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EXPERIMENTAL ARTICLES

Description of *Anaerobacillus alkalilacustre* gen. nov., sp. nov.—Strictly Anaerobic Diazotrophic Bacillus Isolated from Soda Lake and Transfer of *Bacillus arseniciselenatis*, *Bacillus macyae*, and *Bacillus alkalidiazotrophicus* to *Anaerobacillus* as the New Combinations *A. arseniciselenatis* comb. nov., *A. macyae* comb. nov., and *A. alkalidiazotrophicus* comb. nov.[1]

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Abstract—An anaerobic, spore-forming bacterium (strain Z-0521) was isolated from the iron-reducing microbial community enriched from sample of bottom sediments from low-mineralized soda lake Khadyn, Tuva upper Yenisey region (Russia). Cells of strain Z-0521 are motile straight Gram-positive rods, 0.7–1.1 (μm in diameter and 3.0–7.0 μm length. It is a mesophilic halotolerante obligate alkaliphilic bacterium with a pH range for growth 8.5–10.7 (optimum at 9.6–9.7). Utilizes carbohydrates. Peptides, organic acids or alcohols are not utilized. In the presence of mannite strain Z-0521 reduces AQDS, arsenate, selenate and selenite. It is capable of N_2 fixation and has nitrogenase gene *nif*H. The dominant cellular fatty acids are $C_{16:0}$, $C_{16:1w7c}$ and C_{a15}. The G+C content in the DNA is 36.2 mol %. 16S rRNA gene sequencing identified strain Z-0521 as a member of rRNA group 6 of the genus *Bacillus*. Its closest relatives are *B. alkalidiazotrophicus* and *B. macyae* (98.3 and 98.1% sequence similarity). On the basis of physiological properties and genetic analysis, it is proposed that strain Z-0521^T should be assigned to a new species of a new genus, *Anaerobacillus alkalilacustre* gen. nov., sp. nov. It is also proposed that *Bacillus arseniciselenatis*, *Bacillus macyae* and *Bacillus alkalidiazotrophicus* should be transferred to this new genus, with *Anaerobacillus arseniciselenatis* (formely *Bacillus arseniciselenatis*) as the type species.

Key words: strictly anaerobic diazotrophic, *Bacillus*, group 6, *Anaerobacillus alkalilacustre*, soda lake, 16S rDNA.

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Microbial diversity of alkaline environments, such as soda lakes, includes anaerobic bacteria, which could reduce certain metals and metalloids. Respiratory reduction of selenate (Se(VI)), selenite (Se(IV)) arsenate (As(V)), Fe(III), Co(III), Cr(VI) was demonstrated for a few microorganisms during last two decade and has geochemical, ecological and environmental significance [1–5]. There are only few described alkaliphilic dissimilatory metal-reducing microorganisms such as *Bacillus macyae* [3], *Alkaliphilus metalliredigens* [4], *Geoalkalibacter ferrihydriticus* [5]. The majority alkaliphilic metal-reducing bacteria have fermentative type of metabolism and reduce metals as an electron sink using different types of organic compounds, as an electron donors.

Enrichment of anaerobic alkaliphilic iron-reducing community oxidizing acetate was obtained from the sediment sample of lake Khadyn (Tuva, upper Yenisey region). Several new microorganisms able to reduce amorphous Fe(III)-hydroxide (AIH) and AQDS were isolated from this enrichment including acetate-utilizing *Geoalkalibacter ferrihydriticus* [5] and fermentative *Natronincola* [6]. Among the non Fe-reducing sattelites, strain Z-0521 was obtained, which represents a new strictly anaerobic, fermentative, obligately alkaliphilic, diazotrophic and arsenate reducing member of bacilli.

Here we present taxonomic description of strain Z-0521, which is proposed as a new species of a new genus *Anaerobacillus alkalilacustre* whithin the group 6 of the genus *Bacillus*. We also propose to transfer into this genus anaerobic members of the rRNA

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group 6 *Bacillus arseniciselenatis*, *B. macyae* and *B. alkalidiazotrophicus* as *Anaerobacillus arseniciselenatis* comb. nov., *Anaerobacillus macyae* comb. nov. and *Anaerobacillus alkalidiazotrophicus* comb. nov. In accordance with the principle of priority we propose *A. arseniciselenati*s, the first described species from this group, as the type species of a new genus.

MATERIALS AND METHODS

Source of sediments, enrichment and isolation. The described bacterium was isolated from an Fe(III)-reducing enrichment with acetate obtained from the bottom sediments and water collected from soda lake Khadyn (Tuve, Russia), with pH 9.5 and salt content 17 g/l [5]. The enrichment was cultivated in strictly anaerobic medium, containing acetate (2 g/l) as electron donor and Fe(III) as electron acceptor, yeast extract (0.1 g/l) as growth factor, under N_2 in the gas phase [5]. Fe(III) was provided in the form of AIH at 90 mM.

