

Microbial life in Bourlyashchy, the hottest thermal pool of Uzon Caldera, Kamchatka

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Abstract Bourlyashchy is the largest and hottest pool in the Uzon Caldera, located in the territory of Kronotsky Nature Reserve, Kamchatka, Russia, with sediment surface temperatures at the margins ranging from 86 to 97 °C, and pH from 6.0 to 7.0. The microbial communities of the pool water and sediments were studied comprehensively from 2005 to 2014. Radioisotopic tracer studies revealed the processes of inorganic carbon assimilation, sulfate reduction, lithotrophic methanogenesis and potentially very active process of acetate oxidation to CO₂. The total number of microbial cells in water was different in different years ranging from 5.2 to 7.0 × 10⁶; in sediments, it changed from year to year between 6.3 × 10⁶ and 1.75 × 10⁸, increasing with a decrease in temperature. FISH with *Archaea*- and *Bacteria*-specific probes showed that the share of *Bacteria* differed with year, changing from

34 to 71 %. According to 16S rRNA gene pyrosequencing data, lithoautotrophs (*Aquificales* and *Thermoproteales*) predominated in water samples, while in sediments they shared the niche with organotrophic *Crenarchaeota*, *Korarchaeota*, and bacteria of the genus *Caldimicrobium* (phylum *Thermodesulfobacteria*). The majority of organisms in water belonged to cultivated orders of prokaryotes; the only large uncultured group was that representing a novel order in class *Thermoprotei*. In sediments, unclassified *Aquificae* comprised a significant part of the bacterial population. Thus, we showed that the hottest of the terrestrial hot pools studied contains numerous and active microbial populations where *Bacteria* represent a significant part of the microbial community, and planktonic and sediment populations differ in both composition and function.

Keywords *Aquificales* · Hyperthermophiles · Pyrosequencing · *Thermoproteales* · Terrestrial hot springs

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Introduction

The intensive exploration of microbial communities that thrive in terrestrial hot springs was started by Brock (1978) and resulted in the subsequent isolation of numerous thermophilic and hyperthermophilic microorganisms (Wiegel 1992; Stetter 1996). Molecular techniques became a powerful tool, allowing the identification of many novel phylogenetic divisions of prokaryotes inhabiting these terrestrial hot springs, as well as the structure of microbial communities (Barns et al. 1994; Reysenbach et al. 1994; Hugenholtz et al. 1998; Yamamoto et al. 1998; Skirnisdottir et al. 2000; Takacs et al. 2001; Nakagawa and Fukui 2002; Purcell et al. 2007; Lau et al. 2009; Hedlund et al. 2012). Recently, the pyrosequencing of variable 16S rRNA regions and metagenomic and

metaproteomic analyses of microbial communities inhabiting terrestrial hot springs have been performed in several geographically remote areas revealing communities' composition and functions (Inskeep et al. 2013; Cole et al. 2013; Sahn et al. 2013; Huang et al. 2013; Chan et al. 2015).

The Kamchatka Peninsula (Far East Russia) is a world-known area where more than a hundred volcanoes and thousands of associated hot springs are located. The Uzon Caldera is situated in the southeastern portion of the peninsula, approximately 180 km NE of Petropavlovsk, on the territory of Kronotsky Nature Reserve, and is a unique location where hundreds of hot springs are situated in five thermal fields (Zhao et al. 2005). Some of the springs have been the focus of microbiological and ecological studies (Gorlenko et al. 1987; Bonch-Osmolovskaya et al. 1990, 1991, 1999; Miroshnichenko et al. 1998, 2008, 2009; Wagner et al. 2013). Molecular studies of the occurrence and diversity of several phylogenetic (Perevalova et al. 2008; Reigstad et al. 2008; Auchtung et al. 2011; Eme et al. 2013) or metabolic (Kublanov et al. 2009; Reigstad et al. 2010; Wemheuer et al. 2013) groups of microorganisms have been performed; however, the works focusing on comprehensive characterization of particular pools or hot springs have just started. Several investigators have studied the microbial community of Zavarzin Pool with moderate temperature of water and predominantly bacterial population (Gumerov et al. 2011; Burgess et al. 2012; Rozanov et al. 2014). The analysis of Uzon thermal groundwater (50 °C, pH 4.0), demonstrated the presence of numerous groups of uncultured archaea in this environment (Mardanov et al. 2011).

Radioisotopic tracing of microbial processes serves for the evaluation of their actual rates in natural environments, as both the radioactivity of the product formed in vitro and the in situ concentration of the corresponding substrate are used for the calculations. Our previous work (Bonch-Osmolovskaya et al. 1999) revealed high rates of inorganic carbon assimilation in Uzon Caldera hot springs. However, the rates of this process were measured in springs with temperatures ranging from 55 to 85 °C, but not in springs with higher temperatures.

Bourlyashchy Pool is the biggest and hottest in Uzon Caldera. Its temperature, changing from year to year, most of the time exceeds 90 °C, which is above the upper temperature level of growth for thermophilic bacteria (Itoh and Iino 2013). The goal of this work was to characterize the microbial community of Bourlyashchy Pool both qualitatively and quantitatively, and to outline the main processes taking place in this extremely harsh environment. The tasks that we considered actual were (1) to test whether the microbial community of Bourlyashchy Pool is active and performs environmentally relevant processes; (2) to find out whether bacteria are still a significant part of

the microbial community, even at such high temperatures, and (3) to understand whether there is a significant difference between planktonic and sediment communities of the pool. To solve the above tasks, we used multiple methods and approaches, such as radioisotopic tracing, fluorescent microscopy, FISH, PCR/DGGE (denaturing gradient gel electrophoresis) analysis of environmental DNA using primers specific to 16S rRNA genes and genes of key metabolic enzymes, as well as pyrosequencing of 16S rRNA gene fragments and qPCR (quantitative PCR). The data, collected over a period of several years, allowed us to present a detailed view of the microbial community inhabiting Bourlyashchy.

