ISSN 0026-2617, Microbiology, 2009, Vol. 78, No. 4, pp. 445–454. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © T.N. Zhilina, D.G. Zavarzina, T.V. Kolganova, A.M. Lysenko, T.P. Tourova, 2009, published in Mikrobiologiya, 2009, Vol. 78, No. 4, pp. 496–505.

EXPERIMENTAL ARTICLES

Alkaliphilus peptidofermentans sp. nov., a New Alkaliphilic Bacterial Soda Lake Isolate Capable of Peptide Fermentation and Fe(III) Reduction

T. N. Zhilina[a,1], D. G. Zavarzina[a], T. V. Kolganova[b], A. M. Lysenko[a], and T. P. Tourova[a]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Bioengineering Center, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 1, Moscow, 117312 Russia*

Received July 1, 2008

Abstract—A novel strain, Z-7036, of anaerobic spore-forming bacteria was isolated from a cellulolytic consortium obtained from the bottom sediments of the low-mineralization soda lake Verkhnee Beloe (Buryatia). The cells of the new strain are short motile gram-positive rods, 1.1–3.0 × 0.25–0.4 μm. The organism is an aerotolerant anaerobe and obligate alkaliphile growing within the pH range of 7.5–9.7 with an optimum at pH 9.1. The strain is mesophilic and halotolerant and grows at NaCl concentrations from 0 to 50 g/l with an optimum at 20 g/l. Carbonates are required. The microorganism ferments peptone, yeast extract, trypticase, tryptone, Bacto Soytone, meat extract, Casamino acids, ornithine, arginine, threonine, and tryptophan. The strain hydrolyzes the bacterial preparations "Gaprin" and "Spirulina". Acetate and formate are the major fermentation products. The strain reduces amorphous ferric hydroxide (AFH), EDTA–Fe(III), anthraquinone-2,6-disulfonate (quinone), $S_2O_3^{2-}$, fumarate, and crotonate. Major fatty acids are $C_{16:0}$, $C_{16:1\omega7c}$, *iso*-C_{17}, *iso*-C_{15}, and *iso*-$C_{17:1}$. The DNA G+C content is 33.8 ± 0.5 mol %. According to the results of the 16S rRNA gene analysis, strain Z-7036 belongs to the genus *Alkaliphilus* within the cluster XI of low G+C gram-positive bacteria of the family *Clostridiaceae*. The novel strain is closely related to *A. transvaalensis* SAGM1^T and *A. crotonatoxidans* B11-2^T (93.3 and 93.9% 16S rRNA sequence identities, respectively). On the basis of the existing genotypic and phenotypic differences, we propose that strain Z-7036 should be classified as a novel species *Alkaliphilus peptidofermentans* sp. nov.

Key words: peptide fermentation, soda lakes, alkaliphiles, anaerobes, iron reduction, *Alkaliphilus*.

DOI: 10.1134/S0026261709040080

Anaerobic organisms that decompose the mortmass of primary and/or secondary producers are possible constituents of the microbial community of soda lakes. They should be involved in the beginning of the peptolytic pathway and be capable of fermenting nitrogen-containing components of the mortmass.

In the cellulolytic community of the low-mineralization soda lake Verkhnee Beloe (Buryatia), where the saccharolytic pathway predominates [1], we discovered a microorganism that did not utilize both carbohydrates or products of their fermentation but was able to grow on soluble protein extracts and individual amino acids; moreover, it could utilize the mortmass of both primary producers ("Spirulina") and secondary producers "(Gaprin", which is dried *Methylococcus* biomass).

Besides, the isolated bacterium was found to be able to reduce iron and to utilize both the ferric iron of the EDTA–Fe(III) complex and amorphous ferric hydroxide (AFH). To date, only one alkaliphilic organism, *Geoalkalibacter ferrihydriticus*, has been reported to be capable of reducing AFH with the resulting formation of magnetite or siderite [2]. In addition to the microorganism described in this paper, we isolated two novel strains belonging to the genus *Natronincola* capable of reducing iron in its insoluble form [4]. Thus, increasingly more information becomes available on this function of alkaliphilic anaerobes, which has recently been considered doubtful due to the low iron activity under alkaline conditions [3].

In this paper, a new representative of the genus *Alkaliphilus*, isolated from a soda lake and capable of reducing iron in its insoluble form during peptide fermentation is described.

MATERIALS AND METHODS

Isolation source. Strain Z-7036 was a constituent of the microbial anaerobic cellulose-decomposing community, with *Clostridium alkalicellulosi* as the cellulolytic microorganism [1] and *Alkaliflexus imshenetskii*

[1] Corresponding author; e-mail: zhilinat@mail.ru

as a microorganism involved in utilization of carbohydrates produced in the course of cellulose hydrolysis [5]. The community was initiated from a sample of the bottom sediments of a near-shore lagoon of the soda lake Verkhnee Beloe (Buryatia) (mineralization, 7.5 g/l; pH 10).

Cultivation conditions. For selective enrichment and obtaining of the new isolate, we used the mineral medium (pH 9.5) containing the following (g/l): KH_2PO_4, 0.2; $MgCl_2 \cdot 6H_2O$, 0.1; NH_4Cl, 0.5; KCl, 0.2; NaCl, 1.0; Na_2SO_4, 3.0; Na_2CO_3, 3.0; $NaHCO_3$, 10; yeast extract (Difco), 0.2; peptone (Difco), 2.0; $Na_2S \cdot 9H_2O$, 0.5; and trace element solution, 1 ml/l [6]. In optimized medium, the concentrations of Na_2CO_3, $NaHCO_3$, NaCl, and yeast extract were 3.0, 12.0, 5.0 (0.28 M Na^+), and 3.0 g/l, respectively. No changes were made in the other medium components. Inoculated media were incubated in the dark at 37°C for a week.

To elucidate the substrates used for catabolism, they were added to a concentration of 3 g/l. Carbohydrates were added to the alkaline medium in the form of concentrated sterile water solutions immediately before inoculation. The medium preparation and cultivation were carried out under strictly anaerobic conditions in a nitrogen atmosphere. AFH and EDTA–Fe(III) used as electron acceptors were prepared as described in [2].