Media and culturing techniques. Optimized medium contained (g/l): 0.2 KH_2PO_4, 0.5 NH_4Cl, 0.2 KCl, 0.1 $MgCl_2 \cdot 6H_2O$, 1.0 NaCl, 10.0 $NaHCO_3$, 3.0 Na_2CO_3, 0.1 yeast extract (Difco), 2.0 mannite, 1 ml of trace element solution [7]. Preparation of anoxic media achieved by heating the culture medium to 100°C under a constant 100% N_2 headspace. Culture media was then cooled to room temperature and subsequently dispensed under anoxic atmosphere into Hungate anaerobic culture tubes and autoclaved. No reducing agent was added. The pH of the medium after sterilization was 9.5. Temperature of incubation was 37°C.

Physiological studies. Growth was determined by measuring the increase in optical density directly in Hungate tubes at 600 nm (Specol-10 Jena, Germany).

Utilization of electron donors was studied in optimized medium, in which mannite was replaced by organic acids, filter-sterilized sugars or peptides at concentration 3 g/l, for alcohols 5 ml/l, molecular hydrogen (100% v/v). A medium in which the energy source had been omitted was used as a control.

Ability of the strain Z-0521 to use various electron acceptors was studied with mannite as preferred electron donor. The electron acceptors were added from autoclaved stock solutions with final concentration (mM): Na_2SO_3 – 2 or 10; $Na_2S_2O_3 \cdot 5H_2O$ – 20; Na_2SO_4 – 20; $NaNO_2$ – 2 or 10; $NaNO_3$ – 20; Fe(III)–EDTA – 20; Fe(III)–citrate – 5; AQDS – 20; $HAsO_4^{2-}$, SeO_4^{2-} and SeO_3^{2-} – 5; S^0 – 2% (w/v).

Temperature, pH and NaCl concentration ranges for growth and susceptibility to antibiotics were determined under an atmosphere of N_2 in optimized medium. The pH was adjusted with sterile stock solutions of HCl (10%) or NaOH (10%) and measured after sterilization and after growth. In this case initial concentration of carbonate was 10-fold decreased and instead of them NaCl was added in equal molar concentration. When NaCl-dependence was studied all salts containing Cl^-, were replaced by sulfate-salts. Same approach was used in case of carbonate dependence. Tris–HCl or CAPS buffers were added in this case to maintain the pH. The optimal carbonate concentration was estimated by using different concentrations of carbonate with constant $Na_2CO_3/NaHCO_3$ ratio = 0.3 with $Na_2CO_3/NaHCO_3$ (g/l) = 0.03/0.1; 0.15/0.5; 0.3/1.0; 1.5/5.0; 3/10; 6/20; 9/30; 12/40; 15/50; 30/100.

Analytical techniques. Routine examinations of cultures were performed under a phase-contrast ZETOPAN Reichert optical anoptral microscope. Phase contrast micrographs were taken using agar-coated slides [8]. Transmission electron microscopy was performed with a model JEM-100 electron microscope (JEOL) as described previously [9].

Fe(II) was measured with ferrozine [10]; ammonium was analyzed after diffusion with Nessler's reagent. Determination of short-chain organic acids, alcohols and gaseous products of metabolism was performed by HPLC-anionic chromatography with HPX-87H column (BioRad) at 60°C, UV/RI detector and 5 mM H_2SO_4 at 0.6 ml/min as a carrier. Sulfide was determined by methylen blue method [11]. Arsenate was detected by ion-chromatography on HPLC chromatograph "Stayer" with conductometric detector and column "Aquline" A 1.2 (4,6 × 250 mm) and postcolumn membrane suppression (Aquilon Ltd, Russia) in 3.5 mM carbonate buffer, eluent flow 1.5 ml/min.

Fatty acid profile was analyzed on chromatograph Microbial Identification System Sherlock (MIDI Inc, Newark, United States) as described previously [12] with its identification on mass-spectrometer Agilent Technologies AT-5971 (SMART).

Catalase activity in washed cells was quantified by iodometric method [13].

DNA analysis. The genomic DNA was isolated according to [14]. Determination of the G+C content of the DNA and the DNA–DNA hybridization were performed by the thermal denaturation/reassociation technique [15].