Materials and methods

Sampling, sample processing and sample characterization

Samples of water and sediments from Bourlyashchy Pool N54 29.982 E160 00.121 were taken in August–September during field research trips in 2005–2014. All samples were collected aseptically and then treated using the protocols described below. ICP-MS analysis was carried out at the Plasma chemical-analytical center (Tomsk) using a Perkin Elmer ELAN model DRC-e mass-spectrometer. The atomizing argon flow rate was 0.92–0.95 l min⁻¹, the auxiliary flow of argon was 1.17 l min⁻¹, and the flow of orifice argon was 15 l/min⁻¹. The plasma generator capacity was 1270 W, and the detector voltage was 1400 V. Samples for radiotracing of microbiological processes were immediately dispensed into 15-ml Hungate tubes. Samples for acridine orange, DAPI and FISH staining were fixed with 4 % formamide (1.5 ml added to 0.5 ml of sample); then, the suspension was carefully mixed by rotation of the tube and incubated at 10–15 °C for 3–6 h. After centrifugation for 1 min at 8000 rpm, the supernatant was removed, and the pellet was washed twice with 1 × PBS buffer. The pellet was resuspended in 0.5 ml of 1 × PBS buffer, and an equal volume of 96 % ethanol was added. For DNA isolation, samples of sediments or water were used. Samples of water were concentrated by using a filter with the pore diameter of 0.2 μm (track membrane, polyethylene terephthalate, Dubna, Russia) and fixed with RNA Later (Ambion). Prior to arrival in the laboratory (several days), the fixed samples were stored at ambient temperature and then at –20 °C for several months until evaluation.

Radiotracing experiments

For the radioisotopic tracing, samples of the upper layer of sediments were collected with 2-ml plastic syringes with

cut-off tips, placed into 15-ml Hungate tubes, covered with spring water, and sealed with rubber stoppers hermetically, without air bubbles. Then, 0.2 ml of labeled substrate in sterile degassed water solutions was injected [$\text{H}^{14}\text{CO}_3^{2-}$ (0.10 mM), $^{14}\text{CH}_3\text{COO}^-$ (0.02 mM)—10 μCi per sample; $^{35}\text{SO}_4^{2-}$ (0.017 μM)—15 μCi per sample]. After that, the samples were incubated in the pool for 12–24 h. Afterwards, all microbial processes were stopped by injecting 0.5 ml of saturated KOH solution into each experimental tube. All experiments were performed in triplicate. Parallel abiotic controls were carried out by injecting 0.5 ml of saturated KOH into one Hungate tube in each of the series prior to incubation. After the end of experiments, the tubes were stored at 5–10 °C. Measurement of the radioactivity of products in both experimental and control tubes was performed after returning to the laboratory according to the methods described by Pimenov and Bonch-Osmolovskaya (2006). The actual rates of substrate transformation to particular products were calculated by the following equation:

$$I = \frac{(r - r_c) C \alpha}{RT},$$

where I is the rate of product formation by microorganisms, r is the radioactivity of the product formed, r_c is the radioactivity of the same product formed in the abiotic control, R is the initial radioactivity of the labeled substrate added to the sample, C is the natural concentration of this substrate in the sample, α is the correction factor for isotope fractionation (1.06 for ^{14}C , or 1.045 for ^{35}S), and T is the incubation time. For acetate, only the potential rates of acetate transformations (acetate oxidation to CO_2 and acetoclastic methanogenesis) were determined, as the concentration of acetate introduced into the sample was approximately one order of magnitude higher than the in situ concentration of acetate in Bourlyashchy Pool.

Microscopy and enumeration of microbial cells

For the staining and enumeration of microbial cells, samples were taken from the surface of sediments, applied to glass slides and air-dried. Ten μl of 0.0001 % (w/v) DAPI solution was added to each well (Diagnostic Slides, <http://www.thermoscientific.com>) and kept in the dark for 5 min. After staining, the slides were washed with distilled water, rinsed with 96 % ethanol for water removal, and air-dried. For acridine orange staining, a 0.1 % water solution was used after the accumulation of cells on black membrane filters (Millipore or Ostonix).

FISH staining

Fluorescence in situ hybridization (FISH) was performed according to standard protocols (Pernthaler et al. 2001).

For the detection of bacteria, probe EUB338 (5'-Cy3-GCT-GCCTCCCGTAGGAGT-3') was used; for the detection of archaea, a mixture of two probes was employed: Arch915 (5'-Cy3-GTGCTCCCCCGCCAATTCCT-3') and Arch344 (5'-Cy3-TCGCGCCTGCTGCICCCCGT-3'). The probes were synthesized by Syntol, Russia.

Hybridization was performed on slides with a Teflon layer with six openings (6 mm in diameter), and covered with 0.1 % gelatin for better cell adhesion. Fixed samples, 10 μl each, were applied to each opening, distributed by pipette, and dried for several minutes at 46 °C. Then, the slides were placed in 50, 80, and 96 % ethanol at room temperature, for 3 min each time. Fifty ml Falcon tubes with a filter paper at the bottom were used as hybridization chambers (Stahl and Amann 1991). Five ml of hybridization buffer (0.9 M NaCl; 20 mM Tris-HCl, pH 8.4; 0.01 % SDS) warmed to 46 °C was applied to the filter paper in the hybridization chamber. For hybridization, 9 μl of warmed buffer and 1 μl of probe (or a mixture of probes) was applied to each hybridization well (final probe concentration of 50 ng/ml). Slides were carefully placed into warmed hybridization chambers and incubated at 46 °C for 1.5–2 h. After hybridization, the slides were removed from the chamber, washed by placing into a washing buffer (1 M NaCl, 20 mM Tris-HCl, 0.01 % SDS), and incubated at 48 °C for 15 min. Slides were then washed with distilled water and examined with epifluorescent illumination under a Zeiss Axio Imager D1 microscope (Zeiss, Germany), using a drop of Vectashield oil (Bioteam, USA) for each well, covered with coverslips.

