*Microbiology, Vol. 74, No. 6, 2005, pp. 695–703. Translated from Mikrobiologiya, Vol. 74, No. 6, 2005, pp. 800–809.*


**EXPERIMENTAL ARTICLES**

# "*Candidatus* Contubernalis alkalaceticum," an Obligately Syntrophic Alkaliphilic Bacterium Capable of Anaerobic Acetate Oxidation in a Coculture with *Desulfonatronum cooperativum*

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Received June 20, 2005

**Abstract**—From the silty sediments of the Khadyn soda lake (Tuva), a binary sulfidogenic bacterial association capable of syntrophic acetate oxidation at pH 10.0 was isolated. An obligately syntrophic, gram-positive, spore-forming alkaliphilic rod-shaped bacterium performs acetate oxidation in a syntrophic association with a hydrogenotrophic, alkaliphilic sulfate-reducing bacterium; the latter organism was previously isolated and characterized as the new species *Desulfonatronum cooperativum.* Other sulfate-reducing bacteria of the genera *Desulfonatronum* and *Desulfonatronovibrio* can also act as the hydrogenotrophic partner. Apart from acetate, the syntrophic culture can oxidize ethanol, propanol, isopropanol, serine, fructose, and isobutyric acid. Selective amplification of 16S rRNA gene fragments of the acetate-utilizing syntrophic component of the binary culture was performed; it was found to cluster with clones of uncultured gram-positive bacteria within the family *Syntrophomonadaceae*. The acetate-oxidizing bacterium is thus the first representative of this cluster obtained in a laboratory culture. Based on its phylogenetic position, the new acetate-oxidizing syntrophic bacterium is proposed in the *Candidatus* status for a new genus and species: "*Candidatus* Contubernalis alkalaceticum."

*Key words*: obligate syntrophy, acetate, sulfate reduction, alkaliphiles, anaerobes, soda lakes.

Acetate is an important terminal product formed and accumulated in the course of fermentation of organic matter by the anaerobic microbial community. Anaerobic acetate oxidation is performed by secondary anaerobes, a group that includes nitrate, sulfate, and iron reducers and methanogens. The acetate-oxidizing representatives of these groups of secondary anaerobes have been isolated from a range of habitats with neutral pH and are presently well studied. For the alkaliphilic microbial communities of soda lakes, however, no aceticlastic anaerobes are known except denitrifiers of the genus *Halomonas* [1].

Predominance of sulfidogenesis over methanogenesis caused by the developed sulfur cycle is characteristic of soda lakes [2, 3]. Sulfate-reducing bacteria (SRB) can therefore be expected to play an important role in anaerobic sulfate oxidation in these environments. The study of the stages of cellulose decomposition by the alkaliphilic microbial community of Khadyn and Verkhnee Beloe soda lakes did in fact demonstrate that acetate produced during the first stage of cellulose decomposition was oxidized via sulfidogenesis [4]. The attempts at direct isolation of an alkaliphilic acetate-oxidizing SRB were, however, unsuccessful.

Acetate oxidation concomitant with the reduction of sulfate to hydrogen sulfide occurred permanently in an enrichment culture with acetate and sulfate obtained from samples of Khadyn Lake (Tuva) sediments. Several forms of rods, including spore formers, and a motile vibrio morphologically similar to alkaliphilic SRB were present in the culture. When isolated in pure culture, the vibrio was incapable of acetate oxidation but utilized hydrogen as an electron donor. It was classified as a new species in the genus *Desulfonatronum*, *D. cooperativum* [5].

The goal of the present work was to investigate syntrophic acetate oxidation by the binary alkaliphilic culture consisting of the hydrogen-oxidizing SRB *D. cooperativum* and an obligately syntrophic acetate-oxidizing bacterium. Among the tasks of the work were verification of the obligatory nature of the syntrophy and identification of the syntrophic component of the binary culture by amplification of the conservative regions of its 16S rRNA gene.

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0026-2617/05/7406-0695 

## MATERIALS AND METHODS

**Source of isolation of the syntrophic culture.** Samples of reduced black sulfidogenic sediments and water from Khadyn Lake (Tuva, Russia) were collected for the purpose of investigating anaerobic acetate oxidation. The water samples had pH 9.5, a total salt content of 10 g/l, and an alkalinity of 450 mg-equiv/l.

**Cultivation conditions.** For the transfers of enrichment cultures, the following medium was used (g/l): $NH_4Cl$, 0.5; $KH_2PO_4$, 0.2; $MgCl_2 \cdot 6H_2O$, 0.1; KCl, 0.2; yeast extract (Difco), 0.2; $Na_2CO_3$, 6.5; $NaHCO_3$, 3.25; NaCl, 4.18; $Na_2SO_4$, 3.0; sodium acetate, 3.0; trace element solution [6], 1 ml/l; $Na_2S \cdot 9H_2O$, 0.5; pH 10.0. The transfers were performed in 120-ml vials with a 70-ml gas phase using anaerobic techniques, in an atmosphere of $N_2$. The incubation was performed at 35°C in the dark.