Physiological properties. The electron acceptors were added to the sterile medium in the form of concentrated NH_4Cl-free water solutions in the following concentrations (mM): $Na_2S_2O_4$, 1.0; Na_2SO_3, 2 and 20; $Na_2S_2O_3 \cdot 5H_2O$, 10.0; Na_2SO_4, 20; $NaNO_2$, 2.0 and 10; $NaNO_3$, 10; anthraquinone-2,6-disulfonate, 20; Mn(IV) (in the form of artificially synthesized MnO_2), 25; EDTA–Fe(III), 20 + cysteine (0.3 g/l); AFH, 90 in terms of Fe(III) + cysteine, 0.3 g/l); Fe(III) citrate, 5.0; S°, 2% (w/v); crotonate, 10; and fumarate, 5.0.

To determine pH dependency, the required pH values were adjusted by titration with 10% HCl or 10% NaOH; sodium carbonate was substituted for by sodium bicarbonate. The concentration of sodium bicarbonate was reduced tenfold and sodium molarity was equalized with NaCl. pH was determined before and after the experiment. At the end of the experiment, the pH values changed insignificantly, by no more than 0.2 toward the low pH region. The requirement for NaCl, the dependence on carbonates and their optimal concentrations, and growth ranges were studied as described in [4]. The temperature dependency was studied in the range from 18 to 60°C at the optimum pH and NaCl concentration values.

The capacity for microbial growth and O_2 utilization was assessed by the addition of O_2% (0.2; 0.4; 1.4; 2.3; 4.2; 7; or 9.3 %) to the medium prepared under anaerobic conditions in the nitrogen atmosphere without a reducing agent. Bacterial biomass (measured as OD_{600} on a spectrophotometer) and O_2 concentrations (measured on gas chromatograph) were determined at the beginning and end of growth.

Catalase activity was assayed from the effect of a 3% hydrogen peroxide solution on bacterial cells.

Sensitivity to antibiotics was determined by adding concentrated sterile water solutions of relevant antibiotics to the medium to the final concentration of 100 mg/l.

Analytical methods. Growth rate was determined from the optical density of the cultures measured directly in Hungate tubes at 600 nm with a Specol-10 spectrophotometer (Jena, Germany) or a UNICO-2100 spectrophotometer. The contents of hydrogen, oxygen, and nitrogen were determined on an LHM-80 gas chromatograph (Russia) equipped with a katharometer detector. Fatty acids and alcohols were determined by HPLC on a Staier high pressure chromatograph (Russia) equipped with a refractometer detector. The separation was carried out on an Aminex HPX-87H column (BioRad, United States); 5 mM H_2SO_4 was used as an eluent. The content of dissolved hydrogen sulfide was determined colorimetrically from the formation of methylene blue using the Pachmayr method with N,N-dimethyl-*p*-phenylenediamine in the modification described in [7]. The reduction of nitrogen compounds was determined from N_2 production in the media with argon as the gas phase; NH_4^+ production from NO_2^{2-} or NO_3^{2-} was determined using the Nessler's reagent. Fe(III) reduction was determined by the colorimetric reaction with ferrocene; the sediment was predissolved in 0.6 N HCl [8]. Reduced iron-containing minerals formed during bacterial growth were determined by Mössbauer spectroscopy [9]. The fatty acid composition of microbial lipids was determined on a Microbial Identification System (Sherlock) chromatograph (MIDI Inc., Newark, United States) according to the technique described in [10]. The separated fatty acids were identified using an Agilent Technologies AT-5971 SMART mass spectrometer.

Morphology. The cell morphology in live specimens was examined under a ZETOPAN phase-contrast microscope (Austria). The ultrathin sections and whole cell specimens contrasted with 1% phosphotungstic acid to reveal flagella were examined under a JEM-100C electron microscope (Japan).

DNA analysis. The DNA G + C content was determined using the thermal denaturation curves according to the technique described in [11].

Isolation of DNA and amplification and sequencing of the 16S rRNA genes. Isolation and purification of the DNA preparations were performed according to the previously described technique [12].

Amplification and sequencing of the 16S rRNA genes employed primers universal for bacteria [13].

Sequencing of PCR products was performed by Sanger's method on an automatic ABI 3730 sequencer (Applied Biosystems, United States) using a Big Dye

Terminator sequencing kit (version 3.1) according to the manufacturer's instructions.

Phylogenetic analysis of the 16S rRNA gene sequences. The sequences were edited using the BioEdit sequence alignment editor [http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html]. The primary comparison of the de novo determined sequences with the sequences available in the GenBank database was performed using the NCBI BLAST software package [http://www.ncbi.nlm.nih.gov/blast]. The newly determined nucleotide sequences were aligned with the corresponding sequences of the most closely related bacteria using the CLUSTALW v. 1.75 software package. The phylogenetic tree of the studied strains was constructed by the methods implemented in the TREECONW [http://bioc-www.uia.ac.be/u/yvdp/treeconw.html] and PHYLIP [http://evolution.genetics.washington.edu/phylip.html] software packages.

Deposition of nucleotide sequences. The 16S rRNA gene sequences of strain Z-7036 were deposited in the GenBank under the accession number EF382660.

RESULTS

Isolation. Strain Z-7036 was as a part of the cellulolytic "consortium Z-7012," which developed on microcrystalline cellulose (MCC) supplemented with 0.2 g/l of yeast extract [1] and contained a minimum amount of bacterial forms. After heating at 90°C for 20 min, this community contained only thermotolerant rodlike cells, varying in length and width; some of them produced spores. The presence of strain Z-7036 in the consortium was revealed by inoculation of a medium containing yeast extract and peptone, where it grew until the sixth tenfold dilution. On peptone-containing medium, thin rods of different lengths were detected, some of which produced terminal spores; some of the cells were arranged in chains of five to six cells or produced long filaments, which indicated the population heterogeneity.

Isolation of a pure culture of the peptolytic strain Z-7036 from the consortium was carried out using agarized (2% Bacto agar) medium with peptone and yeast extract by isolation of single colonies in roll-tubes and then by the serial dilutions method in liquid medium.

The culture purity was confirmed by microscopic examinations, as well as by partial 16S rRNA gene sequencing with the use of various PCR primers.