DNA isolation

Sediment samples (2–4 ml) were placed in centrifuge tubes and precipitated for 10 min at 13900 rpm and 4 °C. Sediment samples, or crushed filters with water filtrates, were resuspended in TNE buffer at pH of 7.4 (Tris 20 mM, 15 mM NaCl, EDTA 20 mM), frozen in liquid nitrogen, ground, and thawed in a water bath at 37 °C. The freezing and thawing cycle was repeated twice. Lysozyme (200 $\mu\text{g ml}^{-1}$) and RNase (DNase-free, 5 $\mu\text{g ml}^{-1}$) were added, and the mixture was incubated for 30 min at room temperature. Proteinase K (5–10 $\mu\text{g ml}^{-1}$) and SDS (0.5 %) were added, and the mixture was incubated for 30 min at 54 °C. After the addition of 1 M NaCl, followed by mixing, and cooling, an equal volume of a cooled phenol–chloroform–isoamyl alcohol (50:50:1) mixture was added, followed by agitation for 10 min and centrifugation for 10 min at 13900 rpm. The water phase was collected, supplemented with an equal volume of chloroform, agitated for 5 min and centrifuged. The water phase was collected, and chloroform extraction was repeated. Then, 0.1 volume of 3 M sodium citrate (pH 5.2) and two volumes

of cooled 96 % ethanol were added to the water phase. For DNA precipitation, the mixture was maintained at $-20\text{ }^{\circ}\text{C}$ for 60 min. DNA was gathered by centrifugation for 5 min at 13900 rpm, washed with 70 and 96 % ethanol, dried and dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.4). Five μl of the preparation was applied to 1 % agarose gel for DNA visualization.

PCR primers and gene amplification

The following primers were used in this work for the amplification of 16S rRNA genes of *Bacteria* and *Archaea*: U515F; 5'-GTGBCAGCMGCCGCGGTAA-3' (this forward primer was used for both archaea and bacteria; 32), Bac 907R: 5'-CCGTCAATTCMTTGTAGTTT-3' (Muyzer et al. 1993), and Arch 915R: 5'-GTGCTCCCCGCCAATTCCT-3' (Casamayor et al. 2000). Twenty μl of the PCR mixture contained 2 μl of 10 \times buffer (Eurogen buffer with 1.5 mM MgCl_2), 12.5 mM of each deoxynucleotide triphosphate (Eurogen, Russia), 20 pmol of each of the U515F and Arch915R or Bac907R primers, 1.2 U of *Taq* polymerase (Eurogen, Russia), and approximately 10 ng of DNA. The PCR mixture was incubated for 5 min at $94\text{ }^{\circ}\text{C}$, followed by 33 cycles of 30 s at $93\text{ }^{\circ}\text{C}$, 30 s at $52\text{ }^{\circ}\text{C}$ (bacterial primer system) or $62\text{ }^{\circ}\text{C}$ (archaeal primer system), and 60 s at $72\text{ }^{\circ}\text{C}$, with a final extension at $72\text{ }^{\circ}\text{C}$ for 10 min.

For identification of representatives of the genera *Thermococcus* and *Pyrococcus*, we used specific primers described previously: TcPc 173F, 5-TCCCCCATAGGYCTGRGGTACTGGAAGGTC'-3' and TcPc 589R, 5-CGGGCATTCAGGGACCGCTTTAGRGTGCCG-3' (Slobodkina et al. 2004). The PCR mixture was incubated for 5 min at $94\text{ }^{\circ}\text{C}$, followed by 33 cycles of 30 s at $93\text{ }^{\circ}\text{C}$, 30 s at $72\text{ }^{\circ}\text{C}$, and 60 s at $72\text{ }^{\circ}\text{C}$, with a final extension at $72\text{ }^{\circ}\text{C}$ for 10 min. For the detection of *Nanoarchaeota*, we used the phylum-specific primer system; A571F, 5'-GCYTAAAGSRICCGTAGC-3'; and N961R, 5'-CMAT-TAAACCGCRCACCC-3' (Casanueva et al. 2008). The PCR mixture was incubated for 5 min at $94\text{ }^{\circ}\text{C}$, followed by 33 cycles of 30 s at $93\text{ }^{\circ}\text{C}$, 30 s at $55\text{ }^{\circ}\text{C}$ and 60 s at $72\text{ }^{\circ}\text{C}$, with a final extension at $72\text{ }^{\circ}\text{C}$ for 10 min. PCR was also used for the detection of 4-hydroxybutyryl-CoA dehydratase (*4-hbd*), ATP citrate lyase, subunit B (*aclB*), citryl-CoA lyase (*ccl*), ammonia monooxygenase subunit A (*amoA*), dissimilatory sulfite reductase, subunits A and B (*dsrAB*), nitrogenase (*nifH*) and methyl-coenzyme M reductase subunit A (*mcrA*) genes using the primers and amplification conditions listed in Table 1. Temperature gradient and touchdown thermocycling programs were used to optimize the annealing temperature for each primer set. Appropriate positive controls were identified for each primer set and used in each PCR.

