *Bar* = 1 μm. **c**Longitudinal and cross sections show that cell-wall structure is Gram-positive type, and the cell is divided by a septum. *Bar* = 0.5 μm

Growth characteristics

Strain Z-79820 was an obligate alkaliphile. Growth occurred from pH 7.2 to 10.2 with a peak of optimum growth at pH 9.0 (Fig. 2a). There was no growth at pH 7.0 or 10.5. The bacterium was halotolerant because it was capable of growing at NaCl concentrations between 0–10% (w/v) with an optimum at 0–4% (w/v) (Fig. 2b). The organism obligately depended on Na^+ ions in the form of carbonates or chlorides. Growth was equally good with only $NaHCO_3 + Na_2CO_3$or an equimolar amount of NaCl in the medium. In laboratory conditions, when optimal pH was maintained with 20 mM Tris-base buffer ($pK_a = 9.0$) and optimal salt concentration was maintained with NaCl, the organism could grow without carbonates. Strain Z-79820 grew in the medium with a broad salt concentration range of 0.023–0.7 M Na^+ with an optimum at 0.46 M Na^+ (Fig. 2c). At optimal values of pH and salt concentration, the temperature optimum was recorded at 35°C, with the growth still occurring in the range 6–50°C. When the bacterium grew at 6 and 15°C, the lag phase lasted 4 days, and maximal optical density was observed on the 17th day after inoculation. The growth at low temperatures might be explained as an adaptation to the Siberian continental climate. Under optimal growth conditions, the generation time was 5 h. Strain Z-79820 is remarkable due to its adaptation to a wide range of ecological factors. It has sharp optima at particular pH (Fig. 2a), temperature values, and salt concentration (Fig. 2c); however, the growth rate remains significant in a broad pH range (7.2–10.2), temperature (6–50°C), and osmotic adaptation (0–10% NaCl). This range indicates that the organism adapted to a highly unstable environment in a cryoarid climate where seasonal rains and evaporation cause wide fluctuations in environmental parameters.

Strain Z-79820 was a catalase-negative obligate anaerobe. There was no growth under strictly aerobic conditions or under microaerobic conditions in the presence of 0.9, 2.7 or 4.5% of O_2 in the N_2 gas phase. The new organism, when grown in glucose medium, apparently did not need Na_2S as a reducing agent or sulfur source because the absence of Na_2S had no influence on the growth rate. The new isolate was incapable of the dissimilatory reduction of NO_2^-, NO_3^- or sulfur compounds (S, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$). The presence of NO_2^-completely inhibited growth while sulfur significantly improved it. Other electron acceptors did not influence the growth rate.

Strain Z-79820 required yeast extract for anabolic needs. The bacterial growth yield was proportional to the concentration of yeast extract (within the range of 50–1,000 mg/l). Yeast extract, along with NH_4Cl or $NaNO_3$, could be the possible source of nitrogen because strain Z-79820 was unable to assimilate nitrogen from the gaseous phase. In addition, yeast extract could be the only source of sulfur, since the bacterium does not require Na_2S. Casamino acids could not substitute for