**Physiological characteristics.** For the experiments on the utilization of various electron donors by the syntrophic culture or by its acetate-oxidizing component, sodium acetate was replaced with 3 g/l of organic acids, sugars, or peptides or with 5 g/l of alcohols or with 100% $H_2$ in the gas phase.

**Microscopic and analytical techniques.** Living cells were examined under a ZETOPAN phase-contrast microscope (Reichert, Austria). Ultrathin cell sections were prepared as described previously [7] and examined under a JEM-100C electron microscope (Jeol, Japan). Dissolved hydrogen sulfide was determined colorimetrically by the methylene blue reaction, as described previously [7]. Acetate consumption was determined as described previously [4].

**DNA isolation** from bacteria was performed according to the procedure described in [8]. With this method, the DNA concentration in the preparations was 30–50 μg/l. In these preparations, RNA was present in trace amounts (less than 1% as determined by electrophoretic analysis). Two independent DNA preparations were obtained.

**Amplification and sequencing of the 16S rRNA gene.** The interference from the SRB DNA, which was preferentially amplified, was the main difficulty in determining the phylogenetic position of the acetate-oxidizing bacterium of the syntrophic culture. For selective amplification of the 5' fragment of the 16S rRNA gene of the gram-positive component of the binary culture, the 11F forward and 685R3 reverse universal primers were used, which allow amplification of this fragment in most gram-positive bacteria and cyanobacteria [9]. However, since no similar primer existed for selective amplification of the remaining part of the 16S rRNA gene of gram-positive bacteria, a new supplementary primer was developed.

The search for the conservative regions suitable for the development of the primer was performed by aligning complete 16S rDNA nucleotide sequences available from the Ribosomal Database Project (RDP) database (http://rdp.cme.msu.edu). Computer analysis of complete sequences (over 1500 nucleotides) of the 16S rRNA genes from the RDP database was performed. The requirements to the fragments suitable for the development of the primer were their highly conservative nature for all of the representatives of the clostridial line of gram-positive bacteria used for the alignment and their difference from similar fragments of the deltaproteobacterial sequences, including the one previously determined for the SRB of this culture. After thorough analysis of the aligned sequences of the 16S rRNA genes, the most conservative region corresponding to *Escherichia coli* positions 290–318 was chosen. On the basis of its consensus sequence, the clostrF primer (5'-GAGGGTGATCGGCCACATTGGRACTGAG-3') was developed; it was used for selective amplification of the distal fragment of the 16S rRNA gene of the investigated organism together with the universal reverse primer 1492R.

For the polymerase chain reaction and for the subsequent sequencing of the PCR fragments of the 16S rRNA gene, both primer pairs were used, 11F–685R3 and clostrF–1492R. The PCR reaction mixture contained primers, 5 pmol each; 10× buffer (17 mM $(NH_4)_2SO_4$, 6 mM *Tris*–HCl, pH 8.8, 1.5 mM $MgCl_2$), 5 μl; 2 mM dNTP, 5 μl; *BioTaq* polymerase (Dialat, Moscow, 5 U/μl), 0.5 μl; template DNA, 50 ng; and $H_2O$, up to 50 μl. The reaction was performed according to the following scheme: first cycle: 94°C, 9 min; 50°C, 1 min; 72°C, 2 min; 30 subsequent cycles: 94°C, 0.5 min; 50°C, 1 min; 72°C, 2 min; final polymerization, 7 min. The PCR products were analyzed electrophoretically in 0.8% agarose gel at 6 V/s.

The isolation and purification of the PCR products corresponding to various fragments of the gene were performed from low-gelling-temperature agarose using the Wizard PCR Preps Promega kit (United States) according to the protocol recommended by the manufacturer.

The nucleotide sequences of the PCR products were determined with an automatic 3730 DNA-Analyzer (Applied Biosystems, United States) using BigDye Terminator Cycle Sequencing Kit v.3.1.1 (Applied Biosystems, United States) according to the protocol recommended by the manufacturer with minor modifications.

**Phylogenetic analysis of 16S rRNA gene sequences.** The preliminary analysis of the 16S rRNA gene sequences was performed using the data and the software available at the RDP site (http://rdp.cme.msu.edu). The sequences were edited using the BioEdit software package (http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html). The sequences were aligned with the corresponding sequences of related bacterial species using the CLUSTALW v. 1.75 software package. The unrooted phylogenetic trees of the investigated bacteria were constructed using the methods implemented in the TREECONW software package [http://bioc-www.uia.ac.be/u/yvdp/treeconw.html].