Morphology. At the stage of exponential growth, the cells of strain Z-7036 are thin short rods, single or arranged in pairs, 1.15–3.0 μm long and 0.25–0.4 μm in diameter (Fig. 1a). Minicells are produced at cell poles or, in the course of division, in the middle of the dividing cells. Some cells were motile; however, we failed to detect any cells with flagella in whole-cell specimens. The cells produce loose slimy capsules (Fig. 1b). At advanced stages of growth, the cells were arranged in chains of up to twenty cells or thin curved filaments. The spores of strain Z-7036 are terminal and spherical. Under a light microscope, the cells are optically dense and exhibit a good adsorptive capacity for glass. The organism reproduces by binary division by constriction. Its cell wall structure is of the gram-positive type (Fig. 1c). The colonies formed by the strain are whitish pink, flat, slightly elevated in the center, moire, often with uneven slimy transparent edges, 0.1–1 mm in diameter.

Growth characteristics. Strain Z-7036 is an obligate alkaliphile growing within the pH range of 7.5–9.7 with an optimum at pH 9.1. At pH values lower than 6.8 or higher than 9.95, no growth occurred (Fig. 2). With respect to temperature, the organism is a typical mesophile growing within the temperature range of 6–40°C with an optimum of 35°C. At 15 and 6°C, growth is weak; the lag phase duration extends to 4 and 15 days, respectively. The isolate is an aerotolerant anaerobe and does not grow under aerobic conditions or in the media that contain 2.3% O_2, with the upper limit for growth of 1.4% O_2 in the gas phase. In the presence of oxygen, the organism does not utilize it and retains the capacity for anaerobic fermentation. During anaerobic growth, it is able to grow in the absence of reducing agents. A decrease in the redox potential due to the addition of cysteine, thioglycolate, or sulfide (0.3–0.5g/l) to the medium resulted in a decrease in the duration of the lag phase.

The organism does not require NaCl; however, it is halotolerant and grows at NaCl concentrations of up to 50 g/l with an optimum at 20 g/l. Carbonates are required for growth and cannot be substituted for by organic buffers. The optimum Na_2CO_3/$NaHCO_3$ concentration is 1.5–3.0/5.0–10.0 g/l (Fig. 3).

The strain, isolated on the medium with peptone and yeast extract, proved to be able to ferment peptone, yeast extract, trypticase, tryptone, Bacto Soytone, meat extract, Casamino acids, ornithine, arginine, and, to a lesser extent, threonine, tryptophan, and pyruvate; however, it was unable to ferment casein or albumin. The microorganism did not dissolve gelatin but showed weak growth on it. Acetate and formate, produced in equal proportions, were the main fermentation products; isobutyrate was detected in trace amounts.

Strain Z-7036 does not utilize carbohydrates, organic acids, or alcohols. On mineral media with vitamins or additionally added 50 mg/l of yeast extract, the organism failed to utilize the following compounds: sugars (arabinose, galactose, glucose, xylose, lactose, maltose, mannose, sucrose, sorbose, rhamnose, ribose, trehalose, fructose, fucose, raffinose, cellobiose, melibiose, and N-acetyl-D-glucosamine); sugar alcohols (inositol and sorbitol); alcohols (ethanol, methanol; ethylene glycol, 1,4-butanediol, and glycerol); organic acids (lactate, formate, propionate, succinate, malonate, betaine, glycolate, oxalate, and citrate); amino acids (glutamate, aspartate, asparagine, glutamine, pro-

