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Planktonic bacterial community composition of an extremely shallow soda pond during a phytoplankton bloom revealed by cultivation and molecular cloning

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Abstract

Böddi-szék is one of the shallow soda ponds located in the Kiskunság National Park, Hungary. In June 2008, immediately prior to drying out, an extensive algal bloom dominated by a green alga (*Oocystis submarina* Lagerheim) was observed in the extremely saline and alkaline water of the pond. The aim of the present study was to reveal the phylogenetic diversity of the bacterial communities inhabiting the water of Böddi-szék during the blooming event. By using two different selective media, altogether 110 aerobic bacterial strains were cultivated. According to the sequence analysis of the 16S rRNA gene, most of the strains belonged to alkaliphilic or alkalitolerant and moderately halophilic species of the genera *Bacillus* and *Gracilibacillus* (Firmicutes), *Algoriphagus* and *Aquiflexum* (Bacteroidetes), *Alkalimonas* and *Halomonas* (Gammaproteobacteria). Other strains were closely related to alkaliphilic and phototrophic purple non-sulfur bacteria of the genera *Erythrobacter* and *Rhodobaca* (Alphaproteobacteria). Analysis of the 16S rRNA gene-based clone library indicated that most of the total of 157 clone sequences affiliated with the anoxic phototrophic bacterial genera of *Rhodobaca* and *Rhodobacter* (Alphaproteobacteria), *Ectothiorhodospira* (Gammaproteobacteria) and *Heliorestis* (Firmicutes). Phylotypes related to the phylum Bacteroidetes formed the second most abundant group. Clones related to the mainly anaerobic and alkaliphilic bacterial genera of *Anoxynatronum* (Firmicutes), *Spirochaeta* (Spirochaetes) and *Desulfonatronum* (Deltaproteobacteria) were also abundant. Further clone sequences showed less than 95% similarity values to cultivated species of the phyla Actinobacteria, Cyanobacteria, Deinococcus-Thermus, Fibrobacteres, Gemmatimonadetes and Lentisphaerae.

Key words: phytoplankton bloom, soda pond, 16S rRNA gene, phylogenetic diversity, planktonic bacteria

Introduction

Phylogenetic diversity of bacterial communities inhabiting athalassohaline lakes around the world has been examined in detail from the end of the twentieth century (Duckworth et al. 1996; Jones et al. 1998). Recently, the cultivation dependent and independent analyses of planktonic and sediment microbial communities include, among others, studying the soda lakes of South-East Siberia, Russia (Foti et al. 2007, 2008), North-East Mongolia (Sorokin et al. 2004), Inner Mongolia, China (Zhang et al. 2001; Ma et al. 2004a), Lonar Lake, India (Wani et al. 2006; Joshi et al. 2008), the East African Rift Valley, Kenya and Tanzania (Rees et al. 2004; Mwirichia et al. 2010, 2011), Wadi An Natrun, Egypt (Mesbah et al. 2007), as well as Mono Lake, California, USA (Hollibaugh et al. 2001; Humayoun et al. 2003) and Soap Lake, Washington, USA (Dimitru et al. 2008).

Previous studies have shown that saline and soda lakes restricted to the arid and semi-arid areas of the Earth can be characterized by a high level of primary production due to high water temperature and light intensity values as well as to the abundant nutrient supply. Besides the oxygen-producing cyanobacteria, the dominant presence of sulfide in the water and the anoxic circumstances can provide favorable conditions for mass production of halo-alkaliphilic phototrophic purple sulfur and non-sulfur bacteria, which may play a role in the development of the reddish-brown discoloration of these lakes (Jones et al. 1998; Mesbah et al. 2007; Milford et al. 2000; Mwirichia et al. 2011).

An earlier research conducted on planktonic algal communities inhabiting the soda ponds located in the plateau between Danube and Tisza rivers (Kiskunság National Park, Hungary) has revealed that approximately 74-99 % of the biomass consists of red-fluorescent coccoid picoalgae with an average of 1 μm in diameter. Following the changes of light intensity and water temperature values, the composition of phototrophic picoplankton shows a characteristic seasonal dynamics. In spring and autumn, picoeukaryotes, but in summer picocyanobacteria dominate the water of these soda ponds. This phenomenon was confirmed by laboratory examinations using picocyanobacterial and picoeukaryotic strains (Felföldi et al. 2009).