The sequence of the 16S rRNA gene of the acetate-oxidizing bacterium from the syntrophic culture was deposited in GenBank under the accession number DQ124682.

## RESULTS

**Obtainment of the binary culture.** After numerous transfers of enrichment cultures from Khadyn Lake, a stable sulfidogenic, acetate-oxidizing microbial association was obtained. A small vibrio and several morphotypes of rod-shaped bacteria were present, including a spore-forming one. Serial dilutions of this enrichment were inoculated on agarized (2% Bacto Agar) basal medium. Colonies of different types were observed after ten days. Their inoculation into the liquid basal medium demonstrated that none of them was capable of acetate oxidation or sulfidogenesis. A possible explanation of this situation was syntrophic acetate oxidation, with one of the components reducing sulfate with hydrogen as the electron donor. In the subsequent inoculation of the agarized medium, acetate was replaced with formate (3 g/l); uniform pink lens-shaped colonies 0.1–0.2 mm in diameter were formed. They were successfully transferred to liquid medium. This pure SRB culture, strain Z-7999, capable of hydrogen utilization, was described as a new species of the genus *Desulfonatronum, D. cooperativum* sp. nov. [5]. Since a spore-forming rod was present in the enrichment culture, the association was heated for 10 min at 100°C. Since no growth was observed when the heated culture was transferred to the basal medium, this bacterium either had heat-sensitive spores or was unable to grow on acetate in the absence of a hydrogenotrophic organism. Inoculation of the culture heated for 10 min at 60, 70, or 80°C did not result in growth. The culture heated at 100°C was inoculated into the basal medium together with *D. cooperativum.* Growth with sulfidogenesis and acetate consumption was observed after two weeks. Light microscopy revealed cells of two types: the cells of *D. cooperativum* surrounded individual cells of a spore-forming rod designated as culture Z-7904. Thus, a stable binary acetate-oxidizing sulfidogenic association was obtained.

**Attempts to isolate the culture Z-7904.** A number of syntrophic organisms are capable of independent growth and have been isolated in pure cultures [10]. Attempts were therefore made to obtain a pure culture of the acetate-oxidizing bacterium. For this purpose, the syntrophic association containing numerous spores of Z-7904 was heated for 10 min at 100°C. The following substrates were used for inoculation: D-arabinose, D-galactose, D-glucose, D-xylose, D-lactose, D-maltose, D-mannose, melibiose, D-raffinose, rhamnose, D-ribose, sucrose, sorbose, trehalose, trypticase, D-fucose, fructose, peptone, yeast and meat extracts, tryptone, *N*-acetyl-D-glucosamine, casamino acids, arginine, betaine, butyrate, histidine, glycogen, glycerol, starch, lactate, malonate, oxalate, ornithine, pyruvate, propionate, serine, succinate, trimethylamine, microcrystalline cellulose, butanol, methanol, propanol, ethanol, butanediol, and ethylene glycol. Growth of some Z-7904 cells (not more than $10^4$ cells/ml) on some substrates was not maintained in the subsequent transfers. Certain acetate-oxidizing syntrophic organisms are known to perform a reverse reaction, homoacetogenesis from hydrogen and carbon dioxide [11, 12]. No growth of the heated culture with acetate was observed with the following electron acceptors (2–2.5 mM): sulfate, thiosulfate, sulfite, nitrate, Fe(III)EDTA, glycine, proline, anthraquinone, desulfonate, fumarate, crotonate, $S^0$ (3 g/l), and $O_2$.

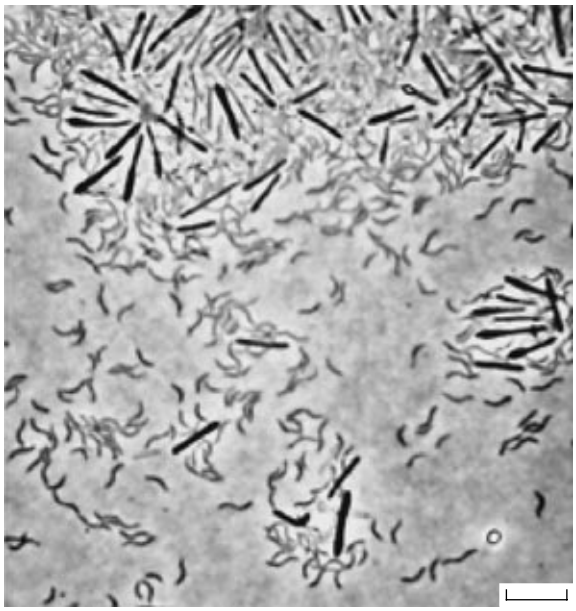

**Fig. 1.** Syntrophic association of culture Z-7904 (rods) and *Desulfonatronum cooperativum* (vibrios) grown on acetate. Phase contrast. Bar, 5 μm.