For DGGE analysis, a 40-bp GC clamp (5'-CGCC-CGCCGCGCCCCGCGC-CCGTCCCCGCGCCCCCGC-CCG-3') was added to the 5' end of the forward primers (Table 1). In some cases, amplification from the environmental DNA samples was performed directly with DGGE-adapted primers, while in other cases, after the first amplification with regular primers, the amplification with DGGE-adapted primers followed. The reaction mixture for PCR was the same in both cases. PCR was performed in a single-channel amplifier (Perkin Elmer Cetus, USA), which made it possible to use the program with the decrease in temperature after every two cycles. The following program was used: initial DNA denaturation at $94\text{ }^{\circ}\text{C}$ for 5 min; followed by one denaturation cycle at $94\text{ }^{\circ}\text{C}$ for 30 s, annealing at $75\text{ }^{\circ}\text{C}$ for 30 and extension at $72\text{ }^{\circ}\text{C}$ for 1 min; then, the annealing temperature was decreased by $1\text{ }^{\circ}\text{C}$ every second cycle to a final temperature of $65\text{ }^{\circ}\text{C}$, at which point 10 additional cycles were performed. The final extension was performed at $72\text{ }^{\circ}\text{C}$ for 10 min. Amplification in the absence of DNA served as the negative control. Amplification products were visualized in a 1 % agarose gel.

Denaturing gradient gel-electrophoresis

Amplification products were applied to a polyacrylamide gel (8 %, v/v) with an acrylamide gradient from 35 to 70 % in 0.5x TAE buffer. Here, 7 M urea (BioRad) and 40 % formamide (Fluca) were used as the denaturing agents. DGGE was performed in the camera produced by SCIE-PLAS (Yorkshire, England), at a voltage of 70 V and $60\text{ }^{\circ}\text{C}$ for 17 h. After electrophoresis, gels were washed with distilled water and stained with SYBR[®] Gold (Molecular probes, Leyden, The Netherlands) for 40 min in the dark. The bands were visualized in a transilluminator, excised, and incubated overnight in tubes with 20 μl of distilled water for DNA elution. Then, the PCR products were re-amplified with corresponding primers, visualized in a 1.5 % agarose gel, and purified using the Wizard[®] SV Gel and PCR Clean-Up System (Promega, USA). PCR products were sequenced using Big Dye Terminator kit v.3.1 and an automatic sequencer ABI 3730 ("Applied Biosystems" Inc., USA), according to the manufacturer's instructions.

qPCR assay

Total prokaryotic and archaeal 16S rRNA genes were amplified with the primer sets Uni515F (5'-GTGBCAGCMGCCGCGGTAA-3') (Kublanov et al. 2009) and 806R (5'-GGACTACHVGGGTATCTAAT-3') (Walters et al. 2011), and Univ515F and Arch915R (5'-GTGCTCCCCGCCAATTCCT-3') (Stahl and Amann 1991), respectively. The following cycling conditions were used: denaturation (3 min at $95\text{ }^{\circ}\text{C}$) was followed by 40 cycles of 20 s

Table 1 PCR primers used for the detection of metabolic genes in this study

Pathway	Gene	Primer (5'–3')	Size (bp)	Amplification conditions	References
4-Hydroxybutyrate cycles of CO ₂ fixation	<i>4-hbd</i>	457F-TTCCARAGVTGYGTIG-GITGGGA	768	94 °C 5 min; 37 cycles of 94 °C for 40 s, 61 °C for 45 s, 72 °C for 1 min 45 s; 72 °C for 10 min	This study
		1270R-GCRTCATIGGRTTIACC-CACCA			
rTCA cycle of CO ₂ fixation	<i>acIB</i>	892F-TGGACMATGGTDGCYGGKGGT 1204R-ATAGTTKGGSCCACCCTCTTC	333	94 °C 5 min; 37 cycles of 94 °C for 40 s, 57 °C for 45 s, 72 °C for 1 min 45 s; 72 °C for 10 min	Campbell et al. (2003)
rTCA cycle of <i>Aquificae</i>	<i>ccL</i>	307F-GCYTWTWGAAGAYGCMATGA 602R-TTCCKATTATRAARAADCCYTT	295	94 °C 5 min; 33 cycles of 94 °C for 30 s, 51 °C for 40 s, 72 °C for 50 s; 72 °C for 10 min	This study
Ammonia oxidation by <i>Crenarchaeota</i>	<i>amoA</i>	a119R-ATGGTCTGGCTWAGACG a616F-GCCATCTGGCTWAGACG	630	94 °C 5 min; 35 cycles of 94 °C for 30 s, 52 °C for 60 s, 72 °C for 60 s; 72 °C for 15 min	Leininger et al. (2006)
Dissimilatory sulfate reduction	<i>dsrAB</i>	F-ACSCACTGGGAAGCACG R-GTGTAGCAGTTACCGCA	1900	95 °C 2 min; 35 cycles of 94 °C for 20 s, 54 °C for 60 s, 72 °C for 150 s; 72 °C for 15 min	Wagner et al. (1998)
Nitrogen fixation	<i>nifH</i>	F-GGHA-ARGGHGGHATHGGNA	400	95 °C 2 min; 35 cycles of 94 °C for 20 s, 55 °C for 40 s, 72 °C for 50 s; 72 °C for 10 min	Mehra et al. (2003)
		ARIC			
		R-GGCATNGCRAANCCVCCRC ANAC			
Methanogenesis	<i>mcrA</i>	F-GGTGGTGMGGATTCA CARTAYGCWACAG R-TTCATTGCRFAGTTWGG RTAGTT	470	95 °C 2 min; 40 cycles of 94 °C for 20 s, 56 °C for 60 s, 72 °C for 120 s; 72 °C for 10 min	Luton et al. (2002)

at 94 °C, 25 s at 55 °C, and 25 s at 72 °C for the primer set Uni515F-806R and 40 cycles of 25 s at 94 °C, 25 s at 66 °C, and 32 s at 72 °C for the primer set Uni515F-Arch915R. For the construction of calibration curves, genomic DNA of *Melioribacter roseus* (Podosokorskaya et al. 2013) and *Thermococcus sibiricus* (Miroshnichenko et al. 2001) was used. The regression coefficient (R^2) for all calibration curves was no <0.99 and the amplification efficiency was no less than 70 %. All qPCR assays were performed using StepOnePlus™ Real-Time PCR System (Life Technologies, USA) and qPCRmix-HS ROX (Evrogen, Russia).