Sequence analysis of the 16S rRNA gene and *nif*H gene. The 16S rRNA genes were amplified using general bacterial primers 11F-1492R [16]. The 470 kb fragment of *nif*H gene was amplified with specially designed and previously tested primer pair F1/R6 (5'-TAYGGIAARGGIGGIATIGGIAARTC-3')/5'-GCCA-TCATYTCICC-IGA-3') [17]. The PCR products were purified from low-melting agarose using the Wizard PCR-Prep kit (Promega, United States). Sequencing was performed using Big Dye Terminator v.3.1 sequencing reaction kit at ABI 3730 DNA automatic sequencer (Applied Biosystems, Inc., United States). Preliminary phylogenetic analysis of the new sequences was done with the NCBI BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/). The nucleotide and inferred amino acid sequences were aligned

with sequences from the GenBank using CLUSTALW. Phylogenetic trees were reconstructed using the TREECON W [18]. Bootstrap analysis (1000 replications) was used to validate the reproducibility of the branching patterns of the trees.

Type strains. *B. macyae* JMM-4^T for comparative studies was obtained from DSMZ collection. *B. alkalidiazotrophicus* MS 6^T was kindly provided by D.Yu. Sorokin.

RESULTS

Enrichment and isolation. For isolation of diverse Fe(III)-reducing species the soda lake enrichment was inoculated into media containing AIH or AQDS as electron acceptors and different electron donors. Motile rods with endospores dominated on the medium with AQDS and mannite as electron donor. After several transfers on the liquid medium culture was inoculated in roll tubes with 1.5% Bacto Agar in the basal medium with AQDS. Single colonies were picked up with glass capillary and subcultured in liquid medium. The purity of isolate designated as strain Z-0521 was checked microscopically, by the absence of growth on control media, and by 16S rRNA gene sequencing.

Cell morphology. Colonies appeared after 24–48 hours incubation in agar roll tube cultures as lense-shaped, 0.1–0.2 mm in diameter white with dark center. Vegetative cells of strain Z-0521 were actively moving rods, 0.7–1.1 μm in diameter and 3.0–7.0 μm in length (Fig. 1a) with slightly tapering end. The cells occurred singly, sometime in pairs. Formation of mini-cells at the end of cell or in the place of division was quite characteristic for this bacterium (see arrow Fig. 1a). Cells had from 1 to 7 peritrichously located flagella (Fig. 1b). Strain Z-0521 formed ellipsoidal, endospores, sometimes two within a single sporangium (Fig. 1a, 1c). After exponential growth phase cells lysed quickly. Ultrathin sectioning of strain Z-0521 revealed a typical Gram-positive structure of the cell wall (Fig. 1c).

Growth characteristics. Strain Z-0521 was obligate alkaliphile with a growth range from pH 8.5 to 10.7 with an optimum at pH 9.7 (Fig. 2a). It was halotolerant, being able to grow 0–110 g/l of NaCl with an optimum at 20–30 g/l of NaCl. It obligately depended on the presence of carbonates with no growth on the medium where carbonate buffer was replaced by CAPS or Tris buffer. The optimal concentration of carbonates was found at 3.0 g/l of Na_2CO_3 and 10 g/l of $NaHCO_3$ (Fig. 2b). The isolate was mesophilic and grew from 18° to 40°C, with an optimum at 30–35°C.

Strain Z-0521 was an obligate anaerobe catalase positive and oxidase negative, utilizing only carbohydrates; peptides, alcohols or organic acids were not used. Fermentative growth was possible with glucose, tregalose, saccharose, cellobiose, fructose, ramnose, xylose, maltose, mannite, dextrin and glycogen; weak growth was observed with ribose, lactose, raffinose, melibiose, sorbitol, *N*-acetyl-D-glucosamine and pullulane. Mannose, galactose, sorbose, fucose, L-arabinose, dulcitol, ornitrine, inosite, starch, casein, hummi-arabic, dextrane, xylane, agarose, alginic acid, aesculine, pectine and cellulose were not fermented.

Growth with electron acceptors. Strain Z-0521 reduced AQDS and arsenate with mannite as electron donor. Concentration of As(V) decreased during the growth from 5 mM to 2.5 mM, but no changes in cell yield was observed. Selenate not inhibited growth of strain Z-0521, but selenite were reduced only in cell suspensions. When selenite was added to the medium before inoculation it inhibited growth of strain Z-0521 and was not reduced. AIH, Fe(III)-citrate, Fe(III)-EDTA, nitrate, nitrite, sulfate, sulfite, thiosulfate, elemental sulfur and fumarate were not reduced by strain Z-0521.

Metabolic products. Formate, lactate, acetate and low concentration of ethanol were the main products from mannite as substrate by strain Z-0521^T. The fermentation products from glucose were ethanol, formate, acetate and lactate. Yeast extract was not required when the medium was supplemented with vitamins. When vitamins were omitted the growth of strain Z-0521 ceased at concentration of yeast extract below 0.05 g/l. The optimal concentration of yeast extract was 0.75 g/l on the basal medium. Doubling time at optimized conditions was 2.1 h.

