

Natronobacillus azotifigens gen. nov., sp. nov., an anaerobic diazotrophic haloalkaliphile from soda-rich habitats

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Abstract Gram-positive bacteria capable of nitrogen fixation were obtained in microoxic enrichments from soda soils in south-western Siberia, north-eastern Mongolia, and the Lybian desert (Egypt). The same organisms were obtained in anoxic enrichments with glucose from soda lake sediments in the Kulunda Steppe (Altai, Russia) using nitrogen-free alkaline medium of pH 10. The isolates were represented by thin motile rods forming terminal round endospores. They are strictly fermentative saccharolytic anaerobes but tolerate high oxygen concentrations, probably due to a high catalase activity. All of the strains are

obligately alkaliphilic and highly salt-tolerant natronophiles (chloride-independent sodaphiles). Growth was possible within a pH range from 7.5 to 10.6, with an optimum at 9.5–10, and within a salt range from 0.2 to 4 M Na⁺, with an optimum at 0.5–1.5 M for the different strains. The nitrogenase activity in the whole cells also had an alkaline pH optimum but was much more sensitive to high salt concentrations compared to the growing cells. The isolates formed a compact genetic group with a high level of DNA similarity. Phylogenetic analysis based on 16S-rRNA gene sequences placed the isolates into *Bacillus* rRNA group 1 as a separate lineage with *Amphibacillus tropicus* as the nearest relative. In all isolates the key functional nitrogenase gene *nifH* was detected. A new genus and species, *Natronobacillus azotifigens* gen. nov., sp. nov., is proposed to accommodate the novel diazotrophic haloalkaliphiles.

The GenBank accession numbers for the 16S rRNA gene of the novel strains are EU143681-EU143690 and EU850814-EU850816; for the *nifH* gene the accession numbers are EU542601, EU563380-EU563386 and EU850817-EU850819.

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Introduction

Soda-rich habitats are unique saline habitats containing substantial concentrations of sodium carbonates among the dominant soluble salts. They include soda (sodic) soils and soda lakes. They are locally distributed in the dry steppe and semidesert areas, such as south Siberia, north-eastern Mongolia and north China in Central Asia, Egypt, Ethiopia, Kenya and Tanzania in Africa as well as in Armenia, India, Pakistan, western Ukraine, Hungary and eastern North America (Bazilevich 1970; Kondorskaya 1965; Grant 1986; Zavarzin 2007). In contrast to the well-studied soda lakes (Jones et al. 1998; Zavarzin et al. 1999; Sorokin and

Kuening 2005), information on microbial diversity and activity in soda solonchaks is very scarce. Most of the studies on saline soils concerned salt-tolerant symbiotic diazotrophs and salt-tolerant plants (Zahran 1997). On top of the high alkalinity and salinity, soda soils are subjected to long periods of desiccation with high aeration, which should lead to a lower diversity of haloalkaliphilic taxa with a domination of spore-forming organisms, such as Gram-positive bacteria, streptomycetes and fungi.

Nitrogen fixation (NF) activity is one of the most important indicators of microbial activity. The evidence for NF activity in haloalkaline habitats is very scarce. A few studies on NF in soda lakes identified haloalkaliphilic cyanobacteria as the only active diazotrophic group (Oremland 1990; Herbst 1998; Zavarzin et al. 1999) in water. Quite recently the potential of NF was also discovered in a few haloalkaliphilic bacteria isolated from soda lake sediments, such as the aerobic *Gammaproteobacteria Thiakalispira microaerophila* and *Alkalilimnicola halodurans* (*nifH* gene detection) (Tourova et al. 2007) as well as the obligate anaerobes *Clostridium alkalicellulosum* (Zhilina et al. 2005) and *Geoalkalibacter ferrihydriticus* (Zavarzina et al. 2006) (activity and *nifH* detection). Molecular analysis of the *nifH* gene in brines of the alkaline saline Mono Lake demonstrated a domination of *Deltaproteobacteria* among the diazotrophic population, although no apparent activity was found under in situ conditions (Steward et al. 2004).

Recently, a potential for nitrogen fixation in soda solonchak soils was detected by our group. The presence of two groups of diazotrophic Gram-positive bacteria in haloalkaliphilic enrichment cultures was identified (Sorokin et al. 2008a). In this paper, a novel branch of obligately fermentative diazotrophic Gram-positive bacteria is described. This branch belongs to the rRNA group 1 of bacilli, which dominate the soda soils and soda lakes of Central Asia and Egypt. The NF potential in this cluster of

low-GC Gram-positive bacteria has never been previously recognized.