**Morphology.** The cells of Z-7904 were straight or slightly curved nonmotile rods, 2–7 μm in length and 0.5–0.7 μm in diameter. The endospores were formed terminally (Fig. 1). They were heat-resistant, surviving heating for 10 min at 100°C. Ultrathin sections revealed a typical gram-positive structure of the cell wall (Fig. 2) and a thick mucous capsule. The ratio of Z-7904 and *D. cooperativum* cells in the associations never exceeded 1 : 10. Single cells of Z-7904 were extremely rare; usually they were surrounded by the SRB cells (Fig. 1). This finding confirms the syntrophic relationship between the two microorganisms.

**Syntrophic oxidation of acetate and other electron donors.** Acetate oxidation occurred only in the binary culture but never in a pure SRB culture or in the sterile control (Fig. 3a). Acetate consumption correlated with an increase in the Z-7904 cell numbers. In the syntrophic association, the exponential growth of sulfate reducers peaked on the 12th–20th day of the experiment, and that of Z-7904, on the 20th–27th day (Fig. 3b).

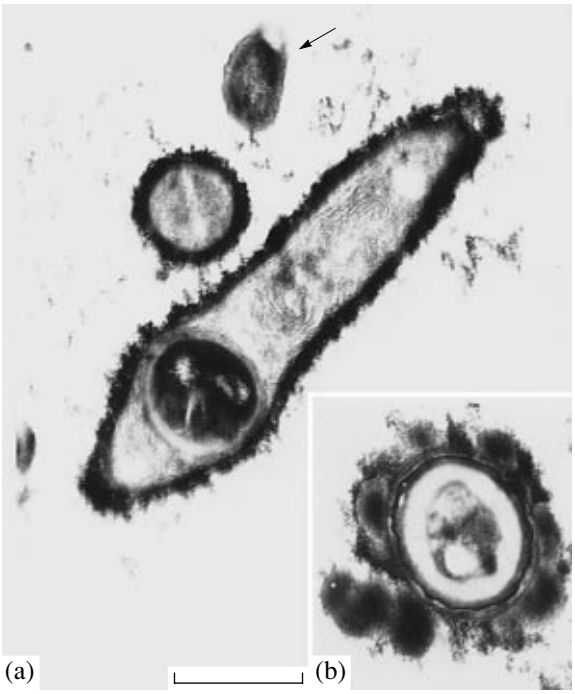


**Fig. 2.** Ultrathin sections of the cells of culture Z-7904: (a) Longitudinal and transverse sections of Z-7904 cells with endospores; the cells possess a thick capsule. Arrow indicates a section of a *D. cooperativum* cell. (b) A section of a Z-7904 mature endospore surrounded with capsule. Bar, 0.5 μm.