Pyrosequencing of 16S rRNA genes and data analysis

Pyrosequencing analyses were performed for sediment and water samples obtained from Bourlyashchy Pool during two different years, 2006 and 2007, respectively. The amplification of the V6 variable region of the 16S rRNA gene was performed with DNA isolated from sediments, with the set of primers described by Huber et al. (Huber JA, Mark Welch DB, Morrison HG, Huse SM, Neal PR, Butterfield DA, Sogin ML. Microbial population structures in the deep marine biosphere 2007). For water analysis, “universal” primers were used for amplification of the V3 variable region of the 16S rRNA gene, U341F: 5-CCTACGGGSGCAGCAG’-3 and U515R: 5’-TTACCGCGGCKGCTGVCAC-3’. The PCR fragments were pyrosequenced on GS FLX (Roche) using standard chemistry. Most of reads covered the full length of the PCR fragment.

The 16S rRNA gene sequences were subjected to the standard filter for environmental pyrosequencing datasets, selected for tags that displayed perfect matches to primers and contained no ambiguous nucleotides. Then, 16S rRNA data were analyzed, employing the RDP Classifier program package (Cole et al. 2009). At first, the sequences obtained with universal primers were attributed either to bacteria, or to archaea using online RDP Naive Bayesian rRNA Classifier Version 2.0 (<http://rdp.cme.msu.edu/classifier/classifier.jsp>). Subsequently, bacterial and archaeal 16S rRNA gene data sets were analyzed separately and subjected to additional filters. First, AmpliconNoise (Quince et al. 2011) was used to account for homopolymer-derived and PCR errors. Then, all remaining singletons (unique sequences occurring only once) were removed, as suggested by Behnke et al. (Behnke et al. 2011).

Complete linkage clustering, rarefaction analysis and the selection of representative sequences for operational taxonomic units (OTUs) were performed using the RDP Classifier (Cole et al. 2009). Then, we assigned OTUs to taxonomic groups (i.e., bacterial and archaeal divisions) on the basis of BLASTN sequence similarity search against

the NCBI database. OTU was assigned to a certain genus if the representative cluster sequence showed more than 97 % identity to that of the 16S rRNA gene of a cultivated microorganism. Otherwise, the taxonomic affiliation was determined following the construction of a phylogenetic tree including the representative sequence of the cluster and a set of 16S rRNA gene sequences of related archaeal or bacterial lineages.

Phylogenetic analyses of gene sequences

Maximum likelihood phylogenetic trees based on the comparison of gene sequences of 16S rRNA, *aclB*, *ccl*, and *4-hbd* genes were constructed using MEGA 6 (Tamura et al. 2013). For *mcrA* gene sequences, the phylogenetic tree was constructed in ARB software package (Ludwig et al. 2004) using maximum likelihood method, Dayhoff PAM model, and the “Fine and Slow” mode of analysis.

Nucleotide sequence accession numbers

The sequences determined in this study were deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov/GenBank/index.html>) under the following accession numbers: KJ801910 to KJ801913 for *4-hbd* sequences, KJ801899 to KJ801907 for *ccl* sequences, KJ801909 for the *aclAB* sequence, KJ801908 for the *mcrA* sequence, and KJ801865 to KJ801898 for 16S rRNA gene sequences.

Results

Characterization of the sampling site

Bourlyashchy Pool is located in the central part of East Thermal Field of Uzon Caldera (Fig.S1). It is the biggest pool in the caldera, about 10 m in diameter (Fig. S2). The name of the pool (Bourlyashchy means “bubbling”) is due to the active emanation of gases in large bubbles occurring continuously. A warm (45 °C) stream enters Bourlyashchy Pool from the north; thus, the water in Bourlyashchy is a mixture of hydrothermal fluid, ground meteoric waters from subsurface horizons, and stream water. Another stream flows out of Bourlyashchy Pool in the south; its temperature rapidly drops along the stream. The water of Bourlyashchy is always reduced and neutral or slightly acidic, with a pH varying from 6.0 to 7.0. The temperature of water and sediments of Bourlyashchy Pool varies from 86 to 97 °C depending on the site and the year. The chemical composition of Bourlyashchy water is typical of Uzon Caldera and indicates that it represents the mixture of hydrothermal fluid and surface ground water of meteoric origin (Table 2).

Table 2 Chemical composition of Bourlyashchy Pool water, Uzon Caldera

Element	Concentration in water, mg l ⁻¹
Ag	0.000250
Al	61.7
As	24.7
B	3.35
Ba	0.0151
Be	0.0188
Ca	607
Cd	0.00187
Cl	474
Co	0.0387
Cr	0.00565
Cs	0.0146
Cu	<0.002
Fe	594
Ga	0.0079
Ge	0.00197
In	<0.00005
K	26.4
La	0.122
Li	2.62
Mg	246
Mo	0.00067
Mn	41.1
Na	467
Ni	<0.002
Nb	<0.0001
P	9.36
Pd	<0.0002
Rb	0.0616
Pd	<0.0002
Rh	<0.00005
Ru	<0.00005
Sb	0.0286
Sc	<0.002
Se	0.0178
Si	138
Sn	0.00097
Sb	0.0286
Sr	1.75
Te	<0.002
Ti	0.118
V	0.944
Y	0.830
Zr	0.00050
Zn	2.64

Rates of microbial processes in the sediments of Bourlyashchy Pool

Radioisotopic methods applied to samples of Bourlyashchy sediments in 2005–2006 registered the processes of inorganic carbon assimilation, acetate oxidation, sulfate reduction and methanogenesis occurring in the sediments of this pool (Table 3). An active process of inorganic carbon assimilation was detected in the sediments of Bourlyashchy Pool. The rate of lithotrophic methanogenesis was rather low, while that of acetoclastic methanogenesis was at the background level. An active microbial process of 2-¹⁴C-acetate oxidation to CO₂ took place (Table 3). The process of sulfate reduction was much more prominent in the streams entering and flowing out of Bourlyashchy Pool, with the maximum rate recorded in the mouth of the stream entering the pool (Table 3). Still, in the pool itself, the process of sulfate reduction also occurred.