Chloramphenicol, penicillin, vankomycin, ampicillin, novobiocin, and streptomycin completely inhibited growth at concentrations 100 μg/ml. Kanamycin at 100 μg/ml did not inhibit growth.

Fatty acid analysis. The dominant fatty acids of the strain Z-0521 included $C_{16:0}$ (31.09%), $C_{16:1w7c}$ (23.24%), C_{a15} (11.99%) and $C_{18:1w7c}$ (8.44%).

G+C content of the genomic DNA. The G+C content of the genomic DNA of strain Z-0521 was 36.2 ± 0.5 mol % (T_m).

Phylogenetic analysis. We determined an almost complete 16S rRNA sequence (1504 nucleotides) for strain Z-0521, corresponding to positions 11–1496 of *E. coli* numbering. According to the preliminary phylogenetic analysis, the highest level of sequence similarity was found with the species of the genus *Bacillus*, rRNA group 6, which accommodate mainly alkaliphilic *Bacillus* species [19, 20]. The closest relative (98.3% sequence similarity) were *B. alkalidiazotrophicus* [21] and *B. macyae* (98.1% sequence similarity) [3] (Fig. 3a). Results of DNA–DNA hybridization between strain Z-0521 and *B. alkalidiazotrophicus* (51% relatedness) and between strain Z-0521 and *B. macyae* (56% relatedness) indicated a separate gene-species status of strain Z-0521 [22].

Fixation of N_2. Since *B. alkalidiazotrophicus* was described as an active diazotroph [21], N_2-fixing potential of strain Z-0521 was tested on a medium with N_2 as the only one N-compound. The result was positive with