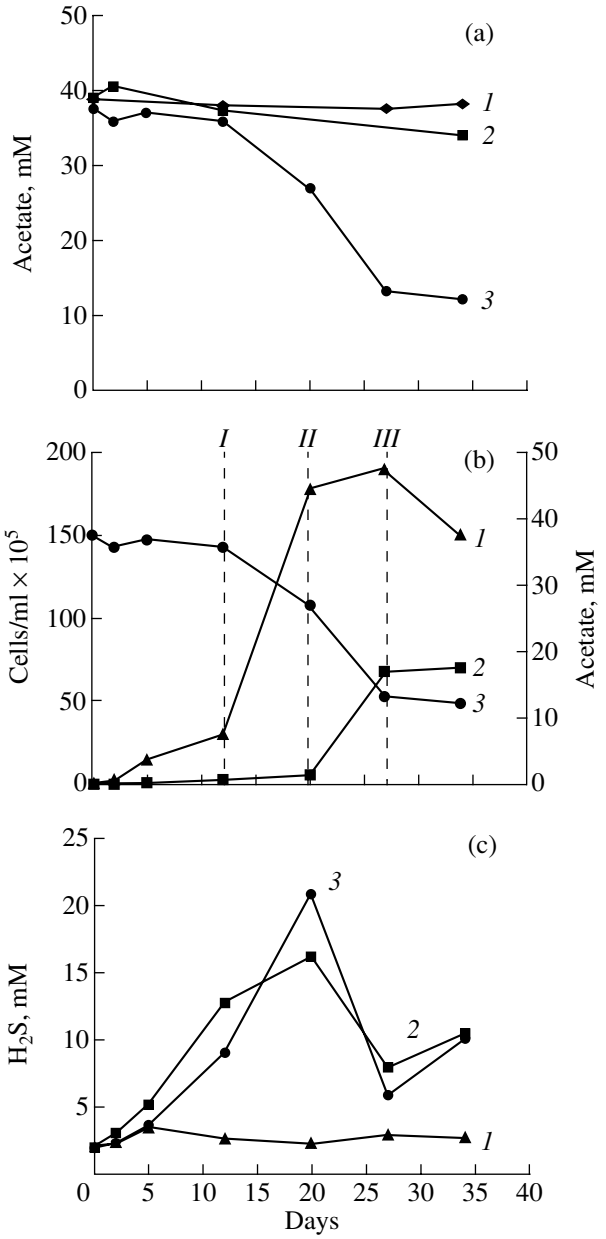


**Fig. 3.** Syntrophic oxidation of acetate by the sulfidogenic association. (a) Acetate consumption (*1*) in sterile control, (*2*) by strain Z-7999, and (*3*) by the syntrophic culture of Z-7999 and Z-7904. (b) Bacterial numbers and acetate consumption during syntrophic growth of Z-7999 and Z-7904: (*1*) cell numbers of Z-7999, (*2*) cell numbers of Z-7904, and (*3*) acetate consumption by Z-7999 and Z-7904. (c) Hydrogen sulfide production by Z-7999 on (*1*) acetate and (*2*) formate and (*3*) in binary culture of Z-7904 + Z-7999 on acetate.

Hydrogen sulfide production was detected either in pure cultures of *D. cooperativum* with hydrogen or formate or with acetate in association with Z-7904, but never in a pure SRB culture with acetate. During the stationary phase, the concentration of hydrogen sulfide in the alkaline medium decreased (Fig. 3c) owing to the formation of polysulfanes, compounds of the $H_2S_n$ type, whose presence was manifested in yellow coloration of the medium.

The culture was inoculated into media with substrates other than acetate in order to determine the possibility of their syntrophic oxidation (table). Apart from acetate, of the substrates tested, ethanol, propanol, and isopropanol were syntrophically oxidized, and to a lesser degree, serine, fructose, and isobutyric acid.

**Species specificity of the syntrophic association.** The possibility of acetate oxidation by Z-7904 in association with other alkaliphilic SRB species of the genera *Desulfonatronum* and *Desulfonatronovibrio* was investigated. For this purpose, a pasteurized culture of strain Z-7904 was incubated together with pure cultures of *Desulfonatronum lacustre* strain Z-7951$^T$ [7], *Desulfonatronum thiodismutans* strain MLF1 [13], or *Desulfonatronovibrio hydrogenovorans* strain Z-7935 [14]. After two weeks, acetate oxidation concomitant with sulfidogenesis was detected in all of the cases.

**Phylogenetic analysis.** A substantial portion of the 16S rRNA gene sequence of strain Z-7904 was determined (1449 nucleotides), corresponding to *E. coli* positions 10 to 1466. The preliminary blast analysis of this sequence using the GenBank database confirmed that the strain belonged to the clostridial lineage of gram-positive bacteria. According to the phylogenetic analysis, the culture Z-7904 belonged to the family *Syntrophomonadaceae*; it was not, however, closely

related to any known genus or species within this family, including the known genera of syntrophic bacteria (Fig. 4).

The clones of uncultured bacteria isolated from the soda Mono Lake [15] and from municipal waste [16] were closest to the culture Z-7904 (91.4–98.2% homology), forming a monophyletic cluster with a maximum level of bootstrap support (100). This cluster appeared to be remotely related to other uncultured clones from different sources (88.6–90.2%) and to the species of the genus *Anaerobranca* (85.6–86.3%), although with a low level of bootstrap support (57). The levels of homology with other representatives of the family Syntrophomonadaceae, including syntrophic species of the genera *Syntrophothermus, Syntrophomonas*, and *Thermosyntropha*, were even lower (76.1–86.0%).

## DISCUSSION

For anaerobic conditions, gradual decomposition of organic matter by the microbial community consisting of specialized bacterial groups is characteristic [17]. The trophic structure of the anaerobic microbial community is known to be similar to a high degree for different ecosystems, including extremely halo- and alkaliphilic ones [1, 18, 19]. In such a community, the first stage of decomposition of complex organic compounds leads to the formation of acetate, formate, hydrogen, and $CO_2$ as the major products. Acetate is thus one of the main products of the anaerobic decomposition of organic matter; its further degradation turns out to be the main problem for the community [17]. Acetate is easily consumed by anoxygenic phototrophic bacteria of the photic zone or oxidized in the oxycline zone [17]. Acetate oxidation in the anaerobic zone occurs only in the presence of an external electron acceptor, e.g., nitrate, sulfate, or iron(III). Acetate fermentation is thermodynamically unfavorable and can only occur in the case of continuous removal of hydrogen from the system by hydrogen-consuming organisms.