Böddi-szék, with its typical grayish-white soda water, is one of the extremely shallow ponds located in the Kiskunság National Park (Hungary) which provide extreme environment for microorganisms as a result of their special light conditions, chemical composition and temporary drying out.

The aim of the present study was to reveal the hitherto unknown planktonic microbial diversity of Böddi-szék soda pond during a phytoplankton bloom, prior to the summer desiccation. Different media were used to cultivate aerobic bacterial strains, and molecular cloning was applied to investigate the uncultured microbial phylogenetic diversity.

Materials and methods

Description of the study site and sampling

Böddi-szék (46°46'N; 19°08'E) located in the Kiskunság National Park (KNP) is a shallow soda pond (characterized by the dominance of Na^+, HCO_3^-, CO_3^{2-}, and Cl^- ions) and has a fragmented (non-continuous) water surface area with a maximum of 1.88 km^2 (Boros et al. 2008, Felföldi et al. 2009). In June 2008, following a few weeks of warm and dry period, the water level of Böddi-szék pond strongly decreased, the water temperature was high (33°C). The average water depth of the pond is approximately 15-20 cm, but at that time, it was no more than 5-6 cm. The detected conductivity (15 700 µS cm^{-1}) and salinity (12.34 g l^{-1}) values were extremely high. The level of dissolved oxygen in the water was 32.7 mg l^{-1} (458%). The physical and chemical parameters of the water were determined using a MultiLine P 8211 (WTW, Weilheim, Germany) portable field instrument.

At the time of the sampling the color of the upper part of the water layer was yellow-green, while the lower part was purple. Due to the extremely shallow water level, separation of water samples with different colors could not be accomplished, therefore approximately 500 ml of mixed water was collected into a sterile bottle.

The water sample was kept cool (6-8°C) until laboratory processing performed within 24 hours. Water physical and chemical data obtained in 2004 following a detailed survey (conducted monthly basis) on the Böddi-szék were used for reference data.

Cultivation of bacterial strains and DNA isolation

The homogenized and serially diluted water sample was plated onto sea water agar (DSMZ 246) and R2A medium (DSMZ 830) supplemented with 5% (w/v) NaCl (http://www.dsmz.de/media/media.htm). The pH of both media was adjusted to 9.5 by adding 1 M Na-sesquicarbonate solution (4.2 g $NaHCO_3$, 5.3 g Na_2CO_3 anhydrous in 100.0 ml distilled water). Colonies with different morphology were isolated following a 7-14 day incubation period at 28°C. The pure cultures were maintained on the isolation media.

The pH range for growth was determined in nutrient broth (3.0 g beef extract and 5.0 g peptone l^{-1}, supplemented with 50 g NaCl). The pH was adjusted from 7.0 to 12.0 with 1 M Na-sesquicarbonate solution at intervals of 1.0 pH units. The NaCl requirement for growth was studied in nutrient broth adjusted to pH 9.0 and supplemented with 0, 5, 7, 10 and 15 % (w/v) NaCl. All physiological tests were performed in duplicate at 28°C.

For amplification of the 16S rRNA gene, DNA from each bacterial strain was extracted using the Bacterial Genomic DNA Miniprep kit (V-GENE, Biotechnology Limited, Hangzhou, China) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR with universal bacterial primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3') (Lane 1991). The following temperature protocol was used: initial denaturation at 95°C for 3 min, followed by 32 amplification cycles of 30 s at 94°C, 30 s at 53°C and 1 min at 72°C, followed by final extension at 72°C for 30 min. The PCR products were purified using the Viogene PCR-M Clean Up System (Proteogenix SA, Illkirch Cedex, France) following the manufacturer's instructions.

Community DNA isolation

In order to obtain dense microbial biomass for DNA extraction, approximately 100 ml of the water sample was centrifuged (3200 rcf for 10 minutes) and then DNA isolation was performed using FastDNA^{TM} Kit (BIO 101 Inc., CA, USA). Isolated DNA was purified with Geneclean Spin Kit (BIO 101, Inc., CA, USA) according to the manufacturer's instructions. 16S rDNA was amplified by PCR using bacterial 27f and 1492r primers (Lane 1991). The following temperature protocol was used: initial denaturation at 98°C for 5 min, followed by 32 amplification cycles of 30 s at 94°C, 30 s at 52°C and 1 min at 72°C, followed by final extension at 72°C for 30 min. The PCR reaction mixture contained 200 mM of each deoxynucleoside triphosphate, 1 U of LC *Taq* DNA Polymerase (recombinant) (Fermentas, Lithuania), 1 x Taq buffer with $(NH_4)_2SO_4$ (Fermentas, Lithuania), 2 mM $MgCl_2$, 0.65 mM of each primer, and about 20 ng of genomic DNA template in a total volume of 50 µL. 16S rDNA PCR products were purified and concentrated using PCR-M Clean Up Kit (Viogene-Biotek Corp., CA, USA) following the manufacturer's instructions.