Methods

Samples

Soda solonchak samples used for the enrichments (top 5 cm) were obtained from the north-eastern part of Mongolia (Choibalsan Province, 1999), from the Kulunda Steppe (Altai, Russia, 2003) and from Wadi Natrun valley in the Lybian desert (Egypt, 2001). The samples were kept in sterile plastic Petri dishes and stored at 4°C. The soda lake sediment samples (top 10 cm of the soil) were taken from three lakes in the south Kulunda Steppe (2007). The chemical properties of the samples are given in Table 1.

Potential nitrogen fixation (NF) activity

Potential NF activity in soils was measured by the acetylene reduction method (Stewart et al. 1968) using 5 g of sample flooded with 3 ml water in 15 ml serum bottles at 0, 2 and 20% O₂ in the gas phase. After 2 days of preincubation, 10% of acetylene in the gas phase and 5 mg of glucose were supplied to start the experiment. The sediments were mixed with near bottom brines or (after removal of the pore brines by centrifugation) with soda buffer (pH 10, same sodium concentration as in the lake brines) at 1:2 ratio and incubated either aerobically or anaerobically with 10% acetylene and 10 mM of various electron donors. Ethylene production, measured daily by GC, was taken as an indication of the NF potential. The same method was used to measure NF activity in pure cultures, except that in this case acetylene was added after replacement of the gas phase with argon.

Table 1 Characteristics of the samples

Habitat	Sample	Location	pH ^a	Total soluble salts ^b (%)	Total soluble alkalinity ^c (mM)
Soda solonchak soils	13KS	Kulunda Steppe (Altai, Russia)	9.85	5.3	50
	24KS		10.21	7.3	1,150
	12MS	n-e Mongolia	10.35	4.8	50
	3ES	Wadi Natrun, Egypt	10.05	8.5	1,740
Soda lake sediments	Lake Picturesque (Zh)	Kulunda Steppe (Altai, Russia)	10.20	10.0	1,340
	Lake Tanatar-5 (T5)		10.35	7.0	770
	Lake Bitter-1 (B1)		10.53	17.5	2,950

^a The pH of the soil samples was measured in a 1:5 water extract

^b (w/w) for the soil samples and (w/v) for the lake brines

^c mmol kg⁻¹ for soils and mmol l⁻¹ for lakes

Cultivation

The enrichment cultures were started from the activated samples using a 10% inoculum under microoxic conditions (10 ml medium in 15 ml static flasks with rubber stoppers) at 25°C using a nitrogen-free soda medium of the following composition (g l⁻¹): Na₂CO₃ 22–95; NaHCO₃ 8–15; NaCl 6–16; K₂HPO₄ 0.5. The range of the medium components represents a total salt concentration from 0.6 to 2 M Na⁺. After sterilization (pH of the medium was 10), the medium was supplemented with 1 mM MgSO₄, 1 ml l⁻¹ of trace metal solution, a vitamin mixture (Pfennig and Lippert 1966), 2 g l⁻¹ filter-sterilized glucose, and 10 mg l⁻¹ of yeast extract. After the appearance of visible turbidity, NF activity was measured and in the case of positive results the cultures were transferred into fresh medium at 1:100 dilution for several times in order to stabilize. Finally the cultures were serially diluted and the most active dilution (5–6 times) was plated onto solid medium with the same composition, which was prepared by 1:1 mixing of double-strength liquid soda medium and 4% agar at 50°C. The plates were incubated in gas-tight jars (Oxoid) under 95% argon/5% N₂ atmosphere for 1–2 weeks at 25°C. Colonies with different morphology were placed into liquid medium and the resulting cultures were checked for NF potential.

Phenotypic characterization of the pure cultures

The phenotypic characterization of the isolates included light and electron microscopy, growth experiments to determine the substrate utilization profile (at pH 10 and 0.6 M total Na⁺) as well as the influence of pH and salt on the growth and NF activity. For the latter, the cells were grown on standard nitrogen-free medium of pH 10 and consequently centrifuged and washed with 0.5 M NaHCO₃ of pH 8 at a protein concentration of 20 mg ml⁻¹. For determining the pH range of growth, the cells were resuspended at a concentration of 0.3–0.4 mg protein ml⁻¹ in a series of buffers (Sorokin 2005) containing 1.3 M total Na⁺, while for the salt range tests soda buffer of pH 10 was used at a concentration of 0.1–4 M Na⁺. The tests were performed in duplicate in 7 ml serum flasks with 3 ml cell suspension under anoxic conditions. The flasks were made anoxic by the replacement of air for argon, after which sodium sulfide (1 mM), glucose (1 mg ml⁻¹) and acetylene (10% in the gas phase) were added. Ethylene production was measured every 4 h by gas chromatography (Chrome-41, Czech Republic; column—Spherosil, 3.2 m; FID detector).