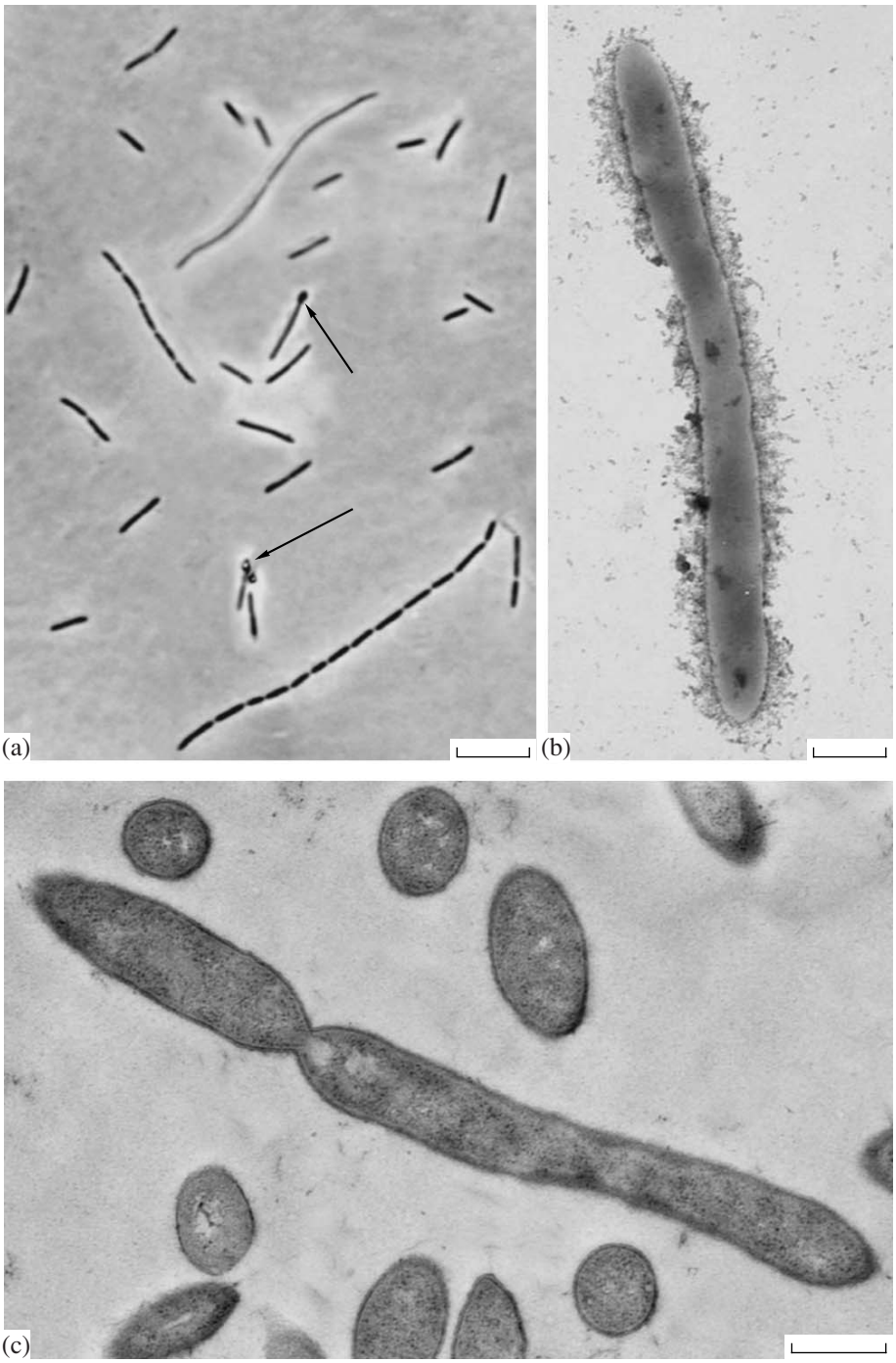

Fig. 1. Cell morphology of strain Z-7036: (a) light microscopy of vegetative cells and cells with spores (indicated by arrows); bar, 10 μm and electron microscopy (b) of a cell in a loose capsule; bar, 0.5 μm; and (c) of a longitudinal ultrathin cell section showing the cell wall structure typical of gram-positive bacteria.

line, lysine, valine, histidine, β-alanine, glycine, leucine, methionine, tyrosine, serine, and uracil); proline + valine, proline + alanine, proline + isoleucine, proline + leucine, proline + histidine, glycine + valine, glycine + alanine, glycine + isoleucine, glycine + leucine, glycine + histidine; and biopolymers (xylan).

The ability of the studied strain to utilize dead algal or bacterial biomass under natural conditions was assessed. The strain grew on the commercial preparations "Gaprin" (dried *Methylococcus* biomass) and "Spirulina" as the sole energy sources. The amounts of the main metabolites produced during growth on

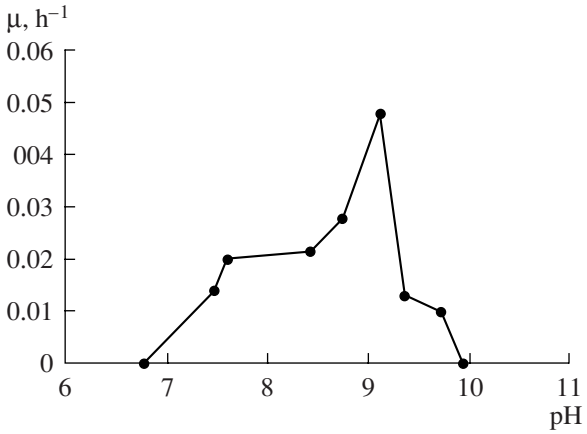

Fig. 2. pH-dependence of the growth rate of strain Z-7036

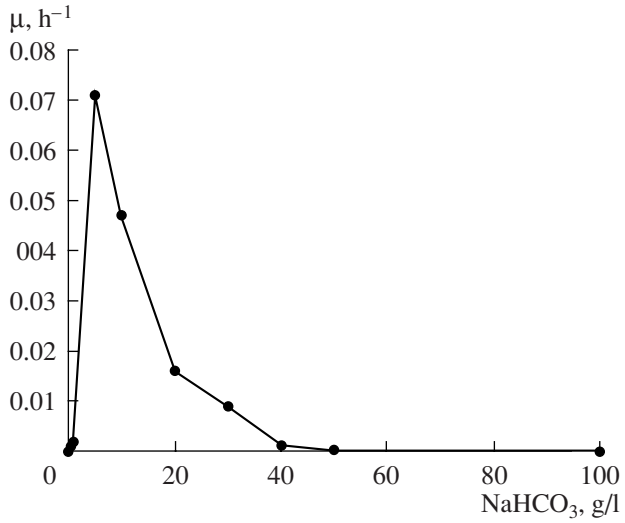

Fig. 3. Dependence of the growth rate of strain Z-7036 on the carbonate concentrations at a $Na_2CO_3/NaHCO_3$ ratio of 0.3. The concentration of Na_2CO_3 at each point is equal to the $NaHCO_3$ concentration × 0.3.

"Gaprin" and "Spirulina," as well as their compositions (3.4 mM acetate and 4.4 mM formate in the case of "Gaprin"; 3.6 mM acetate and 4.0 mM formate in the case of "Spirulina") were comparable to those produced during growth on yeast extract. During growth of strain Z-7036 on "Gaprin" and "Spirulina," *Methylococcus* and *Spirulina* cells were lysed and became transparent compared to the control with optically dense cells. The optical density of the experimental cell suspension decreased significantly as compared to the control (Table 1). Strain Z-7036 did not grow on sterilized cell biomass of *"Euhalotheca," Microcoleus*, and *Clostridium alkalicellulosi*). The lack of growth on the biomass of theses microorganisms sterilized by autoclaving indicates the requirement for the more advanced processing of the substrate attained by steam drying of the commercial preparations "Gaprin" and "Spirulina."

Growth in the presence of external electron acceptors. When studying the possibility of utilization of external electron acceptors with yeast extract as an electron donor, it was demonstrated that strain Z-7036 does not reduce S^0 and sulfur oxides (SO_4^{2-}, and SO_3^{2-}), with the exception of the weak reduction of $S_2O_3^{2-}$ with the resulting formation of 1.6 mM H_2S. Sulfur compounds do not produce any inhibitory effect on the growth of strain Z-7036. The strain does not reduce nitrogen oxides. Growth in the presence of NO_3^- (10 mM) and NO_2^- (2 mM) was comparable to the control; however, 10 mM NO_2^- completely inhibited growth. Fumarate and crotonate were utilized as electron acceptors; 4.2 mM succinate, 4.9 mM acetate, and 2.9 mM formate were formed from 5 mM fumarate (in the case of its complete utilization). The strain dismutated 10 mM crotonate to 6.4 mM acetate and 3.2 mM butyrate, with a significant shift towards enhanced acetate production, as compared to fermentation of yeast extract.

The strain reduced AFH with the resulting formation of black solid sediment consisting of iron sesquioxides, siderite; it reduced EDTA–Fe(III), but not citrate–Fe(III). Moreover, growth was observed on quinone, a humic acid analogue. The reduction of quinone was judged from changes in the medium pigmentation (from light yellow to deep brown); the reduction of EDTA–Fe(III) was judged from the medium discoloration. The strain did not reduce Mn(IV). In the presence of 90 mM AFH and 0.3 g/l of cysteine, the formation of the products of yeast extract fermentation was twice as high, with a significant shift towards enhanced acetate production, as compared to the values obtained in the case of the AFH-free control (Fig. 4); by day 9 of incubation, the production of 24.3 mM reduced Fe(II) was observed.