16S rRNA gene clone library construction

The purified PCR product was cloned into *E. coli* JM109 competent cells (Promega, WI, USA) using the pGEM®-T Easy Vector kit (Promega, WI, USA) following the manufacturer's

instructions. Plasmids were extracted by boiling a loopful of bacterial cells in 50 µl water (5 min at 98°C) and pelleting the debris by centrifugation (4500 rcf for 5 min). The supernatant was transferred into fresh tubes. The inserts from the recombinant plasmids were amplified by PCR using plasmid specific M13f (5'-GTAAAACGACGGCCAG-3') and M13r (5'-CAGGAAACAGCTATGAC-3') primers (Messing 1983). Samples were first denatured at 96°C for 3 min, followed by 32 amplification cycles of 94°C for 30 sec, 52°C for 30 sec and 72°C for 1 min, and a final extension at 72°C for 10 min. To obtain the original inserts without the vector's flanking regions, a nested PCR was carried out with the original primers (27f, 1492r), and the following thermal protocol: 96°C for 3 min initial denaturation, followed by 32 amplification cycles at 95°C for 30 sec, 52°C for 30 sec, 72°C for 1 min, and a final extension at 72°C for 10 minutes.

ARDRA, 16S rRNA gene sequencing and phylogenetic analysis of bacterial strains and clones

PCR products from all strains and clones were subjected to Amplified Ribosomal DNA Restriction Analysis (ARDRA) using *Msp*I and *Bsu*I (Fermentas, Vilnius, Lithuania) enzymes as described by Massol-Deya et al. (1995). The fragments were electrophoresed in 1.5% ethidium bromide stained agarose gel and visualized by UV excitation. Representatives from each unique ARDRA fingerprint group were chosen and subjected to partial sequencing.

The PCR products were subsequently purified and concentrated using PCR-MTM Clean Up System (Viogene, Sunnyvale, USA). Sequencing reaction was performed using Big Dye Terminator Cycle Sequencing Ready Reaction Kit v 3.1 according to the manufacturer's protocol with primer 519r (5'-GWATTACCGCGGCKGCTG-3') (Lane 1991). An ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, USA) at our own facility was used for automated sequence analysis. Approximately 500 bp in the 5′ end of the 16S rRNA gene was sequenced. Clone sequences that were identified as presumptive chimeras with Pintail (Ashelford et al. 2005) were eliminated from further analysis.

The obtained sequences were aligned in NCBI (National Centre for Biotechnology Information) (www.ncbi.nlm.nih.gov) using the BLAST (Basic Local Alignment and Search Tool) program (Altschul et al. 1997) and by EzTaxon-e identification service (Kim et al. 2012) against the database of type strains with validly published prokaryotic names. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al. 2011) with the Kimura two-parameter model (Kimura 1980) and the neighbor-joining algorithm (Saitou and Nei 1987). The sequences of representative strains and clones

were submitted to the EMBL (European Molecular Biology Laboratory) Nucleotide Sequence Database (http://www.ebi.ac.uk/embl/), under accession numbers presented in Figs 1-4.

Results

Phylogenetic analysis and ecological tolerance of bacterial strains

From the water sample of Böddi-szék, altogether 110 aerobic bacterial isolates with different colony morphology were obtained, of which 57 and 53 were retrieved from sea water agar and R2A medium, respectively. All representatives from the 15 ARDRA groups were sequenced. Strains showed the closest relation to the genera *Erythrobacter* and *Rhodobaca* within the class Alphaproteobacteria, *Alkalimonas*, *Halomonas, Marinospirillum* and *Nitrincola* within the class Gammaproteobacteria, *Algoriphagus* and *Aquiflexum* within the phylum Bacteroidetes, furthermore *Bacillus* and *Gracilibacillus* within the phylum Firmicutes (Figs 1-3). Almost half of the strains showed 97% or less affiliation with known bacterial taxa, therefore, they may represent novel species. Strains affiliated with genera of the phylum Bacteroidetes, *Rhodobaca* and *Nitrincola* were isolated only from R2A medium, while those related to the genera *Halomonas* and *Gracilibacillus* were recovered only from Sea water agar. Others were cultivated from both media (Table 1).