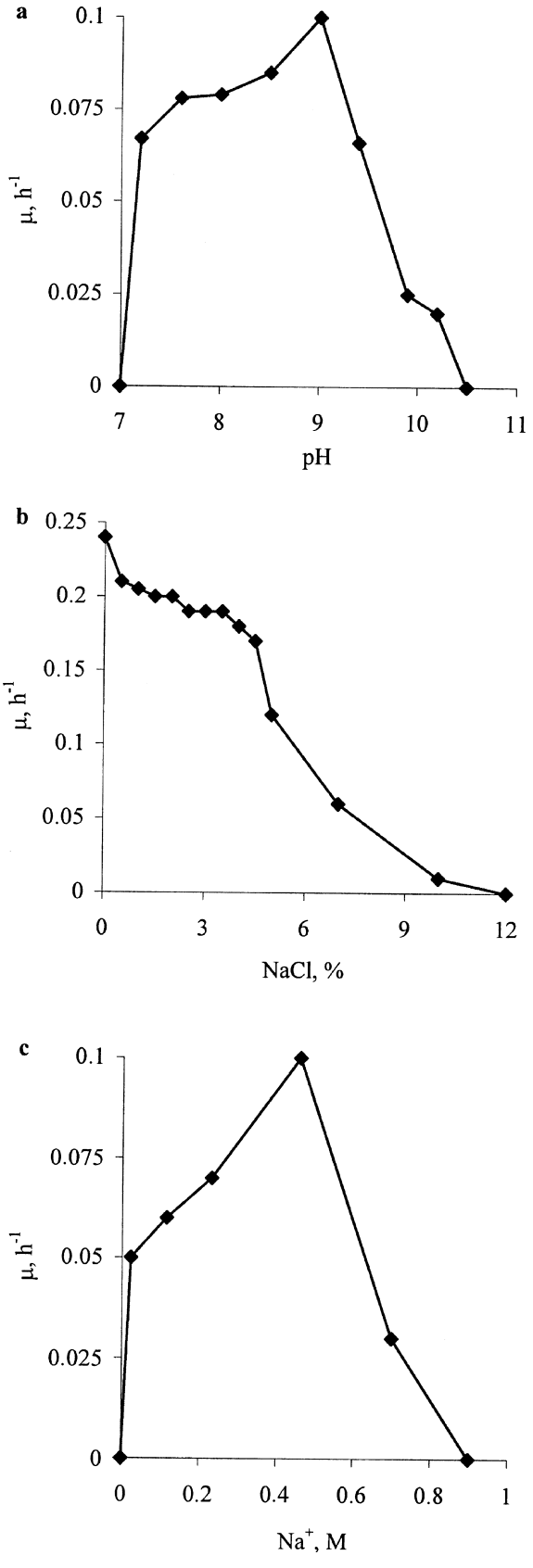

Fig. 2a–c Dependence of the specific growth rate of strain Z-79820 on **a** pH of the medium, **b** NaCl%(w/v), **c** salt concentration of the medium

yeast extract even in the presence of a vitamin solution. Strain Z-79820 thus clearly required some unknown compounds from the yeast extract for growth. The organism was not obligately dependent on the vitamin solution. However, its addition improved the growth rate.

The metabolism of the new saccharolytic bacterium was fermentative. The spectrum of energy sources utilized by strain Z-79820 was limited to ribose, xylose, glucose, mannose, fructose, mannitol, sucrose, tryptone, and peptone. Ammonium production was not detected during peptone and tryptone consumption. The following substrates were not utilized: H_2/CO_2, arabinose, galactose, sorbose, glycerol, sorbitol, dulcitol, inositol, fucose, lactose, maltose, trehalose, cellobiose, erythritol, formate, acetate, propionate, butyrate, glycolate, lactate, pyruvate, malonate, succinate, methanol, ethanol, choline chloride, betaine, trimethylamine, N-acetyl-D-glucosamine, carboxymethylcellulose, microcrystalline cellulose, Casamino acids, yeast extract, gum arabic, avicel, and *Spirulina*dead biomass. Starch, glycogen, xylan, pectin, and chitine were not hydrolyzed. Gelatin and agar were not liquefied.

Strain Z-79820 fermented glucose to formate, acetate, ethanol, H_2, and CO_2. The concentration of products was measured at the late stationary phase when glucose consumption was stopped. At that time the stoichiometry of glucose conversion to products was calculated as follows:

$$5\,\text{mM glucose} \rightarrow 5\,\text{mM acetate} + 4.8\,\text{mM ethanol} + 7.6\,\text{mM formate} + 1.4\,\text{mM } H_2.$$

Based on these measurements, the prediction for the equation was:

$$3C_6H_{12}O_6 + 3H_2O \rightarrow 3CH_3COOH + 3C_2H_5OH + 5CHOOH + H_2 + CO_2$$

The C balance was 96.3%; the H_2balance was 100%; and the O_2 balance was 100%.