Sensitivity to antibiotics. The growth of strain Z-7036 was not affected by the addition of 100 mg/l of ampicillin, rifampicin, kanamycin, chloramphenicol, penicillin, neomycin, or novobiocin, whereas streptomycin, vancomycin, and bacitracin completely inhibited growth.

Analysis of the fatty acid (FA) composition. The lipids of the cells membranes of strain Z-7036 contained the following fatty acids (% of the total): $C_{i15:1}$

Table 1. Lytic effect of strain Z-7036 on *Spirulina* and *Methylococcus* cells during growth on the commercial preparations (1 g/l)

Preparation suspension	Initial OD_{540}	Final OD_{540}
"Spirulina"	1.04	0.78
"Gaprin"	0.87	0.51

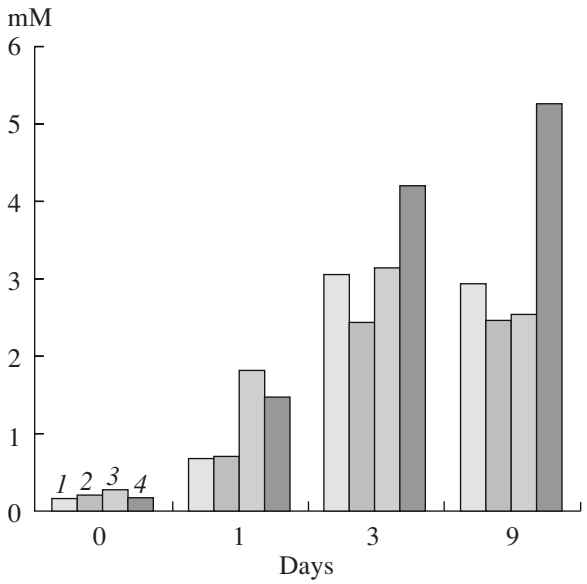

Fig. 4. Effect of AFH on the formation of the products of yeast extract oxidation by strain Z-7036: production of (*1*) formate and (*2*) acetate on medium with yeast extract and production of (*3*) formate and (*4*) acetate on medium with yeast extract and AFH.

0.6; C_{i15} 10.52; C_{ai15} 0.64; $C_{15:0}$ 0.85; $C_{i16:1d9t}$ 0.68; C_{i16} 0.53; $C_{16:1\omega7c}$ 14.5; $C_{16:1\omega7t}$ 1.37; $C_{16:0}$ 20.11; C_{i16a} 0.76; $C_{16:1acis9}$ 2.31; $C_{16:1atrans}$ 0.21; $C_{i17:1}$ 8.84; C_{16a} 5.59; C_{i17} 11.26; C_{ai17} 1.4; $C_{17:1\omega6}$ 0.23; $C_{i17:1a}$ 1.72; $C_{ai17:1a}$ 0.68; $C_{17:0}$ 0.56; C_{i17a} 4.14; C_{ai17a} 0.16; $C_{18:1\omega9}$ 4.89; $C_{18:1\omega7}$ 1.37; $C_{18:0}$ 1.88; $C_{18:1\omega9a}$ 1.15; $C_{18:1\omega7a}$ 0.27; $C_{18:0a}$ 0.3.

Phylogenetic analysis. According to the phylogenetic analysis of the 16S rRNA gene sequences, strain Z-7036 clustered with strains of the genus *Alkaliphilus* within cluster XI of the low G+C gram-positive bacteria of the family *Clostridiaceae*. Within this cluster, the novel strain formed a separate branch. The level of similarity with the closest species of the genus *Alkaliphilus* was 93.9–94.2%, which confirms the affiliation of strain Z-7036 to the new species of the genus *Alkaliphilus*. Among members of this cluster, *"A. metalliredigens"* [3] (94.2% similarity) and two effectively published species, *A. transvaalensis* SAGM1^T [14] (93.3% similarity) and *A. crotonatoxidans* B11-2^T [15] (93.9% similarity) were closely related to the new strain. At the same time, the similarity level between the 16S gene nucleotide sequences of strain Z-7036 and that of the other member of this cluster, *Tindallia magadiensis* [16], did not exceed 89%. The constructed phylogenetic tree (Fig. 5) illustrates the taxonomic position of strain Z-7036 within the genus *Alkaliphilus*.

The G+C content of the DNA of strain Z-7036 is 33.8 ± 0.5 mol %.

DISCUSSION

The bacterium isolated from the cellulolytic community was found to be incapable of hydrolyzing cellulose or fermenting carbohydrates produced in the course of cellulose degradation. A small amount of yeast extract, which was present in the medium as a growth factor was sufficient to stimulate the development of the new strain within the community.

The studied strain is an anaerobic alkaliphilic halotolerant peptide-utilizing organoheterotroph; it is involved in the proteolytic pathway within the anaerobic community. Strain Z-7036 does not utilize sugars or nitrogen-free fermentation products as substrates, except for pyruvate as a substrate of the central metabolism. It is capable of weak growth on an amino acid mixture (Casamino acids) and does not drive the Stickland reaction. The organism does not ferment most amino acids, except for ornithine, arginine, and, to a lesser extent, threonine and tryptophan. Thus, peptides are possible substrates of its trophic specialization in the microbial community. There is a widespread opinion that oligopeptides are more available to bacteria

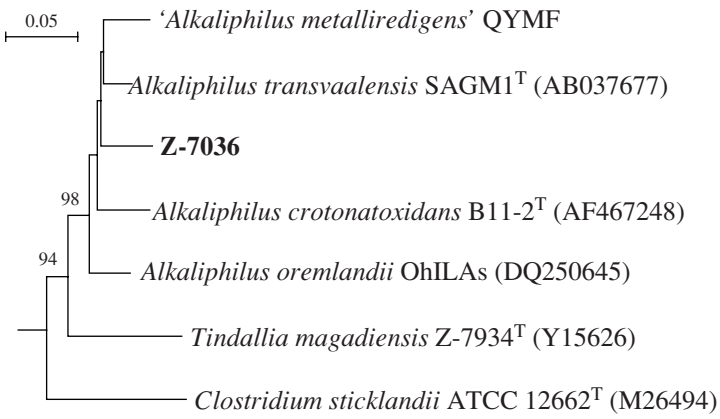

Fig. 5. Phylogenetic position of the new strain Z-7036 among members of the genus *Alkaliphilus* within the cluster XI of low G + C gram-positive bacteria. The phylogenetic tree was constructed based on comparative analysis of 16S rRNA gene sequences. Bar corresponds to five nucleotide substitutions per 100 nucleotides.