All bacterial strains were tested for their pH and NaCl tolerance using nutrient broth. The pH values of the broths were adjusted between 7.0 and 12.0 while the NaCl tolerance was studied at 0, 5, 7, 10 and 15% (w/v) NaCl concentrations (Table 1). Regarding the results of the pH tolerance tests, most bacterial strains (members of genera *Erythrobacter, Halomonas, Marinospirillum, Nitrincola, Alkalimonas, Algoriphagus*) proved to be facultative alkaliphilic as they grew best at pH 9.0 or above but were able to grow at neutral pH as well. Members of genera *Aquiflexum* and *Gracilibacillus* showed optimal growth at pH 11.0 and could not grow at neutral pH so they can be considered obligate alkaliphilic. However, strains belonging to genera *Rhodobaca* and *Bacillus* showed optimal growth in the pH range of 7.0 and 9.0, therefore they are alkalitolerant. With respect to NaCl tolerance, all strains grew best at 5% (w/v) NaCl concentration. Most strains (members of *Erythrobacter, Rhodobaca, Halomonas, Algoriphagus, Aquiflexum* and *Gracilibacillus*) were not able to grow in the absence of NaCl. Strains belonging to genera *Marinispirillum, Nitrincola* and *Bacillus* had wide salt tolerance (between 0 and 15% (w/v) NaCl concentration), whereas those belonging to genera *Erythrobacter, Rhodobaca* and *Aquiflexum* showed narrow salt tolerance (between 5 and 7% (w/v) NaCl concentration).

Phylogenetic analysis of clones

Altogether 157 out of the 192 bacterial clones resulted in 62 ARDRA groups (5 clones were removed as they comprised putatively chimeric sequences). In order to estimate the diversity within the clone library, rarefaction analysis was used to calculate the appropriateness of screening for clones. Rarefaction curve (Supplementary Fig. 1) represented the number of processed clones against the number of different ARDRA patterns detected in the Böddi-szék clone library. The calculated rarefaction curve did not reach asymptote, indicating that the analysis of a greater number of clones would have revealed further diversity. Representative clones from Böddi-szék water were distributed among the phyla Deinococcus-Thermus, Cyanobacteria, Proteobacteria, Firmicutes, Actinobacteria, Spirochaetes, Fibrobacteres, Bacteroidetes, Gemmatimonadetes and Lentisphaere (Supplementary Fig. 2). According to the GenBank database used for sequence alignments, the Böddi-szék clone sequences showed 85.5 to 99.5% similarities to environmental clone sequences (Figs 1-4).

Discussion

In case of shallow soda lakes, the phenomenon called purple bacteria mass production has been reported only from those located in Siberia and Mongolia (Bryantseva et al. 1999b; Sorokin et al. 2004). A similar event has not been observed in Böddi-szék so far. However, in the course of the present study, bacterial phylogenetic diversity similar to that found in the north-eastern Mongolian soda lakes was revealed from the water of a shallow soda pond located in Kiskunság National Park (Hungary, Europe) during a phytoplankton bloom using a combination of cultivation based and molecular cloning methods.

Due to the known selectivity of the applied methods (e.g. sample collection, medium composition, cultivation conditions, nucleic acid extraction, PCR amplification and cloning), the results obtained by cultivation and molecular cloning complemented each other rather than overlapped. Among the Böddi-szék sequences originated from different ARDRA patterns, bacterial strains represented only three, whereas the molecular clones altogether ten phyla but the phylogenetic distributions were unequal. Members of Gammaproteobacteria were the most abundant groups in case of both cultivation and cloning methods. However, very few matches (e.g. *Nitrincola* and *Aquiflexum*) have been found at levels of bacterial genera or species. A similar result was also found by studying the bacterial diversity of

Phragmites australis periphyton communities in two other Hungarian soda ponds (Rusznyák et al. 2008).