The concentration of cell protein was determined by the Lowry method (Lowry et al. 1951). The catalase activity in washed cells was quantified by using the iodimetric method (Sumner and Dounce 1963). The phospholipid composition

was detected by TLC according to Govorukhina and Trotsenko (1989). The fatty acids of the membrane polar lipids were extracted with a mixture of methanol and chloroform and the composition was characterized by GC-MS according to Zhilina et al. (1997). The isomers of diaminopimelic acid (DAP) in the peptidoglycan were determined by TLC on cellulose (Hasegawa et al. 1983). The comparison of total protein content was performed using denaturing gradient electrophoresis according to Laemmli (1970). The final products of the glucose fermentation were detected by HPLC-anionic chromatography using a HPX-87-H column (BioRad) at 60°C. The products were detected by a UV and RI detector and a 5 mM H₂SO₄ solution at 0.6 ml min⁻¹ was used as eluent. The samples were neutralized to pH 7 before analysis.

Genetic analysis

The isolation of the genomic DNA was performed according to Marmur (1961) and the subsequent determination of the G + C content of the DNA and the DNA–DNA hybridization were carried out by using the thermal denaturation/reassociation technique (De Ley et al. 1970; Marmur and Doty 1962). The 16S rRNA genes were amplified by using the universal bacterial primers 11F-1492R (Lane 1991) and the fragments of the *nifH* gene were amplified with the specially designed and previously tested primer pair F1-R6 (Marusina et al. 2001). The PCR products were purified from low-melting agarose using the Wizard PCR-Prep kit (Promega, USA). Sequencing was performed using the Big Dye Terminator v.3.1 sequencing reaction kit using an ABI 3730 DNA automatic sequencer (Applied Biosystems, Inc., USA). Preliminary phylogenetic analysis of the new sequences was performed using 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 Neighbor-Joining algorithm within the TREECONW program package (Van de Peer and De Wachter 1994).

Results and discussion

Potential NF activity

The acetylene reduction test demonstrated the presence of a diazotrophic community in all the four investigated soda solonchak samples with potential NF activity ranging from 16 to 80 nmol N (g h)⁻¹. However, the activity started to manifest itself only after a 2–3-day lag phase indicating either a low initial number of active diazotroph cells or

their dormant state (spores). The activity was inhibited at fully aerobic conditions and was almost equal at microoxic (2% oxygen) and anoxic conditions.

The NF potential was measurable directly only in the soda lake sediments of Lake Picturesque (low sulfide sediments), while in the other two lakes (high sulfide sediments) the activity manifested itself only after removal of the pore brines. A qualitative ammonium test (Merck) was positive in the latter and negative for the Picturesque Lake sediments, which might explain the results. Perhaps, the absence of the NF activity reported recently for the Mono Lake anoxic waters (Steward et al. 2004) was also the result of a blockade by bound nitrogen. The potential NF activity in the unblocked lake sediments under dark anaerobic conditions with glucose was within the range of 2–30 nmol N (g h)⁻¹. The NF activity in the sediments clearly belonged to an obligately alkaliphilic population (Fig. 1a) with only a moderate salt tolerance (Fig. 1b).