In a trophic system of anaerobic community of soda lakes, strain Z-79820 belongs to a group of fermentative microorganisms, which utilize mono- and disaccharides released after hydrolysis of polymers (in particular cellulose). The fermentation products of the strain, especially formate, could serve as substrates for methanogens and sulfate reducers, which were detected in the soda lakes of the southeastern Transbaikal region (Zavarzin et al. 1996; Namsaraev et al. 1999; Gorlenko et al. 1999). Strain Z-79820, as well as other saccharolytics studied—*Halonatronum saccharophilum* (Zhilina et al. 2001a) and *Amphibacillus fermentum* (Zhilina et al. 2001b)—produce formate as a major product, which can substitute H_2 in interspecies transfer in the trophic system of alkaliphilic community; in addition, strain Z-79820 and *H. saccharophilum* produce H_2. Complete removal of H_2 is needed for a full decomposition of organic matter in the anaerobic community. In the soda lake community this function is performed by hydrogenotrophic, alkaliphilic sulfate reducers (Zavarzin et al. 1996) such as *Desulfonatronovibrio* (Zhilina et al. 1997) and *Desulfonatronum* (Pikuta et al. 1998), both of which are capable of utilizing either formate or H_2. Formate is easily converted to H_2 by formate lyase, and it is likely that sulfate reducers metabolize it and thus decrease the reducing power of the system. High tolerance of *H. saccharophilum*, *A. fermentum*, and Z-79820 to H_2S allows them to develop in a zone of active sulfidogenesis and create product–substrate relationships with sulfate reducers.

Cooperative sulfidogenesis with carbohydrates as substrate was demonstrated under laboratory conditions in binary cultures of *Desulfonatronovibrio hydrogenovorans* and *Desulfonatronum lacustre* with saccharolytic anaerobes (Table 1). Products of fermentation had no inhibitory effect on the growth of *H. saccharophilum* or *A. fermentum* since their elimination by sulfate reducers had no stimulatory effect on glucose consumption (as compared to pure cultures). In contrast, when Z-79820 products were eliminated by sulfate reducers, the strain consumed twice as much glucose as compared to growth in pure culture. This fact indicates the existence of a cooperative development between saccharolytic and sulfate-reducing bacteria. H_2 was the preferable substrate for sulfate reducers, and it was immediately utilized. Formate served as the main substrate for sulfidogenesis due to its significant production by saccharolytics (Table 1). This example of cooperative behavior of Z-79820 and hydrogenotrophic sulfate reducers demonstrates that saccharides can be easily utilized for sulfidogenesis in very short trophic chain, and formate may serve as an effective interspecies

Table 1 Glucose consumption saccharolytic alkaliphiles Z-79820, *Amphibacillus fermentum*, and *Halonatronum saccharophilum* in pure culture and coculture with alkaliphilic sulfate-reducing bacteria *Desulfonatronovibrio hydrogenovorans* and *Desulfonatronum lacustre*

–, Does not produce as a product

Cultures	Glucose consumed (mM)	Products of fermentation (mM)		
		Formate	H_2	H_2S
Z-79820	15.6	25.9	2	–
Z-79820 + *D. hydrogenovorans*	26.6	0.13	0	33.0
Z-79820 + *D. lacustre*	26.0	0	0	24.4
A. fermentum	26.0	19.6	–	–
A. fermentum + *D. lacustre*	25.8	0	–	20.5
A. fermentum + *D. hydrogenovorans*	25.7	0.7	–	14.9
H. saccharophilum	28.6	32.0	0.33	–
H. saccharophilum + *D. hydrogenovorans*	20.5	0.13	0	19.1

mediator between saccharolytics and sulfate-reducing organisms.

DNA and phylogenetic analysis

The G+C content in the DNA of strain Z-79820—determined from the thermal denaturation curves—was 42.1 mol% (Tourova et al. 1999).