than free amino acids due to transport limitations [17]. The organism, like the previously described *Tindallia magadiensis* [16], was found to be highly specialized with respect to amino acid utilization; arginine and ornithine are preferential. Although, in the cellulolytic community, the organism was a satellite of the main culture, *C. alkalicellulosi* [1], it was found incapable of lysing both living cells and the sterilized mortmass of the primary producers. Hence, it utilizes soluble low-molecular-weight protein products, but it is capable of growth on the insoluble bacterial preparations "Gaprin" and "Spirulina," being thus involved in solid-phase fermentation.

With respect to the pH dependency, obligate requirements for carbonates and low salinity, the new isolate is an obligate, but moderately alkaliphilic halotolerant microorganism. Judging from its osmotic characteristics, the organism is an inhabitant of moderately mineralized waters with a relatively low (less than 50 g/l) upper limit of mineral salt content.

The strain is a moderately aerotolerant microorganism. It is able to grow in the presence of 1.4% O_2; however, it does not utilize oxygen as an electron acceptor and performs fermentation, which allows us to identify the studied strain as an obligate anaerobe.

Utilization of some organic and inorganic electron acceptors, fumarate, crotonate, quinone, AFH, or EDTA–Fe(III), indicates that this organism is able to switch from the fermentative to the respiratory type of metabolism.

Many alkaliphilic bacteria with a fermentative type of metabolism are capable of reducing various inorganic compounds; some of them are able to reduce soluble forms of ferric iron, carrying out the so-called facilitated fermentation [4, 11, 16, 18]. The new isolate, being incapable of reducing nitrogen and sulfur compounds, with the exception of weak reduction of thiosulfate, actively reduced, in addition to soluble forms of ferric iron, AFH, with the resulting formation of 27% Fe(II) of the initial AFH content. In the course of iron reduction, the rate of product formation increased significantly as compared to the Fe(III)-free medium, like in the case of other peptide-utilizing species of the genus *Natronincola* [4], which presents a strong argument for the assumption that these organisms get additional energy via iron reduction.

According to the phylogenetic position of the isolated organism, it belongs to the genus *Alkaliphilus*, which currently includes four species; only two of them, *A. transvaalensis* and *A. crotonatoxidans*, have been validly described [14, 15]. The genus includes three microorganisms with a fermentative type of metabolism, one alkalitolerant microorganism (*A. crotonatoxidans*) [15], one facultatively alkaliphilic bacterium (*"A. oremlandii"*) [19], as well as the obligately and extremely alkaliphilic microorganism *A. transvaalensis* [14]; only for *"A. oremlandii"* [19] has the capacity for the metal reduction been demonstrated. Another moderately alkaliphilic microorganism, *"A. metalliredigens"* [3], is incapable of fermentation and is a chemoorganotroph that utilizes Co(III)–EDTA, Cr(VI), and ferric iron as electron acceptors and yeast extract, lactate, or acetate as electron donors. *"A. metalliredigens"*, like *Geoalkalibacter ferrihydriticus* [2], is capable of the dissimilatory reduction of Fe(III), which it utilizes in the form of Fe(III)–citrate and Fe(III)–EDTA complexes. *"A. metalliredigens"* does not reduce AFH, which allowed Ye et al., [3] to doubt the possibility of such a process under alkaline conditions. Unlike *"A. metalliredigens"*, strain Z-7036 is able to reduce AFH, but not the Fe(III)–citrate complex.

As in the case of *"A. metalliredigens"*, the main fatty acids of strain Z-7036 are hexadecanoic ($C_{16:0}$) and *cis*-9-hexadecenoic ($C_{16:1\omega7c}$) acids, which make up 34.6% of the total fatty acids. At the same time, relatively high concentrations of branched acids (*iso*-pentadecanoic (C_{i15}), *iso*-heptadecanoic (C_{i17}), and *iso*- heptadecenoic ($C_{i17:1}$), which were not detected in the other *Alkaliphilus* species make up 30.6% of the total fatty acids. *Iso*-pentadecenoic acid ($C_{i15:1}$), which was detected in the other *Alkaliphilus* species and prevailed in *A. transvaalensis* (42.6%) was absent. The proportion of tetradecanoic acid ($C_{14:0}$), the main fatty acid of *A. crotonatoxidans* (45.6%) and *A. transvaalensis* (24.4%), was only 2.02%. The presence of a large amount of *cis*-9-hexadecenoic acid ($C_{16:1\omega7c}$) in the FA profile of the studied strain corresponds well with the previous suggestion [3] that this acid may be a usual constituent of membranes of metal-reducing bacteria. The fact that this amino acid is predominant in the FA profile of the previously isolated by us two alkaliphilic species of *Natronincola* [4], which are capable of reducing AFH, supports the idea of using this acid as a marker for metal reduction.

In addition to differences in the FA profiles, *A. transvaalensis* and *A. crotonatoxidans* differ from the studied strain in some phenotypic characteristics. *A. transvaalensis* is an extreme alkaliphile; it reduces crotonate, fumarate, and some sulfur compounds, but not iron compounds. Unlike strain Z-7036, *A. transvaalensis* does not utilize Casamino acids. The moderate alkaliphilic *A. crotonatoxidans*, isolated from waste waters, is the only sugar-fermenting representative of *Alkaliphilus. A. crotonatoxidans* is incapable of chemoorganotrophic growth in the presence of electron acceptors other than crotonate [15]. *A. oremlandii*, the microorganism that was recently described but not yet validated should be mentioned as well. This is a moderately alkaliphilic microorganism (optimum pH 8.4) with a fermentative type of metabolism, capable of fermenting lactate, fructose, and glycerol and utilizing external electron acceptors (arsenate and thiosulfate) [19]. This brief description indicates that there are significant phenotypic differences between *A. oremlandii* and strain Z-7036.