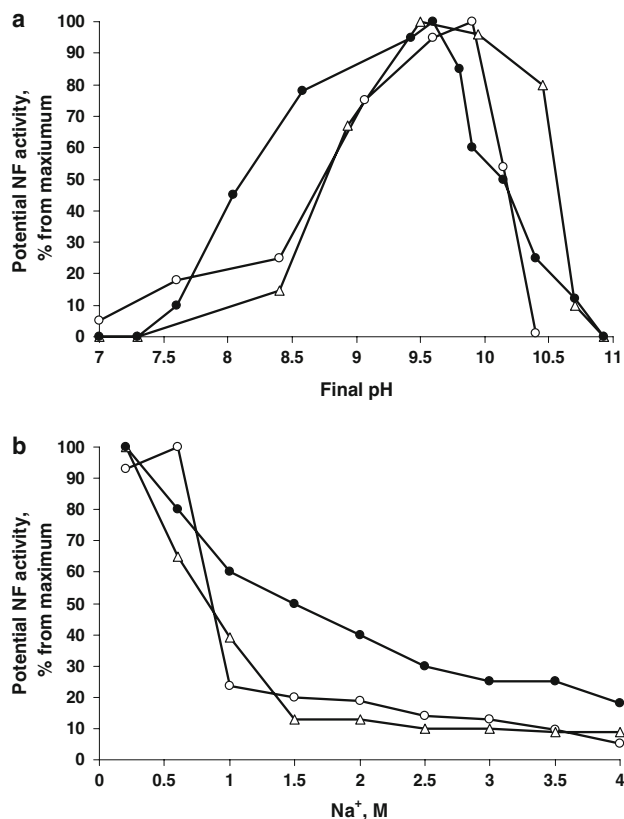


Fig. 1 The influence of pH at native salinity (a) and sodium carbonates at pH 10 (b) on potential NF activity under dark anoxic conditions in sediment slurries (1 part sediment/2 parts buffer) from soda lakes. The activity was measured after removal of the pore brines and resuspension of the solids in anaerobic buffers. 0.1 M HEPES/NaCl was used for the pH range from 6 to 8 and NaHCO₃/Na₂CO₃ for the pH range from 8 to 11.5. The pH was adjusted by 1 M HCl or NaOH before the experiment and the final pH values were taken as the incubation pH. Incubation time was 24 h. 100% activity, nmol N/(cm³ h)⁻¹: Picturesque lake (closed circles) = 9; Tanatar-5 (open circles) = 2; Bitter-1 (open triangles) = 37

Enrichment and isolation of the diazotrophic haloalkaliphiles

In the microoxic (soils) and anoxic (lake sediments) enrichment cultures (pH 10, salt concentration equivalent to 0.6–2 M total Na⁺) with glucose, Gram-positive endospore-forming bacteria were obviously dominating. However, most of them were apparently not diazotrophic, despite the absence of bound nitrogen in the medium. In the serial dilutions made from the primary enrichments, growth was observed up to (–9)–(–10) dilution, while the acetylene reduction stopped already at (–6)–(–7) dilution. Hence, the isolation of pure cultures on plates was not straightforward. Of more than a hundred colonies obtained from seven enrichments only eleven resulted in active diazotrophic cultures.

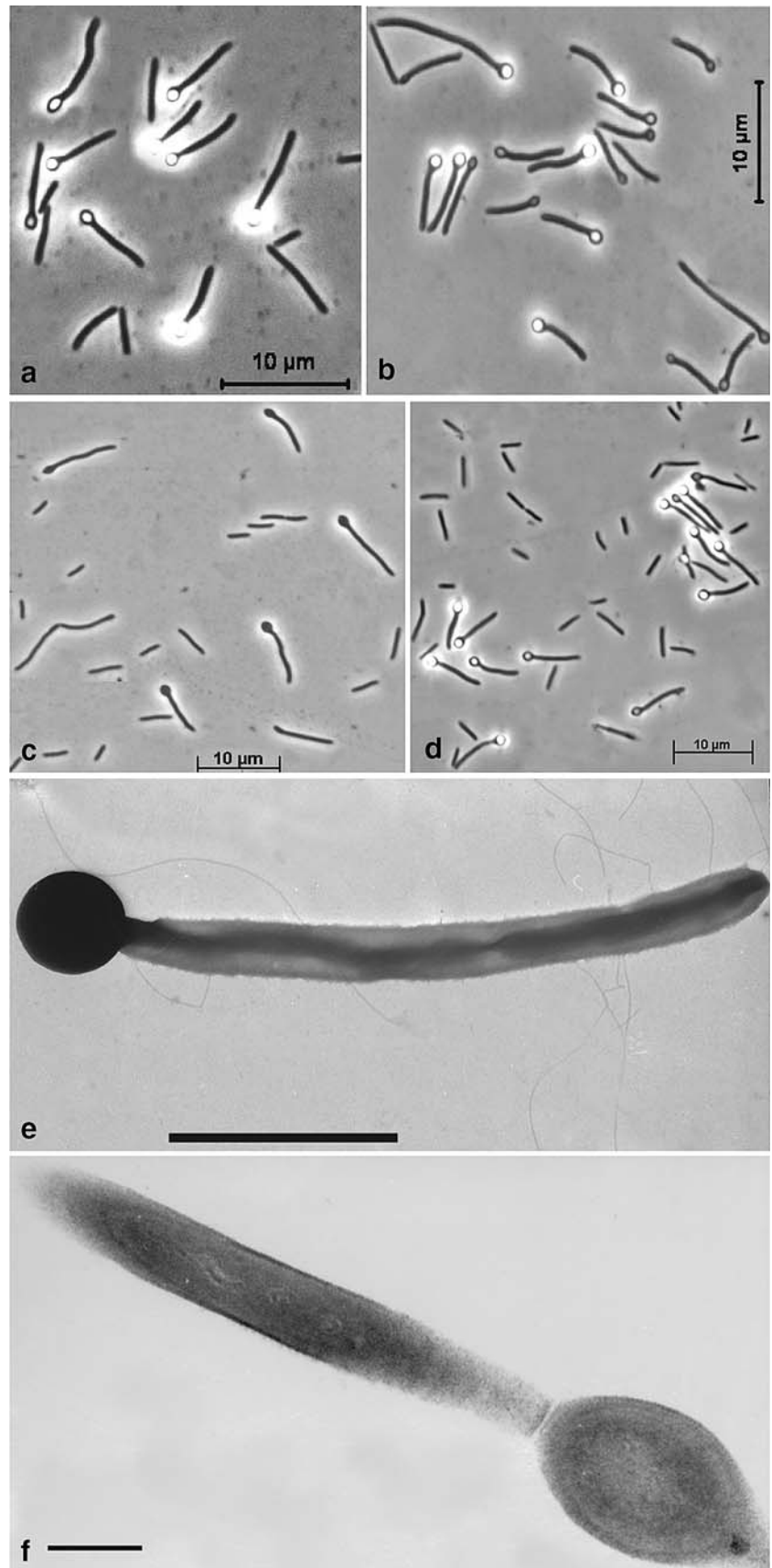
Properties of the haloalkaliphilic diazotrophs