An almost-complete sequence of the 16S rRNA gene of the strain Z-79820 was obtained; it consisted of 1,432 nucleotides between positions 48 and 1,510 (*Escherichia coli* numbering). Comparative analysis of this sequence with the complete database of the 16S rRNA gene sequences within the GenBank BLAST program confirmed our preliminary conclusion (Tourova et al. 1999) that strain Z-79820 is a member of 16S rRNA gene cluster XV, the low G+C containing Gram-positive group (Collins et al. 1994). The tested strain formed a distinct phylogenetic branch in the 16S rRNA gene cluster XV that included neutrophilic species of the genera *Acetobacterium*, *Eubacterium*, and *Pseudoramibacter* (Willems and Collins 1996; Fig. 3). High bootstrap values confirm the validity of such position (Fig. 3). Strain Z-79820 exhibits rather a high degree of 16S rRNA gene sequence divergence from species of the genera *Acetobacterium* (9.1–9.7%), *Eubacterium* (8.9–12.5%), and *Pseudoramibacter* (11.9%), which also indicates the separate taxonomic position of the new bacterium.

As all the representatives of the 16S rRNA gene cluster XV, alkaliphilic strain Z-79820 is an obligately anaerobic, Gram-positive, non-spore-forming, chemoorganotrophic rod. The new organism is different from homoacetogenic species of genera *Acetobacterium*, *Eubacterium limosum* (Moore and Holdeman Moore 1986), and *E. aggregans* (Mechichi et al. 1998) in its inability to perform autotrophic growth, homoacetogenesis, and fermentation of another spectrum of carbohydrates. *E. limosum* and *E. aggregans* produced different products from that of the new bacterium when they fermented carbohydrates during chemoorganotrophic growth. *E. barkeri*, *E. callanderi* (Mountfort et al. 1988), and *Pseudoramibacter alactolyticus* (Willems and Collins 1996), as well as strain Z-79820, are able to grow only chemoorganoheterotrophically. However, alkaliphilic strain Z-79820 differs from the other three in the spectrum of substrate utilization and products of catabolism. It is the first true alkaliphilic representative in the 16S rRNA gene cluster XV. Unlike other members of this phylogenetic group, the new strain was isolated from a contrasting habitat (continental soda lake). Ecophysiological features of the strain Z-79820 are in accordance with the habitat in soda lakes, and thus the bacterium possesses some characteristics that are unusual for other representatives of the 16S rRNA gene cluster XV: it grows at high pH, obligately depends on Na^+ ions, and it is able to grow at high salt concentrations.

On the basis of distinct position of strain Z-79820 on phylogenetic tree, together with its physiological and genotypic characteristics, the strain—as well as its closest relative strains Z-7980 and Z-7983—represents a new genus and species, for which we propose name *Alkalibacter saccharofermentans* gen. nov., sp. nov. with the type strain Z-79820.

Description of Alkalibacter gen. nov.

Alkalibacter, Garnova, Zhilina, Tourova [al·ka·li·bac·ter *n.* NLat. *alkali* (from Arabic *al qaliy* soda ash); Med.Lat. *masc. n. bacter* equivalent Greek *neut. n. bakterion* rod or staff; *Alkalibacter* alkaliphilic rod-shaped cells,