Many cases of syntrophic relationships between microorganisms oxidizing acetate, propionate, butyrate, and other organic compounds are presently known [10, 12, 20]. Since methane tanks have been the main source of the isolation of syntrophic cultures, hydrogenotrophic methanotrophs usually act as the hydrogen-oxidizing component of the known syntrophic associations that oxidize acetate in parallel with aceticlastic methanogens predominant in methanetanks [10]. Apart from methanogenic syntrophic associations, syntrophic associations with iron- and sulfate-reducing bacteria are known. The possibility of syntrophic acetate oxidation under mesophilic conditions was initially demonstrated for an anaerobic, sulfate-reducing bacterial association [21, 22]. More recent discoveries are a syntrophic acetate-oxidizing culture consisting of an iron-reducing bacterium *Geobacter sulfurreducens* and a fumarate- or nitrate-reducing bacterium *Wolinella succinogenes* [23] and the culture *Syntrophus aciditrophicus*, capable of oxidizing benzoate and certain other organic acids in association with sulfate reducers, iron reducers, or methanogens [24].

Oxidation of certain substrates by strain Z-7999 and by the syntrophic association of Z-7904 and Z-7999

| Substrates + $SO_4^{2-}$ | $H_2S$ production, mM | |
|---|---|---|
| | Z-7999 | Z-7904 + Z-7999 |
| Alanine | 3.10 | 4.76 |
| Aspartate | 1.27 | 1.55 |
| Acetate | 3.75 | 11.55 |
| Betaine | 4.03 | 4.09 |
| Butyrate | 2.36 | 2.75 |
| Glycine | 3.43 | 4.84 |
| Glutamate | 1.17 | 1.20 |
| Glucose | 1.30 | 1.85 |
| Yeast extract | 2.23 | 3.05 |
| Isobutyric acid | 2.85 | 6.17 |
| Isopropano | 3.28 | 11.98 |
| Casamino acids | 1.50 | 1.45 |
| α-Ketoglutarate | 2.20 | 1.60 |
| Lactate | 9.38 | 10.75 |
| Malate | 1.82 | 1.40 |
| Malonate | 1.27 | 1.87 |
| Methanol | 3.15 | 3.23 |
| Oxalate | 1.22 | 1.70 |
| Pyruvate | 3.83 | 4.60 |
| Pyruvate without $SO_4^{2-}$ | 1.05 | 1.45 |
| Proline | 3.40 | 5.03 |
| Propanol | 3.50 | 15.53 |
| Propionate | 3.15 | 3.10 |
| Sucrose | 5.13 | 4.00 |
| Serine | 3.35 | 8.21 |
| Succinate | 1.22 | 1.67 |
| Formate | 15.2 | 15.28 |
| Fructose | 3.70 | 7.17 |
| Fumarate | 1.25 | 1.60 |
| Cholin | 3.45 | 3.30 |
| Citrate | 3.10 | 4.40 |
| Ethanol | 3.68 | 15.60 |
| $H_2$ | 19.25 | 17.05 |
| Control | 3.60 | 4.30 |

Syntrophic bacteria are rather diverse phylogenetically, although most of the syntrophic propionate-, ethanol-, and benzoate-oxidizing bacteria isolated so far belong to *Deltaproteobacteria*, and the β-oxidation of fatty acids and acetate is performed mainly by the syntrophic bacteria of the low G+C gram-positive lineage [10]. The majority of the known syntrophic organ-