Total number of microbial cells and relative abundance of Archaea and Bacteria in water and sediments of Bourlyashchy Pool

The total number of microorganisms in water samples of Bourlyashchy Pool determined in different years by counting of DAPI-stained cells was 5.2–7.0 × 10⁶ cells per ml (Table 4). The share of archaea in the water determined by FISH staining depended on temperature and decreased from 56 % at 97 °C (2007) to 31 % at 94 °C (2014; Fig S4).

In order to analyze the microbial community of water in Bourlyashchy Pool in 2007, we used deep pyrosequencing of the V3 variable region of 16S rRNA genes (Sogin et al. 2006) amplified with universal primers. The filtered 16S rRNA dataset contained 26021 bacterial and 20087 archaeal 16S rRNA gene sequences. Subsequent analysis showed that 552 “bacterial” reads actually represented pine chloroplast rRNA gene sequences; thus, bacteria accounted for 56 % of all of the detected microorganisms. Thus, according to two independent approaches at temperatures as high as 97 °C, bacteria represent about half of the microbial community in the water of Bourlyashchy Pool.

In sediments, the total number of microbial DAPI-stained cells also changed from year to year, increasing with the decrease of temperature from 6.3 × 10⁶ cells per ml (2007, 97 °C) to 1 × 10⁸ (94 °C, 2014) and to 1.75 × 10⁸ (2008, 86 °C). In 2014 (94 °C); the relative share of archaea in sediments was 30 % according to FISH data (Fig. S3), and 39 % according to quantitative PCR with universal and archaea-specific primers (as the total amount of prokaryotic 16S rDNA was 5.46 × 10⁵

Table 3 Rates of microbial processes in Bourlyashchy Pool, Uzon Caldera

Process	Rate ^a of the process, nmol cm ⁻³ day ⁻¹
Carbon dioxide assimilation	1.6 ± 0.4
Lithotrophic methanogenesis	0.17 ± 0.03
Potential rate of acetate oxidation ^a	5.8 ± 0.7
Sulfate reduction, 86 °C	0.21 ± 0.05
Sulfate reduction, 60 °C ^b	2.1 ± 0.4
Sulfate reduction, 45 °C ^c	7.7 ± 0.7

^a In all cases, the actual rates of processes were determined, except for the rate of acetate oxidation. The natural acetate concentration in Bourlyashchy Pool is less than 0.01 mM; and the final concentration of C¹⁴-acetate in the incubation tube was 0.08 mM

^b Measurement was performed at the outflow of the stream at the southern margin of Bourlyashchy Pool

^c Downstream the same spring

(±3.97 × 10⁴) copies per ng DNA and *Archaea* were represented by 2.08 × 10⁵ (±7.4 × 10⁴) 16S rDNA copies per ng DNA). The staining of Bourlyashchy sediments with acridine orange revealed that the rod-shaped organisms were dominant, some with attached coccoid mini-cells (Fig. S4).

Therefore, both in water and sediment samples of Bourlyashchy Pool, *Archaea* and *Bacteria* share the niche, with each group representing, depending on the year, between one-third and two-thirds of the total microbial population.

The rarefaction analysis of bacterial and archaeal communities in water (Fig. 1) revealed that when a sequence dissimilarity of 0.03 or 0.05 was used to define OTU, the rarefaction curves reached the plateau phase, suggesting that the entire microbial community was sufficiently covered (Fig. 1). The diversity of the bacterial community was estimated to be about 250 species (cluster distance 0.03), while archaea were less diverse and represented fewer than 50 species (Fig. 1).

Table 4 Number of all microorganisms, bacteria and archaea (×10⁷ cells/ml) in water and sediments of Bourlyashchy Pool, Uzon Caldera

Year, T (°C)	Total counts			Bacteria (FISH staining) counts	Archaea (FISH staining) counts
	Phase contrast microscopy	Acridine orange staining	DAPI staining		
2007, 97 °C	0.65 ± 0.06 (water),	ND	0.70 ± 0.10 (water),	0.40 ± 0.10 (water)	0.50 ± 0.10 (water)
	1.1 ± 0.35 (sediment)		0.63 ± 0.11 (sediment)		
2008, 87 °C	30.5 ± 3.0 (sediment)	25.5 ± 9.5 (sediment)	17.5 ± 3.5 (sediment)	ND	ND
2014, 94 °C	10.8 ± 3.0 (sediment)	10.1 ± 3.2 (sediment)	10.0 ± 2.1 (sediment)	0.36 ± 0.03 (water), 8.0 ± 3 (sediment)	0.16 ± 0.02 (water), 3.5 ± 2.0 (sediment)