All of the diazotrophic cultures were represented by Gram-positive, thin and slightly curved rods of variable length. The rods were motile with peritrichous flagella and usually formed round terminal endospores (Fig. 2). They had a fluffy polysaccharide matrix in the concentrated cell pellets and a yellow pigmentation of a varied intensity. The latter was due a presence of methanol-extractable pigment, which on basis of its spectrum (maximum at 435 nm and shoulders at 407 and 465 nm) resembled a novel C₂₀ xanthine-like chromophore recently described for the extremely natronophilic strains of the genus *Thioalkalivibrio* (Takaichi et al. 2004).

All of the strains were strictly fermentative anaerobes using various simple carbohydrates, but they could tolerate fully aerobic conditions and continued to ferment glucose even after being placed in shaking flasks. This observation is probably due to a relatively high catalase activity in the organism [0.2–0.4 U (mg protein)⁻¹]. The growth rate under aerobic conditions was equal to that of the anaerobic cultures but the final growth yield was two times lower at forced aeration, indicating that some inhibition of growth had taken place. The respiration test with washed cells showed no glucose-stimulated oxygen consumption. The final fermentation products from glucose were ethanol, acetate and formate. Cytochromes were not detectable by spectrophotometer in the cell-free extracts. One of the striking features of the novel isolates was their extraordinary sulfide tolerance. During the anaerobic growth at pH 10 with glucose all strains tolerated up to 100–120 mM of sulfide.

The analysis of phospholipids in strain 24KS-1^T grown at 0.6 M of total Na⁺, pH 10 and 30°C demonstrated the presence of phosphatidic acid, phosphatidylethanolamine and phosphatidylglycerol (cardiolipin) as major components as well as several minor unidentified phospholipids. Fatty acid analysis of the polar lipids in the same strain showed a profile typically found in bacilli with a domination of

Fig. 2 The cell morphology of diazotrophic strains from soda soils grown with glucose at pH 10. **a–d** Phase contrast micrographs (**a** strain 24KS-1^T; **b** strain MS4-1; **c** strain T5-1; **d** strain B1-3d); **e** electron micrograph of a total preparation, strain 24KS-1^T (*bar* 1 μm); **f** thin section, strain 24KS-1^T (*bar* 0.5 μm)



saturated species, such as anteisoC_{15:0}, isoC_{15:0} and C_{16:0} (supplementary Table). Analysis of the respiratory quinones in strain 24KS-1^T gave negative results, probably due to the strict fermentative nature of this bacterium. The cell wall analysis demonstrated a presence of *meso*-DAP as a diagnostic diaminoacid in its peptidoglycan.

The behavior under different pH and salt conditions, especially of the nitrogenase activity, of the new isolates was of special interest. The growth and activity experiments demonstrated that all of the isolates were obligately alkaliphilic and extremely natrono(soda)-tolerant (Fig. 3). However, the isolates did not depend on chloride, which is a typical trait for the bacteria isolated from soda-rich habitats. Surprisingly, despite the fact that both the nitrogenase and fermentation enzymes are intracellular, both systems reacted to changes in pH in the same way as the growing cultures, i.e. exhibited alkaliphilic profiles (Fig. 3a). However, the nitrogenase activity was much more sensitive to salt inhibition than the cultures growing in the presence of organic nitrogen (Fig. 3b). Both the pH profile and the salt profile for the nitrogenase activity corresponded well to that which was observed in the sediment slurry experiments (see Fig. 1).