According to the applied cultivation conditions, the largest proportion of bacterial strains were identified as obligate and facultative alkaliphilic and moderately halophilic or halotolerant species with chemoorganotrophic metabolism. In addition to *Bacillus* species, strains with 98 % sequence similarity to *Marinospirillum alkaliphilum* (Zhang et al. 2002) described from the Hailer soda lake (Inner Mongolia, China) and *Alkalimonas delamerensis* (Ma et al. 2004a) described from Lake Elmenteita soda lake (Kenya) were found in large proportions. It is interesting to note that the species *Bacillus aurantiacus* previously isolated and described from Böddi-szék (Borsodi et al. 2008), was not detected during this study. The different type of the sample (water and not sediment) and/or the dissimilar medium used for cultivation could be the reason for this. The sequence similarities of some Böddi-szék bacterial strains to the closely related species of *Belliella balticum* and *Aquiflexum balticum* ranged between 90-95 %. The type strains of both species were isolated from the surface water of the Baltic Sea during the decay of a plankton bloom (Brettar et al. 2004a, 2004b). These isolates through their chemoorganotrophic metabolism may participate in the aerobic decomposition of organic materials in the water of the soda pond.

Among the Böddi-szék isolates, those capable of photosynthesis showed 95-95 % sequence similarities to the halophilic species of *Erythrobacter longus* (Csotonyi et al. 2008) and to the alkaliphilic species of *Rhodobaca bogoriensis* (Milford et al. 2000). These purple non-sulfur bacteria are mixotrophs which use dissolved organic matter under aerobic conditions but are able to use the light and inorganic nutrients for their anoxic photosynthesis as well.

At phylum level, the dominance and phylogenetic affiliation of bacterial clones from the water sample of Böddi-szék (Supplementary Fig. 2) were consistent with the results of bacterial diversity revealed from the water of four Swedish lakes during cyanobacterial blooms using molecular biological methods (Eiler and Bertilsson, 2004). However, many Böddi-szék clones showed the highest sequence similarities to uncultured environmental clones originating from similar saline and alkaline aquatic environments (Dimitru et al. 2008; Humayoun et al. 2003; Mesbah et al. 2007). In accordance with our results, studying the prokaryotic communities inhabiting the water and sediment of Wadi an Natrun lakes (Egypt), Mesbah et al. (2007) also revealed an unexpected phylogenetic diversity and found that none of their rarefaction curves obtained from the analysis of clone libraries reached plateaus.

Among the Böddi-szék clones, phylotypes affiliated with different sequences of the phylum Proteobacteria were the most abundant (38 %), whereas those members of the phyla Bacteroidetes, Firmicutes and Spirochaetes were present in almost the same proportions.

Altogether 18 % of the Böddi-szék clones were affiliated (with 84-99 % sequence similarities) to different Bacteroidetes species, among which only *Belliella pelovolcani* could be identified at species level. Sequences related to the species *Aquiflexum balticum* were also found by cultivation. The detection of phylotypes related to Bacteroidetes as one of the predominant groups is in agreement with the previous cultivation independent studies performed on the bacterial communities inhabiting soda lakes (Humayoun et al. 2003; Mesbah et al. 2007; Rees et al. 2004).

Using the cloning method, sequences related to extremophile bacterial species with strictly anaerobic metabolism were also revealed. Some clones were related to the alkaliphilic, obligate anaerobic and saccharolytic fermentative bacterium *Anoxynatronum sibiricum* (Garnova et al. 2003) described from the Belo Nizhnee soda lake (Russia, Siberia). Approximately 12 % of the Böddi-szék clones were affiliated with different *Spirochaeta* species, among others, the alkaliphilic and obligate anaerobic fermentative species *Spirochaeta alkalica* and *Spirochaeta asiatica* described from the sediments of the alkaline lakes Magadi (East African Rift, Kenya) and Khatyn (Tuva, Russia) (Zhilina et al. 1996). These fermentative bacteria may participate in the anaerobic decomposition of the organic materials formed during the primary production. The end products of fermentation (e.g. organic acids and/or hydrogen) may serve as substrates for thiosulfate- or sulfate-reducing bacteria. Sequences related to thiosulfate-reducing species of *Dethiosulfatibacter aminovorans* and *Fusibacter paucivorans* as well as the sulfate-reducing species *Desulfobotulus sapovorans* and *Desulfonatronum lacustre* were also found among the Böddi-szék clones. In previous studies, the presence of sulfate-reducing phylotypes was reported mainly from the sediment of saline and soda lakes (Foti et al. 2007, 2008; Mesbah et al. 2007).