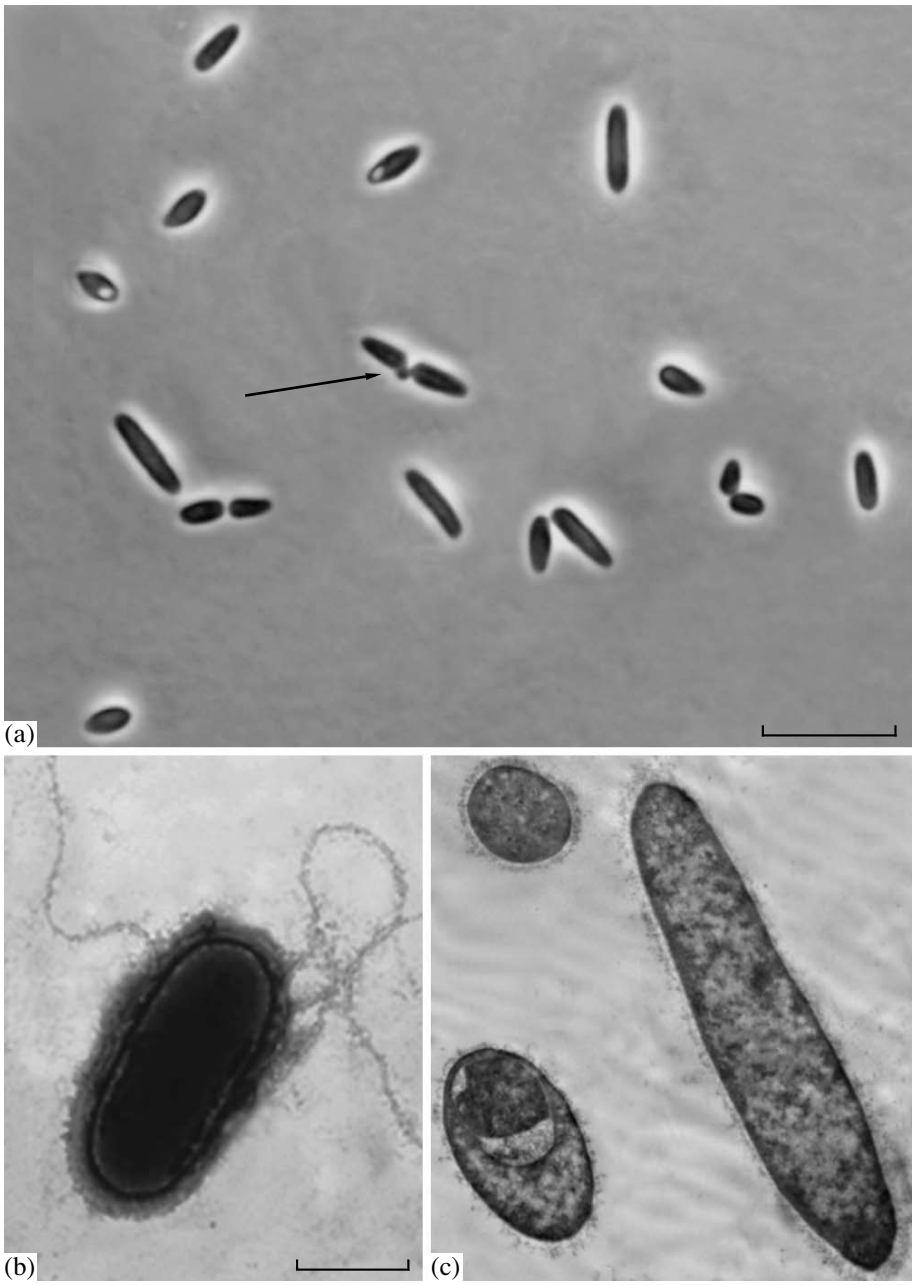

Fig. 1. Cell morphology of strain Z-0521^T grown in basal medium with mannite. (a) Phase-contrast micrograph. Mini-cells are pointed by arrow. Bar, 10 μm. (b) Electron micrograph of negatively stained cell with peritrichous flagella. (c) Ultrathin sections of strain Z-0521^T showing cell wall layers. Bar, 1 μm.

acetylene-reducing activity 16.7 μmol of C_2H_2 l/h on 5.5×10^6 cells.

Analysis of *nif*H gene. PCR amplification with specific primers detected a presence of a *nif*H gene fragment (nearby 450 nucleotide) in the genomic DNA of strain Z-0521. According to the preliminary BLAST-analysis the sequence of this fragment was closely related to *B. alkalidiazotrophicus* and *B. macyae* (89.5 and 88% accordingly) that corresponds well to 16S rRNA data. Comparison of the translated sequences revealed higher similarity between sequences of strains Z-0521 and *B. macyae* (96%) than with *B. alkalidiazotrophicus* (94.6%). On the phylogenetic tree (Fig. 3b) strain Z-0521 formed a single *nif*H cluster with *B. alkalidiazotrophicus*, *B. macyae* and *B. arseniciselenatis*.

DISCUSSIONS

The genus *Bacillus includes* more than 200 species with extremely diverse metabolism and phylogeny. Ini-

tially, this genus included only gram-positive, spore-forming, aerobic organotrophic bacteria. However, currently this "supergenus" includes aerobic, facultatively and obligately anaerobic, heterotrophic or autotrophic organisms capable of growth practically in extremely diverse ecological niches [23, 24]. Due to a heterogeneity of the genus there is no wonder that since last two decades fifteen phylogenetic groups have been reclassified as a new genera.

Strain Z-0521^T had several phenotypic traits, which separate it from its closest relatives (see table). It was clearly distinct from *B. alkalidiazotrophicus* by its ability to reduce selenate and selenite. Similar to the strain Z-0521, *B. alkalidiazotrophicus* is a strictly fermentative but aerotolerant organism [21]. Both have a relatively high content of $C_{16:1w7}$ that is certainly unusual for bacilli [25], but is presented in cellular fatty acid profile of metal-reducing bacteria [4, 6], Strain Z-0521 fermented pentoses such as xylose and ribose, but could not utilize starch.

From *B. macyae* the novel bacterium differed by strictly fermentative type of metabolism, ability to grow at pH above 8.5, and its ability to hydrolyse polymers.

Similar to *B. alkalidiazotrophicus*, strain Z-0521 was able to fix N_2 and had a functional nitrogenase gene *nif*H.

Most of diazotrophic members of the bacilli are accommodated within the genus *Paenibacillus* [26], but *B. alkalidiazotrophicus* and *B. arseniciselenatis* formed a novel independent lineage of diazotrophs within gram-positive bacteria with low G+C content and the *nif*H family cluster I related to the genus *Paenibacillus* [21]. During this study we also detected *nif*H gene in *B. macyae*. Obtained sequence was closely related to *nif*H of *B. alkalidiazotrophicus*, *B. arseniciselenatis* and strain Z-0521^T that were clearly different from the known *nif*H available in GenBank and confirmed separate phylogenetic position of these four bacilli in the rRNA group 6 of the genus Bacillus.

Based on 16S rRNA gene phylogeny, *B. alkalidiazotrophicus*, *B. macyae*, *B. arseniciselenatis* and strain Z-0521 formed a well-separated branch within the rRNA group 6 of the genus *Bacillus*. As a group, they have less than 95% 16S rRNA gene sequence homology with other bacilli, which is below the standard genus border [22]. Physiology within the group is markedly different from classical bacilli, i.e. obligate anaerobic metabolism. The common properties are spore formation, similar low G+C, halotolerante, obligate or slightly (*B. macyae*) alkaliphily and the potential to reduce various electron acceptors. For *B. alkalidiazotrophicus* the latter was not demonstrated, but we found it capable of arsenate reduction to arsenite during anaerobic growth with glucose.