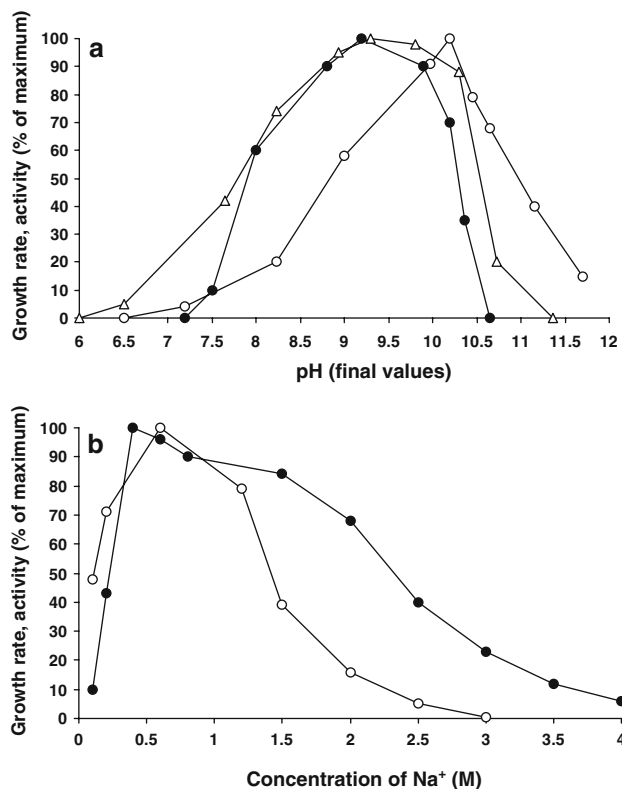


Fig. 3 The influence of pH at 1.3 M Na⁺ (a) and of sodium at pH 10 (b) on the fermentative growth and the activity of washed cells of strain 24KS-1^T with glucose. Closed circles growth rate, open circles nitrogenase activity in washed cells, open triangles activity of glucose uptake by washed cells

Identification of the haloalkaliphilic diazotrophs

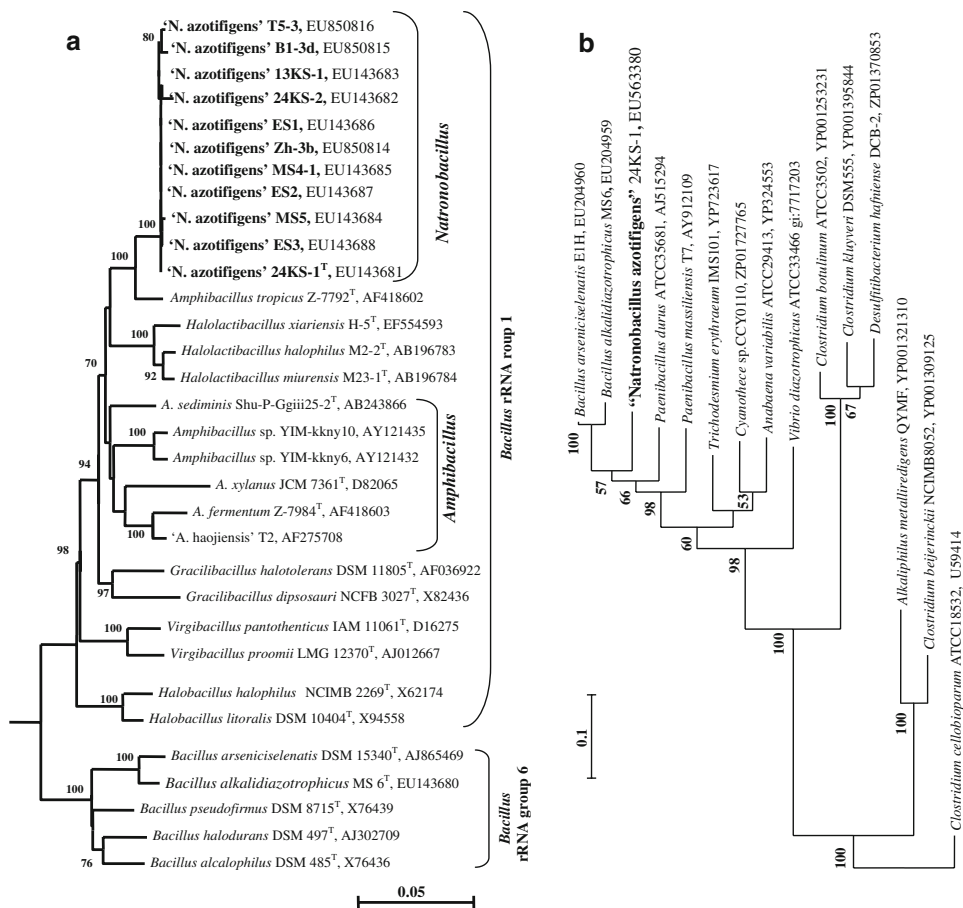
Phylogenetic analysis based on sequencing of the 16S-rRNA gene placed the isolates into the rRNA group 1 of bacilli (Nielsen et al. 1994) (Fig. 4a). The strains formed a compact group with almost identical sequences (99–99.7%) and were clustering (96.0–96.6% of sequence similarity) with *Amphibacillus tropicus*, which is an aerotolerant fermentative haloalkaliphile from soda lakes (Zhilina et al. 2001). The diazotrophic isolates had a 70–93% DNA–DNA similarity as well as similar, but not identical, total protein profiles (supplementary Fig. 1), which indicates that they belong to a single genetic species. On the other hand, the isolates had only a 30–35% DNA similarity (strains 24KS-1^T, MS5, ES3 and T5-3) with *A. tropicus*.