Several different phototrophic bacterial groups may take part in the primary production of soda lakes. Sequences related to the phylum Cyanobacteria were present (3 %), but did not dominate the Böddi-szék clone library, contrary to the bacterial communities of the shallow haloalkaline Lake Elmenteita, Kenya (Mwirichia et al. 2011).

Nevertheless, altogether 28 % of the Böddi-szék clone sequences were affiliated with different anoxygenic phototrophic bacteria of Erythrobacteraceae and Rhodobacteriaceae (Alphaproteobacteria), Ectothiorhodospiraceae (Chromatiales, Gammaproteobacteria) and Heliobacteriaceae (Clostridiales, Firmicutes). Beside the species *Rhodobaca bogoriensis*

identified by cultivation, sequences related with *Rhodobaca barguziensis*, *Rhodobacter capsulatus* and *Rhodovulum strictum* (species of purple non-sulfur bacteria) were also found in the Böddi-szék clone library. The species *Rhodovulum strictum* was described from colored blooms in tidal and seawater pools in Japan (Hiraishi and Ueda, 1995). Among the obligate photolitho-autotrophic purple sulfur bacteria, clones related to the moderately halophilic species *Ectothiorhodospira shaposhnikovii* and alkaliphilic *Thiorhodospira sibirica* described from a Siberian soda lake (Bryantseva et al. 1999b) were detected. In addition, some other Böddi-szék clones showed 96 % sequence similarity to the species *Heliorestis daurensis*, a bacteriochlorophyll-g containing, Gram-positive, alkaliphilic heliobacterium described from microbial mats of a low-salt alkaline Siberian soda lake (Bryantseva et al. 1999a).

Therefore, the results suggest that during the blooming event of the extremely shallow Böddi-szék soda pond, a special sulfur-cycle based microbial community called sulfuretum could develop. The sulfate produced by the metabolism of anoxygenic phototrophic purple sulfur and non-sulfur bacteria was directly converted to hydrogen sulfide by the metabolism of sulfate-reducing bacteria. Thereafter, hydrogen sulfide served again as electron donor for anoxygenic phototrophic bacteria.

Conclusions

The results of this study confirm that the special physical and chemical characteristics of the water of Böddi-szék during the phytoplankton bloom were favorable for a high diversity of bacteria by different metabolism. Due to its spatial-temporal restrictions, the examined astatic soda pond represents extreme environments, and maintains extremophile bacterial communities. The coexistence of bacteria with different types of sulfur metabolism observed during this study is evidence that sulfur cycle can be complete in an extremely shallow soda pond.

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Figure and table captions

Fig. 1 Phylogenetic relationships of Proteobacteria and Cyanobacteria sequences retrieved from Böddi-szék soda pond. (The neighbor joining phylogenetic tree was based on the 16S rRNA gene. Bootstrap values <50 were removed from the nodes. Bar, 2 nucleotide substitutions per 100 nucleotides. Sequences determined in this study appear in bold.)

Fig. 2 Phylogenetic relationships of Firmicutes and Actinobacteria sequences retrieved from Böddi-szék soda pond. (The neighbor joining phylogenetic tree was based on the 16S rRNA gene. Bootstrap values <50 were removed from the nodes. Bar, 5 nucleotide substitutions per 100 nucleotides. Sequences determined in this study appear in bold.)

Fig. 3 Phylogenetic relationships of Bacteroidetes sequences retrieved from Böddi-szék soda pond. (The neighbor joining phylogenetic tree was based on the 16S rRNA gene. Bootstrap values <50 were removed from the nodes. Bar, 5 nucleotide substitutions per 100 nucleotides. Sequences determined in this study appear in bold.)

Fig. 4 Phylogenetic relationships of Spirochaetes and other sequences retrieved from Böddi-szék soda pond. (The neighbor joining phylogenetic tree was based on the 16S rRNA gene. Bootstrap values <50 were removed from the nodes. Bar, 5 nucleotide substitutions per 100 nucleotides. Sequences determined in this study appear in bold.)

Table 1 Percentile positive NaCl and pH tolerance test results of bacterial strains isolated from the water of Böddi-szék

Supplementary Fig. 1 Rarefaction curve for the different ARDRA patterns of 16S rRNA gene bacterial clones originated from the water of Böddi-szék

Supplementary Fig. 2 Distribution of bacterial strains and clones from Böddi-szék within the phylogenetic groups