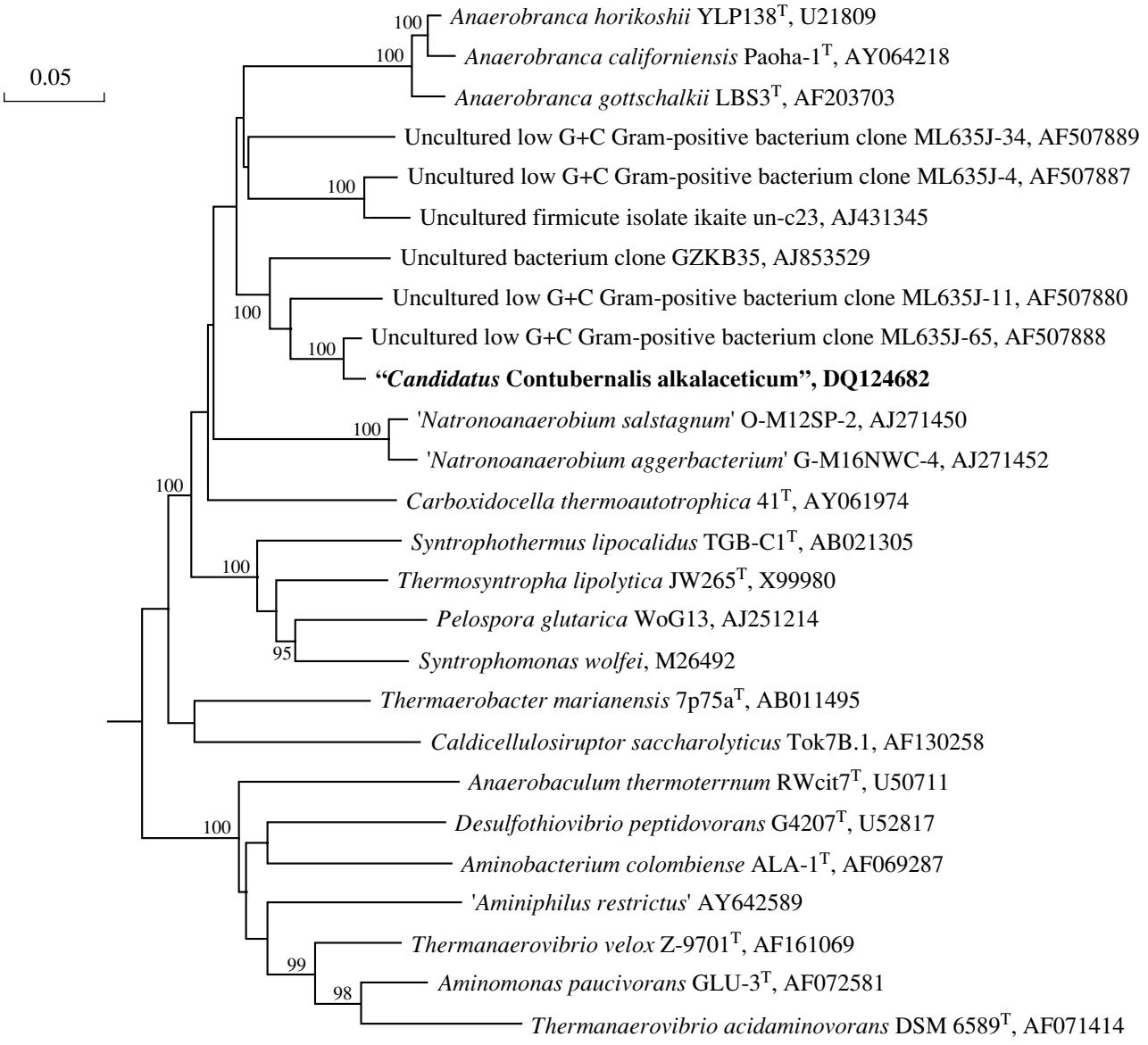


**Fig. 4.** Phylogenetic position of culture Z-7904 on the tree of 16S rRNA genes of the family *Syntrophomonadaceae* of the subdivision of low G+C gram-positive bacteria. Bar corresponds to 5 nucleotide substitutions per 100 nucleotides. Numbers show the reliability of the branching order as determined by bootstrap analysis (the values above 95 were considered significant).

isms are capable of independent growth on certain substrates and were therefore isolated in pure cultures. However, *Syntrophobacter wolinii* [25] and *Pelotomaculum schinkii* [26] were described as obligately syntrophic bacteria; they are as yet not available as pure cultures.

The syntrophic acetate-oxidizing bacterium isolated by us from Khadyn Lake has the following characteristic features:

1. Apart from acetate, it can syntrophically oxidize ethanol, propanol, isopropanol, serine, fructose, and isobutyric acid.

2. Beyond an association, the culture Z-7904 could not grow and oxidize any of the substrates from a broad list of organic compounds tested. It is therefore the first alkaliphilic obligately syntrophic bacterium.

3. The second component in the association can be replaced by another hydrogen-oxidizing species, which is also true for some other syntrophic associations [24, 26]. Our experiments were limited to alkaliphilic SRB since alkaliphilic hydrogen-oxidizing methanogens were not available to us, although their existence had been demonstrated [2]. Furthermore, methane was not found to be produced by our enrichment cultures.

The growth curves of bacteria in the syntrophic association and the curve of acetate consumption (Figs. 3a, 3b) confirm that acetate consumption is mostly related to the growth of Z-7904. The dynamics

of bacterial numbers, although easy to follow owing to the differences in cell morphology, were puzzling. Sulfate reducers were the first to start growth, followed by the spore former (Fig. 3a). The inoculum of nongrowing or weakly growing Z-7904 cells possibly produced enough hydrogen for the exponential growth of the SRB (Fig. 3b, curves *1, 2*); acetate concentration decreased by 10 mM during this stage. The decrease in the acetate concentration was possibly also caused by the anabolism of the hydrogen-oxidizing SRB, in which 2/3 of the carbon source requirements are met by acetate [2]. The exponential growth of Z-7904, which resulted in the consumption of additional 12 mM of acetate, commenced only after the necessary density of the SRB population was achieved (Fig. 3b, curves *2, 3*).