From the tree topology and the sequence distances, it is quite clear that the genus *Amphibacillus* in its present form needs a revision. The type species *A. xylanus*, *A. fermentum*, *A. sediminis* and several undescribed isolates from saline lakes form the core group of the genus, while *A. tropicus* and the novel diazotrophic haloalkaliphiles from soda habitats form a separate generic lineage with only 91–93% sequence similarity to the core group. Another closely related group to the novel isolates with 16S rRNA gene sequence similarity of around 95% is the genus *Halolactibacillus* (Ishikawa et al. 2005), accommodating alkali- and halo-tolerant marine fermentative bacteria. A phenotypic comparison of the novel diazotrophs with their closest phylogenetic relatives is given in Table 2. Assuming the close relation of the novel diazotrophs to the *Amphibacillus* species, the ability to fix N₂ was also tested in the latter (*A. tropicus* and *A. fermentum*), but without success.

nifH detection

Functional gene analysis demonstrated the presence of the *nifH* gene (coding for the nitrogenase-reductase subunit of nitrogenase) in all of the diazotrophic isolates but not in their haloalkaliphilic relatives (*A. tropicus* and *A. fermentum*). The sequences had a very high similarity level (>95%) and formed a separate cluster related to the *nifH* gene of a recently described *Bacillus alkalidiazotrophicus* MS 6, which is a novel diazotrophic representative of the RNA group 6 of bacilli from the Mongolian soda soil, and its closest haloalkaliphilic relative *B. arseniciselenatis* (Sorokin et al. 2008 b) (Fig. 4b). This alkaliphilic cluster formed a novel deep lineage within the *nifH* gene family related to *Paenibacillus* and cyanobacteria. It is remarkable that the only group of bacilli containing diazotrophic species is currently confined within the aerobic genus *Paenibacillus* (Achouak et al. 1999). Apparently, there is more to the ability of the Gram-positive bacteria (other than clostridia) to fix nitrogen than is currently recognized.

Fig. 4 The phylogenetic position based on the 16S rRNA gene sequence analysis (a) and inferred amino acid sequence analysis of the *nifH* gene (b) of the diazotrophic haloalkaliphilic isolates. Tree topography and evolutionary distances are given by the neighbor-joining method with Jukes and Cantor corrections. Numbers at the nodes indicate the percentage of bootstrap values for the clade of this group in 1,000 replications



Concluding, the results indicate the presence of an active population of fermentative diazotrophs in extremely haloalkaline habitats from different geographical locations, which forms a novel phylogenetic lineage within the rRNA group 1 of bacilli. These bacteria are extremely salt tolerant obligate alkaliphiles able to grow in saturated soda brines. The nitrogen fixation activity is active up to 2 M total Na⁺ at pH 10. These conditions were not reported so far for any other diazotrophs. Based on the phenotypic and genetic comparison, the new haloalkaliphilic diazotrophic members of the rRNA group 1 of bacilli from soda-rich habitats are proposed to be accommodated by a new genus and species *Natronobacillus azotifigens* gen. nov., sp. nov.

Description of *Natronobacillus* gen. nov.

(N.Gr. n. *natron*, arbitrarily derived from the Arabic n. natrun or natron, soda; L. masc. n. *bacillus*, a small rod; N.L. masc. n. *Natronobacillus* soda-loving rod)

Rod-shaped, motile, endospore-forming bacterium with obligately fermentative metabolism. Aerotolerant and extremely sulfide-tolerant. Cells contain yellow pigment. Fatty acids of the polar lipids are dominated by saturated

C₁₅-C₁₆ species. Peptidoglycan contains *meso*-DAP. Diazotrophic. Obligately alkaliphilic and extremely salt-tolerant. Natronophilic (soda-loving) and chloride-independent. Utilizes simple carbohydrates and some natural polymers, such as starch, xylan and glycogen. A member of the rRNA group 1 of bacilli with low G + C content. Habitats—soda soils and soda lake sediments. The type species is *Natronobacillus azotifigens*.