Table 2. Comparison of the new isolate with the phylogenetically close species of the genus *Alkaliphilus*

Properties	Z-7036^T	*A. transvaalensis* SAGM^T [14]	*A. crotonatoxidans* B11–2^T [15]	"*A. metalliredigens*" QYMF^T [3]
Morphology: slightly curved rods	+	+	+	+
Size, μm	0.25–0.4 × 1.15–3.0	0.4–0.7 × 3.0–6.0	0.4–0.6 × 2.0–3.0	0.5 × 3.0–6.0
Motility	+	+	+	+
Flagella	–	+	+	–
Obligate anaerobe	+, aerotolerant	+	+	+
Fermentative type of metabolism	+	+	+	–
Electron acceptors	Fumarate, crotonate, $S_2O_3^{2-}$, quinone, AFH, Fe(III)–EDTA	Fumarate, crotonate, S^0, $S_2O_3^{2-}$	Crotonate	Fe(III)-citrate, Fe(III)-EDTA, Co(III)-EDTA, Cr(VI)
Temperature, °C (optimum)	6–40 (35)	20–50 (40)	20–40 (35)	4–45 (35)
NaCl, g/l (optimum)	0–50 (20)	0–33 (5)	0	0–80 (20)
pH (optimum)	7.5–9.7 (9.1)	10 (8.5–12.5)	5.5–9.0 (7.5)	7.0–11.0 (9.5)
Substrates:				
tryptone or peptone	+	+	+	–
yeast extract	+	+	+	+*
casamino acids	+	–	ND	ND
casein	–	+	–	–
Sugars	–	–	+	–
Major fatty acids	$C_{16:0}$, $C_{16:1\,\omega7c}$, C_{i17}, C_{i15}, $C_{i17:1}$	$C_{i15:0}$, $C_{14:0}$, $C_{16:0}$	$C_{14:0}$, $C_{16:0}$	$C_{16:0}$, $C_{16:1\omega7c}$, $C_{14:1}$
DNA G+C content, mol %	33.8	36.4	30.6	ND
Isolation source	Cellulolytic microbial community of a soda lake (Buryatia, Russia)	Deep alkaline waters of a gold mine (South Africa)	Butyrate-degrading methanogenic consortium from agricultural waste waters (Beijing, China)	Alkaline pond with boron-containing waste waters (Boron, California)

Note: *As a donor.

The characteristics that distinguish the new isolate strain from other representatives of the genus are listed in Table 2.

On the basis of the existing phylogenetic and phenotypic differences, we propose that strain Z-7036 should be classified as a novel species of the genus *Alkaliphilus*, *Alkaliphilus peptidofermentans* sp. nov.

Description of *Alkaliphilus peptidofermentans* sp. nov.

N. L. n. *peptidum*, peptides; L. part. adj. *fermentans*, to ferment; N. L. *peptidofermentans*, peptide-fermenting organism.

Rod-shaped, short motile cells measure 1.15–3 × 0.25–0.4 μm. The cells are single or arranged in pairs or short chains. The cells are spore-forming and divide by constriction. The structure of the cell wall is of the gram-positive type. Aerotolerant, catalase-positive anaerobe. Obligate alkaliphile. Growth occurs in a pH range of 7.5–9.7, with an optimum at 9.1. Mesophile. The temperature range for growth is 6– 40°C, with an optimum at 35°C. Halotolerant; growth occurs in a salinity range of 0 to 50 g/l NaCl, with an optimum at 20 g/l. Natronophile. The optimum Na_2CO_3/$NaHCO_3$ concentration is 1.5–3.0/5.0–10.0 g/l. Organoheterotroph. Ferments peptone, yeast extract, trypticase,

tryptone, soytone, meat extract, Casamino acids, ornithine, arginine, and, much less actively, threonine, tryptophan, and pyruvate. Hydrolyzes the bacterial preparations "Gaprin" and "Spirulina." Acetate, formate (1 : 1), and isobutyrate (in trace concentrations) are the products formed in the course of yeast extract fermentation. The organism is incapable of fermentation of sugars, organic acids (with the exception of pyruvate), and alcohols. Capable of chemoorganotrophic growth: utilizing AFH, EDTA–Fe(III), anthraquinone-2,6-disulfonate, $S_2O_3^{2-}$, fumarate, and crotonate as electron acceptors. Does not reduce Mn(IV). Reduces AFH with the resulting formation of Fe^{2+} sesquioxides and siderite. The major fatty acids are $C_{16:1\omega7c}$ (14.56%), $C_{16:0}$ (20.11%), C_{i17} (11.26%), $C_{i17:1}$ (8.84%), and C_{i15} (10.52%).

The DNA G+C content is 33.8 ± 0.5 mol%.

The isolation source is bottom sediments of soda lakes. The type strain is Z-7036^T (= VKM B-2502 = DSMZ 18978), isolated from the low-mineralization soda lake Verkhnee Beloe (Transbaikal Region).

ACKNOWLEDGMENTS

The authors thank N.I. Chistyakova for Mössbauer spectroscopy of solids, N.A. Kostrikina for electron microscopic research, and V.V. Kevbrin for help in determination of metabolites.

This work was supported by the programs of the Presidium of the Russian Academy of Sciences "Origin of the Biosphere and Evolution of Geobiological Systems" and "Molecular and Cell Biology" and the program NSh–1221.2008.4 (state contract no. 02.740.11.0023 with Russian Federal Agency of Science and Innovation (Rosnauka).