The main characteristic feature for the group is their inability to use O_2. Three of them are strict anaerobes

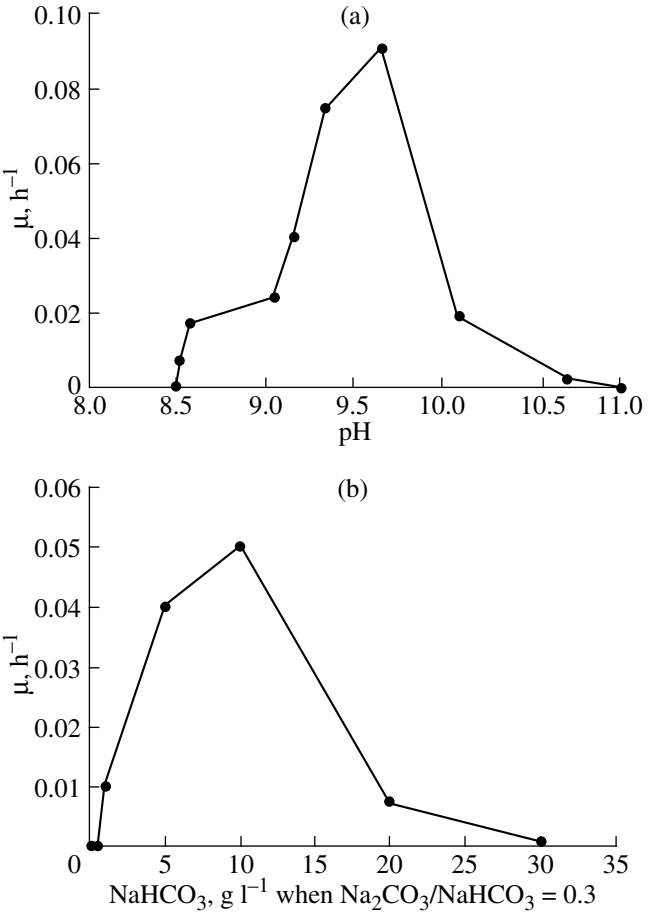

Fig. 2. Effect of pH (a) and carbonate concentration (b) on the growth of strain Z-0521^T.

and only *B. alkalidiazotrophicus* could grow under aerobic condition but it retained strictly fermentative type of metabolism. Besides these bacilli only thermophilic *B. infernus* was described as strict anaerobe [27], but phenotypicaly and phylogeneticaly it is rather distant from the group 6 anaerobes.

Thus, on the basis of physiological characteristics, the results of DNA-DNA hybridization studies, 16S rRNA gene and *nif*H gene analysis, we propose a new genus, *Anaerobacillus* gen. nov., containing new species *Anaerobacillus alkalilacustre* sp. nov., with strain Z-0521^T as a type. We also propose to transfer anaerobic species of the bacilli rRNA group 6, *B. arseniciselenatis*, *B. alkalidiazotrophicus* and *B. macyae*, to this new genus, with *Anaerobacillus arseniciselenatis* (formely *Bacillus arseniciselenatis* E1H) as the type species. The description of genus is based on our studies, as well as on previous descriptions of the anaerobic species [2, 3, 21].