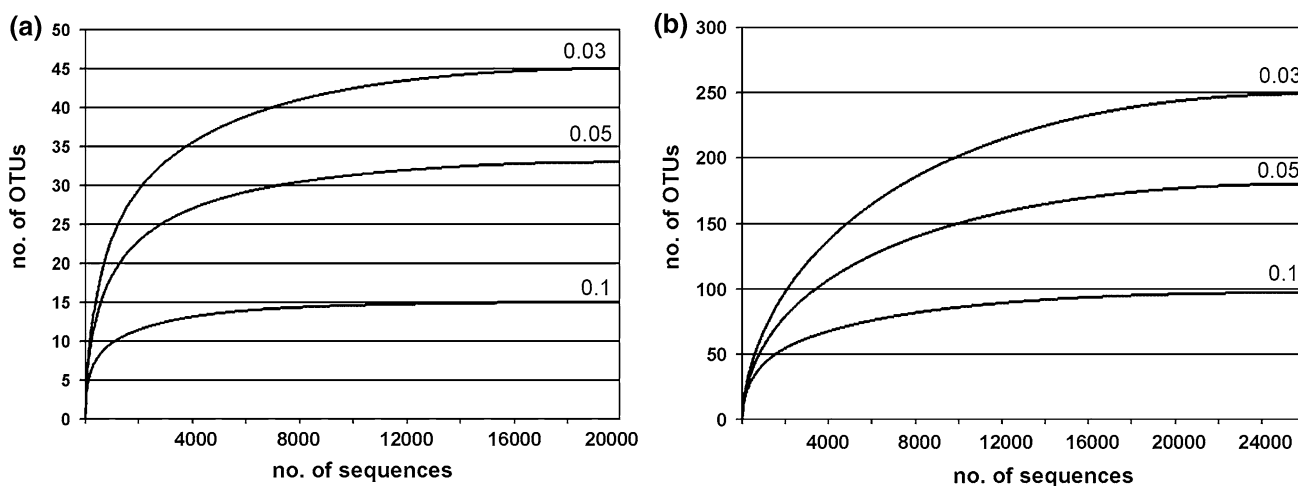
**Fig. 1** The results of rarefaction analysis of archaeal and bacterial populations in the water of Bourlyashchy Pool

Fig. 2 Relative abundance (%) of the groups of *Bacteria* determined by pyrosequencing of V6 and V3 regions of 16S rRNA genes in the Bourlyashchy Pool sediments and water, respectively. *Unc.* uncultured

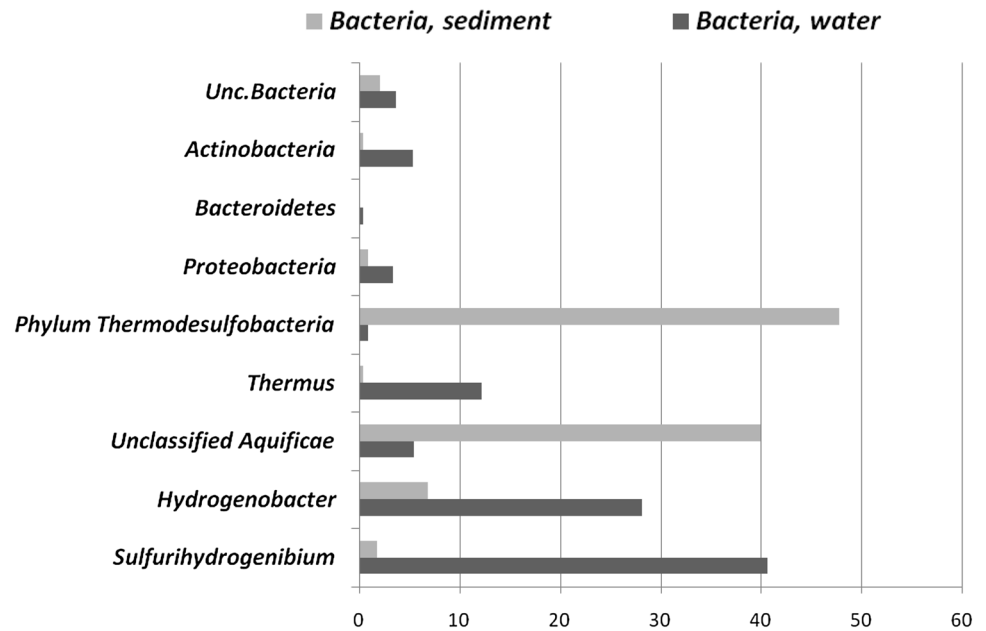
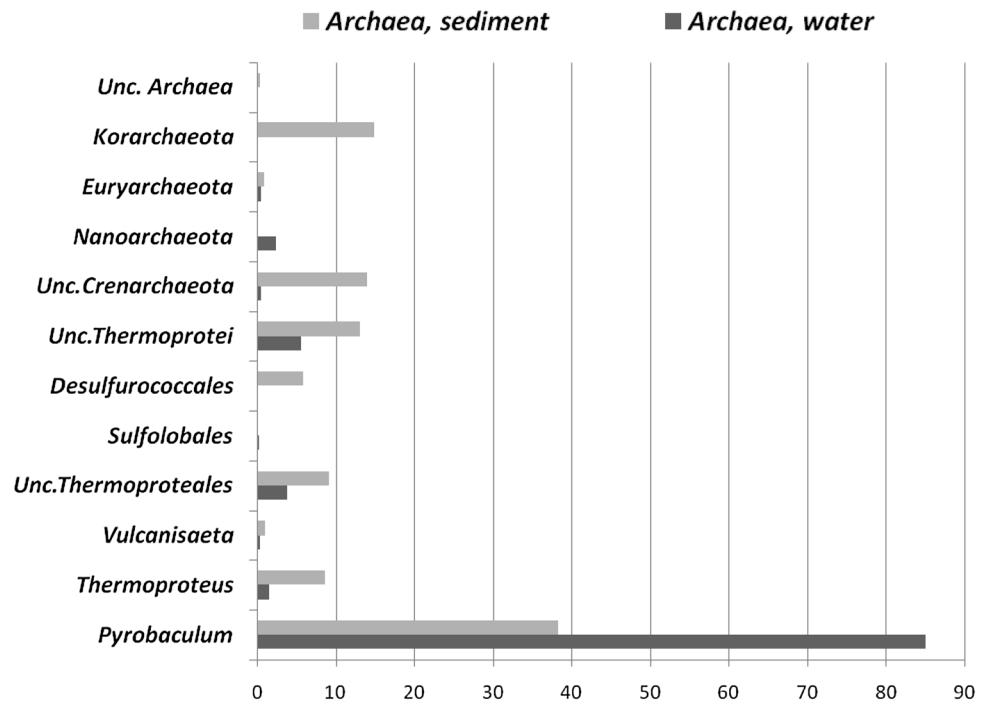