REFERENCES

1. Zhilina, T.N., Kevbrin, V.V., Tourova, T.P., Lysenko, A.M., Kostrikina, N.A., and Zavarzin, G.A., *Clostridium alkalicellum* sp. nov an Obligately Alkaliphilic Cellulolytic Bacterium from a Soda Lake in the Baikal Region, *Mikrobiologiya*, 2005, vol. 74, no. 5, pp. 642-653 [*Microbiology* (Engl. Transl.), vol. 74, no. 5, pp. 557–568].
2. Zavarzina, D.G., Kolganova, T.V., Boulygina, E.S., Kostrikina, N.A., Tourova, T.P., and Zavarzin, G.A., *Geoalkalibacter ferrihydriticus* gen. nov. sp. nov., the First Alkaliphilic Representative of the Family *Geobacteraceae*, Isolated from a Soda Lake, *Mikrobiologiya*, 2006, vol. 76, no. 6, pp. 673–678 [*Microbiology* (Engl. Transl.), vol. 76, no. 6, pp. 673–682].
3. Ye, Q., Roh, Y., Carroll, S.L., Blair, B., Zhou, J., Zhang, C.L., and Fields, M., Alkaline Anaerobic Respiration: Isolation and Characterization of a Novel Alkaliphilic and Metal-Reducing Bacterium, *Arch. Microbiol.*, 2004, vol. 70, pp. 5595–5602.
4. Zhilina, T.N., Zavarzina, D.G., Osipov, G.A., Kostrikina, N.A., and Tourova, T.P., *Natronincola ferrireducens* sp. nov. and *Natronincola peptidovorans* sp. nov., New Anaerobic Alkaliphilic Peptolytic Iron-reducing Bacteria Isolated from Soda Lakes, *Mikrobiologiya*, 2009, vol. 78, no. 4 [*Microbiology* (Engl. Transl.), vol. 78, no. 4].
5. Zhilina, T.N., Appel, R., Probian, Ch., Brossa, E., L., Harder, J.,Widdel, F., and Zavarzin, G.A. *Alkaliflexus imshenetskii* gen. nov., sp. nov.-a New Alkaliphilic Gliding Carbohydrate-Fermenting Bacterium with Propionate Formation from a Soda Lake, *Arch. Microbiol.*, 2004, vol. 182, pp. 244–253.
6. Kevbrin, V.V. and Zavarzin, G.A., Effect of Sulfur Compounds on the Growth of the Halophilic Homoacetic Bacterium *Acetohalobium arabaticum, Mikrobiologiya*, 1992, vol. 61, no. 5, pp. 812–817.
7. Trüper, H.G. and Schlegel, H.G., Sulfur Metabolism in *Thiorhodaceae.* Quantitative Measurements on Growing Cells of *Chromatium okenii, Antonie van Leeuwenhoek*, 1964, vol. 30, pp. 225–238.
8. Lovley, D.R. and Phillips, E.J.P., Organic Matter Mineralization Is the Reduction of Ferric Iron in Anaerobic Sediments, *Appl. Environ. Microbiol.*, 1986, vol. 51, pp. 683–689.
9. Chistyakova, N.I., Rusakov, V.S., and Zavarzina, D.G., Mössbauer Study of the Kinetics and Formation Conditions of Iron Minerals by Thermophilic Iron Reducers, *Izv. Akad. Nauk, Ser. Fiz.*, 2003, vol. 67, no. 9, pp. 1354–1358.
10. Stead, D.E., Sellood, J.E., Wilson, J., and Viney, I., Evaluation of a Commercial Microbial Identification System Based on Fatty Acid Profiles for Rapid, Accurate Identification of Plant Pathogenic Bacteria, *J. Appl. Bacteriol.*, 1992, vol. 72, pp. 315–321.
11. Garnova, E.S., Zhilina, T.N., Tourova, T.P., and Lysenko, A.M., *Anoxynatronum sibiricum* gen. nov., sp. nov., Alkaliphilic Saccharolytic Anaerobe from Cellulolytic Community of Nizhnee Beloe (Transbaikal Region), *Extremophiles*, 2003, vol. 7, pp. 213–220.
12. Boulygina, E.S., Kuznetsov, B.B., Marusina, A.I., Tourova, T.P., Kravchenko, I.K., Bykova, S.A., Kolganova, T.V., and Galchenko, V.F., A Study of Nucleotide Sequences of *nifH* Genes of Some Methanotrophic Bacteria, *Microbiology* [Engl. Transl. of *Mikrobiologiya*,] 2002, vol. 71, no. 4, pp. 425–432.
13. Lane, D.J, 16S/23S rRNA Sequencing, *Nucleic Acid Techniques in Bacterial systematics*, Stackebrandt, E.. and Goodfellow, M., Eds., Chichester,: UK: John Wiley & Sons, 1991, pp. 115–177.
14. Takai, K., Moser, D.P., Onstott, T.C., Spoelstra, N., Pfiffner, S.M., Dohnalkova, A., and Fredrickson, J.K., *Alkaliphilus transvaalensis* gen. nov., sp. nov., an Extremely Alkaliphilic Bacterium Isolated from a Deep South African Gold Mine, *Int. J. Syst. Evol. Microbiol.*, 2001, vol. 51, pp. 1245–1256.
15. Cao, X., Liu, X., and Dong, X., *Alkaliphilus crotonatoxidans* sp. nov., a Strictly Anaerobic, Crotonate-dismutating Bacterium Isolated from a Methanogenic Environment, *Int. J. Syst. Evol. Microbiol.*, 2003, vol. 53, pp. 971–975.
16. Kevbrin, V.V., Zhilina, T.N., Rainey, F.A., and Zavarzin, G.A., *Tindallia magadii* gen. nov., sp. nov.: an Alkaliphilic Anaerobic Ammonifier from Soda Lake Deposits, *Curr. Microbiol.*, 1998, vol. 37, pp. 94–100.

17. Sallam, A. and Steinbüchel, A., Anaerobic and Aerobic Degradation of Cyanophycin by the Denitrifying Bacterium *Pseudomonas alkaligenes* Strain DIP1 and Role of Three Other Isolates in Mixed Bacterial Consortium, *Appl. Environ. Microbiol.*, 2008, vol. 74, pp. 3434–3443.
18. Pollock, J., Weber, K.A., Lack, J., Achenbach, L.A., Mormile, M.R., and Coates, J.D. Alkaline Iron (III) Reduction by a Novel Alkaliphilic halotolerant, Bacillus sp. Isolated from Salt Flat Sediments of Soap Lake, *Appl. Microbiol. Biotechnol.*, 2007, vol. 77, pp. 927–934.
19. Fisher, E., Dason, A.M., Polshyna, G., Lisak, J., Crable, B., Perera, E., Ranganathan, M., Thangavelu, M., Basu, P., and Stolz, J.F., Transformation of Inorganic and Organic Arsenic by *Alkaliphilus oremlandii* sp. nov. Strain OhILAs, *Ann. N. Y. Acad. Sci.*, 2008, vol. 1125, pp. 230–241.