Description of *Natronobacillus azotifigens* sp. nov.

(*a.zo.ti.fi'*gens N.L. n. azotum (from Fr. n. azote), nitrogen; L. part. adj. *figens*, fixing, attaching; N.L. part. adj. *azotifigens*, nitrogen-fixing)

Cells are Gram-positive, thin and curved rods of 0.4–0.5 × 2.0–6.0 μm, motile with peritrichous flagella. Cells contain yellow membrane-bound pigment with an absorption maximum at 435 nm. The dominant phospholipids include phosphatidic acid, phosphatidylethanolamine and phosphatidylglycerol. The dominant cellular fatty acids are aiC_{15:0}, iC_{15:0}, aiC_{13:0}, C_{16:0}, aiC_{17:0} and iC_{13:0}. Strictly fermentative but aerotolerant anaerobe. Extremely sulfide-tolerant. The following carbohydrates can be

Table 2 The properties of the diazotrophic strains from soda habitats compared to those of the closest relatives from *Bacillus* rRNA group 1

Property	1	2	3	4
Cell morphology	Long curved rods	Long curved rods	Short straight rods	Long curved rods
Endospores	+	+	–	–
Yellow pigment	+	–	–	–
Dominant fatty acids in polar lipids	aiC _{15:0} , iC _{15:0} , aiC _{13:0} , C _{16:0}	aiC _{15:0} , iC _{15:0} , C _{16:0} , iC _{13:0}	C _{16:0} , aiC _{13:0} , C _{15:0}	aiC _{15:0} , C _{16:0} , iC _{15:0} , aiC _{17:0} , C _{15:0}
Fermentative growth	Obligate	Obligate	Obligate	Aerobe
Main fermentation products	Acetate, formate, ethanol	Acetate, formate, ethanol	Lactate, acetate, formate, ethanol	–
Catalase	+	+	–	+
Nitrogen fixation	+	–	ND	ND
Alkaliphility	Obligate	Obligate	Facultative	Tolerance
Na ⁺ M range, (optimum)	0.2–4.0 (1.0–1.5)	0.2–3.6 (1.0–1.9)	0–4.1 (0.5)	0–2.7 (0)
Na ⁺ -dependence	+	+	–	–
Cl [–] -dependence	–	–	+	+
Nitrate reduction	–	–	+	+
Utilization of pentoses	D-Ribose, D-xylose	D-Xylose	D-Ribose	D-Xylose
Utilization of <i>N</i> -acetyl-glucosamine	–	–	ND	+
Polymer hydrolysis	Starch, xylan, glycogen	Starch, xylan, glycogen	Starch	Starch, glycogen, gelatin
Hydrolysis of Tween-80	–	–	ND	+
G + C in the DNA, mol% (Tm)	36.1–37.2	39.2	38.5	38.0
Isolated from:	Soda soils and lakes	Soda Lake Magadi (Kenya)	Sea water	Great Salt Lake

1 *Natronobacillus azotifigens* 24KS-1^T/ES1/T5-1, 2 *Amphibacillus tropicus* (Zhilina et al. 2001), 3 *Halolactibacillus halophilus* (Ishikawa et al. 2005), 4 *Gracilibacillus halotolerans* (Wainø et al. 1999)

ND not determined

fermented: glucose, fructose, maltose, xylose, galactose, sucrose, cellobiose, raffinose (some strains), and weakly: ribose, lactose, mannose, melibiose, melizitose and arabinose. Able to hydrolyze and utilize starch, xylan and glycogen. Glucose is fermented to acetate, ethanol and formate. Diazotrophic. Obligate alkaliphile with a pH range for growth between 7.5 and 10.6 (optimum 9.5–10) and a salt range from 0.2 to 4 M Na⁺ (optimum 1.0–1.5 M). Mesophilic with an optimum temperature for growth at 36–38°C. The G + C content in the genomic DNA is 36.1–38.5 mol% (Tm). Isolated from soda solonchak soils and soda lake sediments in Central Asia and Egypt.

The type strain is 24KS-1^T (NCCB100215^T = UNIQEM U378^T). Isolated from soda soils in the Kulunda Steppe (Altai, Russia). The G + C content in the genomic DNA of the type strain is 36.1 mol% (Tm). The Gen Bank accession number of the 16S-rRNA gene and *nifH* gene sequences of the type strain are EU143681 and EU563380, respectively.

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