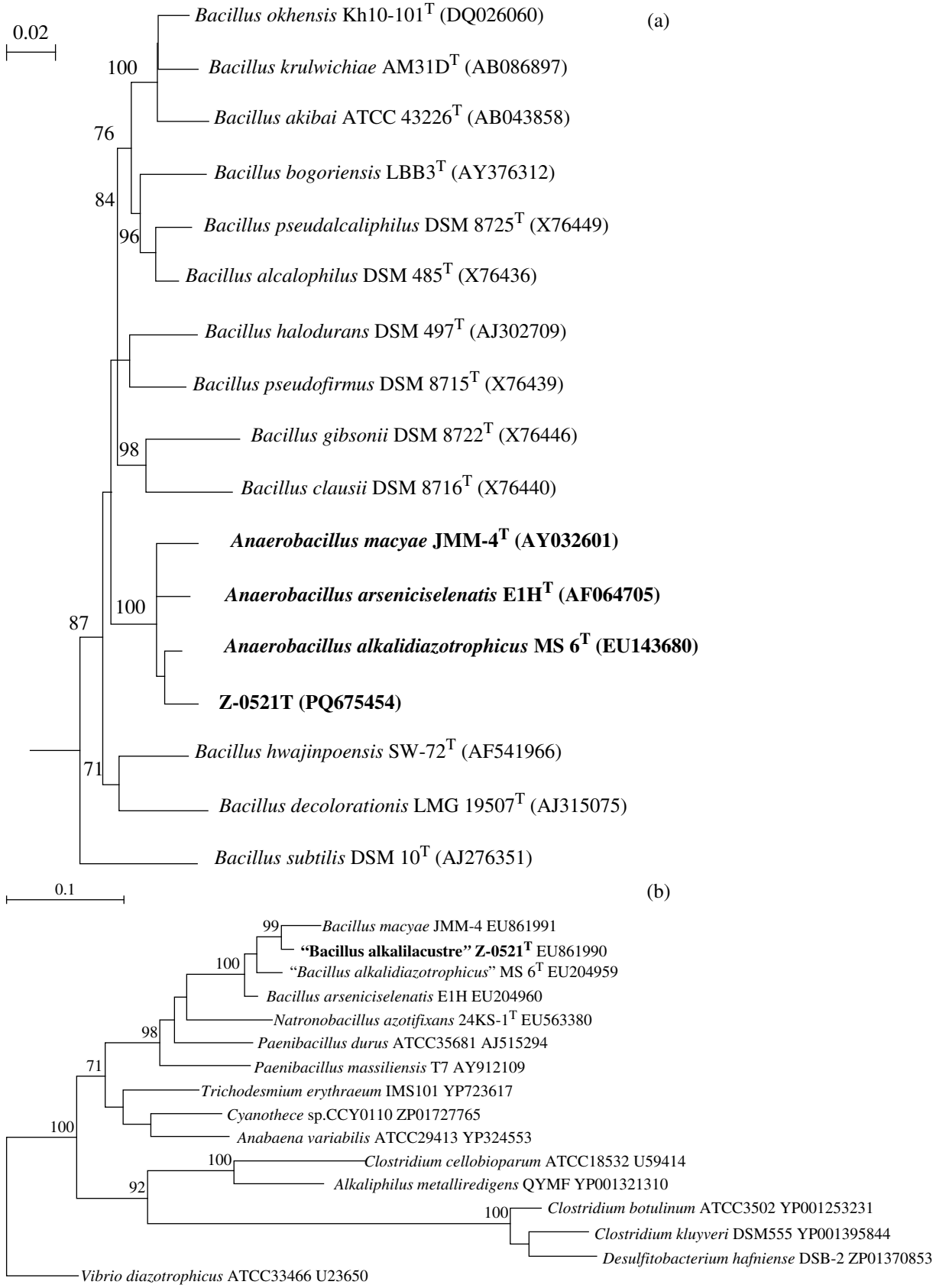

Fig. 3. Phylogenetic position of the new strain Z-0521 and other anaerobic diazothrophic species among closest neighbour species of the genus *Bacillus* based on 16S RNA gene sequence analysis (a) and *nif*H gent sequence analysis (b). Tree topography and evolutionary distances are given by the neighbour-joining method with Jukes–Cantor corrections. Numbers at the nodes indicate the percentage of boodstrap values for the clade of this group in 1000 replications (values more than 70 are shown). Bar, 2 and 10 substitutions per 100 nt.

Comparative characteristics of strain Z-0521 and related species of genus *Bacillus*

Characteristic	Z-05211	*B. alkalidiazotrophicus*, MS 6 [21]	*B. arseniciselenatis*, E1H[2]	*B. macyae*, JMM-44 [3]
Motility	+	+	–	+
Fermentation type of metabolism	+	+	+	–
pH growth range (optimum)	8.5–10.7 (9.6–9.7)	7.8–10.6 (9.5)	7.0–10.2 (9.8)	7.0–8.4 (7.8)
NaCl growth range, g/l (optimum)	0–110 (20–30)	5.0–70.0 (10–20)	20–120 (60)	1.2–30 (1.2)
Aerotolerance	–	+	–	–
Catalase	+	+	+	+
Oxidase	–	–	+	–
Electron acceptors:				
O_2	–	–	–	–
AQDS	+	ND	ND	ND
Arsenate	+	+*	+	+
Selenate	+	–	+	–
Selenite	+	–	–	–
Fe(III)	–	ND	+	–
Nitrate	–	–	+	+
Hydrolysis of polymers	glycogen	starch, glycogen	starch	–
Utilization of pentoses	xylose, ribose	–	–	–
Anaerobic respiration	–	–	+	+
Diazotrophy	+	+	ND	ND
*nif*H	+	+	+**	+*
G+D content of DNA, mol %	36.2	37.1	40.0	37.0
Habitat	Soda soil	Soda soil	Soda lake	Gold mine

Note: ND – not determined; * our date; ** date from paper Sorokin et al., 2008 [21].