In our earlier work on cellulose decomposition by the alkaliphilic microbial community of the Khadyn and Verkhnee Beloe soda lakes, acetate utilization followed by sulfidogenesis was attributed to the *Desulfotomaculum*-like spore-forming rods which appeared at the concluding stages of decomposition [4]. The attempts to isolate them on acetate were, however, unsuccessful. Our present findings indicate that acetate oxidation in such communities probably occurs syntrophically; it should be noted that the rod observed previously was morphologically similar to Z-7904.

The culture Z-7904 is the first cultured organism in the cluster of uncultured bacterial clones within the family *Syntrophomonadaceae*. The uncultured bacterial clones obtained from a similar environment, the alkaline hypersaline Mono Lake [15], were the closest relatives of Z-7904. Among cultured relatives of Z-7904, the closest were representatives of the genus *Anaerobranca* (85.6–86.3% homology), which are alkaliphilic, thermophilic, gram-positive bacteria with fermentative metabolism able to reduce various sulfur compounds [27]. The representatives of the genera *Syntrophothermus, Syntrophomonas*, and *Thermosyntropha* were the closest among syntrophic bacteria (76.1–86.0% homology). Unlike culture Z-7904, these gram-negative bacteria syntrophically oxidize fatty acids with four or more carbon atoms [28–30]. They are all capable of independent growth by crotonate oxidation; *Thermosyntropha lipolytica* can also oxidize yeast extract, tryptone, casamino acids, and betaine. Apart from the alkali-tolerant *Thermosyntropha lipolytica*, which is able to grow up to pH 9.5, all representatives of these genera are neutrophiles [30].

Since the acetate-oxidizing bacterium Z-7904 is phylogenetically remote from other bacteria (and clones) of the family *Syntrophomonadaceae* and differs from them phenotypically, its presentation as a new genus of this family is well grounded.

In accordance with the suggestion of Murray and Schleifer [31], the International Committee on Systematics of Procaryotes recommends using the *Candidatus* taxonomical category [32–34] for the description of well-characterized but as yet uncultured microorganisms, as well as of the microorganisms lacking complete phenotypic or phylogenetic description required for their validation as new taxa according to the *International Code of Nomenclature of Bacteria, 1990 Revision* [35]. Based on this recommendation, we suggest that the new alkaliphilic acetate-oxidizing bacterium described by us in a syntrophic association should be classified as a new taxon of generic level in a status of *Candidatus: "Candidatus* Contubernalis alkalaceticum."

**"*Candidatus* Contubernalis"** *Con.tu.ber.na.lis* N.L.n. companion

Gram-positive rod. Spore-forming. Alkaliphilic. Strictly anaerobic. Obligately syntrophic. When grown together with a hydrogen-consuming organism, is capable of syntrophic oxidation of acetate, ethanol, propanol, isopropanol, serine, fructose, and isobutyric acid. Does not grow in pure culture without a hydrogen-consuming organism. The only species is "*Candidatus* Contubernalis alkalaceticum."

**"*Candidatus* Contubernalis alkalaceticum."** *Al.kal.ace.ti.cum*, n.N.Lat. *alkali* from Arabic adjective *al qaliy*, alkaline; n.N.Lat. *aceto* from Latin noun *acetum*, vinegar; *alkalaceticum*, utilizing vinegar under alkaline conditions.

Cells are straight or slightly curved rods measuring 0.5–0.7 × 2–7 μm. Nonmotile. Spore-forming. Round endospores are located at the cell pole. Heat-resistant. The cell wall structure is of the gram-positive type. Cells possess a thick mucous capsule. Strict anaerobe. Mesophile. Alkaliphile. Does not require NaCl. Obligately syntrophic, oxidizes acetate, ethanol, propanol, isopropanol, serine, fructose, and isobutyric acid in cooperation with the alkaliphilic hydrogen-utilizing sulfate-reducing bacterium *Desulfonatronum cooperativum.* Can oxidize acetate together with bacteria of the genera *Desulfonatronum* or *Desulfonatronovibrio.* The culture Z-7904, in syntrophic association with *Desulfonatronum cooperativum*, is deposited in the VKM culture collection under the designation B-2362.

The site of isolation is Khadyn soda lake.

## ACKNOWLEDGMENTS

We thank N.A. Kostrikina for the analysis of ultrathin sections and E.V. Pikuta for providing the type culture of *Desulfonatronum thiodismutans.* This work was supported by the fundamental research programs of the Presidium of the Russian Academy of Sciences "Molecular and Cellular Biology" and "The Origin and Evolution of the Biosphere" and by the Russian Federation President's grant "Scientific Schools" NSh-1101.2003.4.

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