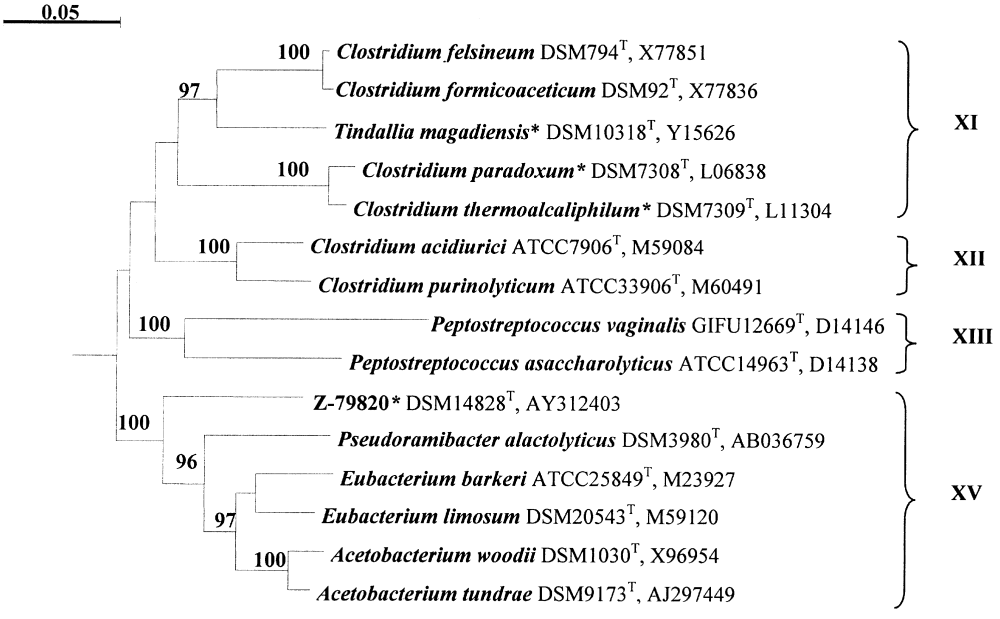

Fig. 3 Phylogenetic tree of Z-79820 group of *Clostridium* 16S rRNA gene cluster XV constructed, based on the comparison of nucleotide sequence of the 16S rRNA genes. *Bacillus subtilis* was taken as an outgroup. The *bar* corresponds to five nucleotides substitution per 100 nucleotides. Bootstrap values (expressed as percentage of 100 replications) are shown at *branch points*; values >95 were considered significant. *Asterisks* indicate alkaliphiles

inhabits soda environments] are strictly anaerobic, Gram-positive rods and alkaliphilic, fermentative organoheterotrophs. They obligately depend on Na^+ ions. They are members of the 16S rRNA gene cluster XV of Gram-positive bacteria with low G+C content. They are monotypic, the type species being *A. saccharofermentans* (Z-79820^T).

Description of A. saccharofermentans sp. nov.

A. saccharofermentans Garnova, Zhilina (sac·cha·ro·fer·men·tans. Latin *n. saccharum* sugar; Latin *n. fermentum* to ferment; Latin *part. adj. saccharofermentans* sugar-fermenting) are Gram-positive and rod-shaped cells, 0.5×1.5–2.5 μm, with pointed ends; they form singly or in pairs, or rarely, form short chains. They are nonmotile cells divided by a septum. Spores are not observed. The cells are thermosensitive; there is no growth after heating for 10 min at 80°C.

A. saccharofermentans is an obligate anaerobe. It is catalase-negative. Growth occurs without reducing agents in the presence of glucose.

It is chemoorganoheterotrophic and nonhydrolytic. It utilizes the monosaccharides ribose, xylose, glucose, mannose, fructose; the disaccharide sucrose; and the sugar alcohols mannitol and peptone, and tryptone for growth. Glucose is fermented to acetate, ethanol, formate, H_2, and CO_2. Sulfate, sulfite, thiosulfate, dithionite, NO_3^-, or NO_2^- is not reduced. For anabolism it uses yeast extract, which cannot be replaced by Casamino acids. Yeast extract is sufficient as a source of nitrogen and sulfur. It does not require a vitamin solution, but its addition improves the growth rate.

It is obligately alkaliphilic; the optimum pH is 9.0, with the growth range from pH 7.2 to 10.2. Halotolerant, it is able to grow at 0–10% NaCl with the optimum between 0 and 4% NaCl. *A. saccharofermentans* obligately depends on Na^+ ions in the form of carbonates or chlorides. The optimal salt concentration for growth is 0.46 M Na^+, with good growth between 0.023–0.7 M Na^+.

A. saccharofermentans is a mesophile; growth occurs at temperatures from 6 to 50°C, with an optimum at 35°C. Under optimal growth conditions the generation time is 5 h. The G+C content of the genomic DNA for the type strain is 42.1 mol% (determined by thermal denaturation).

Its habitat is saline–carbonate lakes. The 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 (southeastern Transbaikal region, Russia).

The type strain is Z-79820^T(=DSM14828), (=Uniqem-218).

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