Description of Anaerobacillus gen. nov.

Anaerobacillus (An.ae.ro.ba.cilílus. Gr. pref. *an* not; Gr. n. *aer* air; L. dim. n. *bacillus* small rod; M. L. masc. n. *Anaerobacillus* anaerobic rod).

Cells are rods producing ellipsoidal endospores occuring singly or in pairs, motile by peritrichous flagella or non-motile. The cell wall structure is gram-positive. Chemo-organotrophs. Strictly anaerobic or aerotolerant. Type of metabolism is fermentative or anaerobic respiration. Mesophilic. Obligate or moderately alkaliphilic. Halotolerant or moderately halophilic. Catalase positive. Most of the species are capable of hydrolysis and utilization of polymeric carbohydrates. Able to reduce arsenate. Diazotrophic, nitrogenase gene *nif*H is present. The G+C content of DNA is in the range 36.2–40 mol % (T_m). Most of the strains are obtained from high-pH habitats.

The type species is *Anaerobacillus arseniciselenatis* (basonym *Bacillus arseniciselenatis* Switzer–Blum et. al, 1998).

Description of Anaerobacillus alkalilacustre sp. nov.

Anaerobacillus alkalilacustre (al.ka.li.la.cu's.tre. N. L. at. n. *alkali* (from Arabic *al gaily* soda ash); N. L. gen. n. *lacus* of lake; N. L. adj. *alkalilacustre* alkaliphile from lake).

Cells are large rods with tapered edges, 0.7–1.1 × 3.0–7.0 μm, motile by peritrichous flagella. Forms one or two ellipsoidal endospores per the cell. Gram-positive. Strictly anaerobic. Obligate alkaliphile growing within a pH range from 8.5 to 10.7 with an optimum at pH 9.6–9.7. Mesophilic, with growth range from 18 to 40°C and optimum at 30–35°C. Halotolerant, with salt range from 0 to 110 g/l NaCl and an optimum at 20–30 g/l. Carbonate dependent. Type of metabolism is fermentative. Utilizes carbohydrates, but not peptides, organic acids or alcohols. The following carbohydrates can be fermented: glucose, tregalose, saccharose, cellobiose, fructose, ramnose, xylose, maltose, mannite, dextrin, glycogen. Weak growth with ribose, lactose, raffmose, melibiose, sorbitol, *N*-acetyl-D-glucosamine and pullulane. Reduces AQDS, arsenate, selenate and selenite with mannite as an electron donor. Diaz-

otrophic. Catalase positive and oxidase negative. Requires yeast extract or vitamins for growth. The dominant cellular fatty acids include $C_{16:0}$ (31.09%), $C_{16:1w7c}$ (23.24%), C_{a15} (11.99%) and $C_{18:1w7c}$ (8.44%). The G+C content of DNA is 36.2 mol % (T_m). Isolated from low-salt soda lake Khadyn (Tuva, Russia). The type strain is Z-0521^T (=VKM B-2403^T = DSMZ 18345^T). The GenBank accession number for the 16S rRNA sequence and *nif*H gene sequence is DQ675454 and EU861990, respectively.

Description of Anaerobacillus arseniciselenatis Zavarzina et al. comb. nov. [basonym Bacillus arseniciselenatis Switzer-Blum et al., 1998 (Validation List no. 80, 2001)]

In addition to the characteristics given for *A. arseniciselenatis* by Switzer Blum et al. (1998) gene *nif*H was detected by Sorokin et al. (2008). The type strain is E1H^T (=ATCC 700614^T). The GenBank accession number for the 16S rRNA sequence and *nif*H gene sequence is AF064705 and EU204960, respectively.

Description of Anaerobacillus alkalidiazotrophicus Zavarzina et al. comb. nov. [basonym Bacillus alkalidiazotrophicus Sorokin et al., 2008]

In addition to the characteristics given for this species by Sorokin et al. (2008), *A. alkalidiazotrophicus* was found to be able to reduce arsenate during anaerobic growth with glucose. The type strain is MS 6^T (= NCCB100213^T = UNIQEM U377^T). The GenBank accession number for the 16S rRNA sequence and *nif*H gene sequence is EU143680 and EU204959, respectively.

Description of Anaerobacillus macyae Zavarzina et al. comb.nov. [basonym Bacillus macyae Santini et al., 2004]

In addition to the characteristics given for the new genus and those given for *A. macyae* by Santini et al. (2004), a presence of *nif*H gene was detected in this study. The type strain is JMM-4^T (=DSM 16346^T = JCM 12340^T). The GenBank accession number for the 16S rRNA sequence and *nif*H gene sequence is AY032601 and EU204960, respectively.

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