Fig. 3 Relative abundance (%) of the groups of *Archaea* determined by pyrosequencing of V6 and V3 regions of 16S rRNA genes in the Bourlyashchy Pool sediments and water, respectively. *Unc.* uncultured



Most numerous groups of prokaryotic sequences in the water and sediments of Bourlyashchy Pool

The composition of the microbial communities of Bourlyashchy Pool water and sediments was determined by the pyrosequencing of 16S rRNA gene fragments—V3 and V6, respectively. In the planktonic part of the community, sequences of *Aquificales* were most numerous (68.7 % of all bacterial sequences); most of them belonged to the

genera *Sulfurihydrogenibium* (40.6 %) and *Hydrogenobacter* (28.1 %; Fig. 2). The second most abundant group of bacterial sequences was that of *Deinococcus-Thermus* (12.2 %), mainly represented by the genus *Thermus*. The phylum *Thermodesulfobacteria* accounted for only 0.9 % of bacterial sequences in the water fraction. About 5.5 % of the sequences formed several lineages that are distantly related to *Aquificales*, but they could not be reliably classified, even at the phylum level.

Two groups of sequences were found to be most numerous in the bacterial part of the sediment community (Fig. 2). The phylum *Thermodesulfobacteria* (47.6 % of all bacteria) was only represented by the genus *Caldimicrobium*, the first and the only cultured representative which was isolated from another spring (Treshchinny) of Uzon Caldera (Miroshnichenko et al. 2009). The other abundant group was that of *Aquificae* (48.5 %), the uncultured members of which were most numerous (39.9 % of all bacteria). Parallel studies of bacterial diversity in the sediments using PCR with bacteria-specific primers and subsequent DGGE analysis also revealed the presence of *Aquificales*, but the sequences identified were affiliated with the genera *Sulfurihydrogenibium*, *Hydrogenobacter*, and *Hydrogenobaculum* (Fig. S5).

The most numerous archaeal sequences both in water and sediment communities were those of *Thermoproteales*: 85 % and 62.6 %, respectively. In the water, the archaeal sequences were represented mostly by the genus *Pyrobaculum* (85 %) (Fig. 3). About 5.6 % of archaea found in the water were assigned to a separate order-level lineage of thermophilic *Crenarchaeota* (class *Thermoprotei*). *Sulfophobococcus* represented 0.1 % of all archaeal sequences. In sediments, the sequences of the genus *Pyrobaculum* were again most numerous (32.2 %), but here they were accompanied by those of the obligate organotrophs *Sulfophobococcus* (9.7 %) and *Korarchaeota* (14.9 %); the share of *Thermoproteus* sequences in the sediments increased to 8.6 % in comparison with 1.5 % in water.

Sequences of *Nanoarchaeota* comprised 1.7 and 2.4 % of all archaeal sequences in sediments and water, respectively, showing a correlation with the number of *Pyrobaculum* sequences (38.2 and 85.0 %, respectively). PCR-DGGE with *Nanoarchaeota*-specific primers also demonstrated the presence of diverse *Nanoarchaeota* in sediments of Bourlyashchy Pool (Fig. S6).

Minor components of the microbial communities of sediments and water

In addition to dominant groups of bacterial and archaeal sequences, there were many microorganisms in the water and sediments of Bourlyashchy Pool that were represented only by a small number of sequences. In the water, a minor but still significant fraction of bacteria revealed by pyrosequencing was assigned to the lineages of non-thermophilic aquatic and soil bacteria representing *Actinobacteria* (5.3 %), *Alphaproteobacteria* (3.3 %), *Bacteroidetes* (0.4 %), and *Gammaproteobacteria* (0.1 %). Some bacteria in sediments were only revealed in PCR-DGGE experiments (genera

Deferribacter and *Thiobacillus*, and phylum *Firmicutes*, Fig. S5). Representatives of the archaeal orders *Sulfolobales* and *Thermoplasmatales* were found only in the water and in minor amounts (0.3 and 0.5 % of all archaeal sequences, respectively). A few *Thermococcales* sequences were also identified in the water population; the presence of *Thermococcales* in sediments was confirmed by amplification with the *Thermococcus*-specific primers followed by DGGE and sequencing (Fig. S7). In sediments, 201 sequences of representatives of *Methanopyrales* were identified; only two such sequences have been revealed in water samples.

Detection of genes encoding key metabolic enzymes

All tests for the presence of key functional genes were performed with the mixture of water and sediment samples. The genes for ATP citrate lyase and citryl-CoA lyase, the key enzymes of the reductive tricarboxylic acid cycle of CO₂ assimilation (Hügler et al. 2011), were shown to belong to bacteria of the genera *Sulfurihydrogenibium*, *Hydrogenobacter*, and *Hydrogenobaculum*, which is in agreement with the results of 16S rRNA gene analysis (Fig. S8).

4-Hydroxybutyryl-CoA dehydratase is the key enzyme of the 3-hydroxypropionate/4-hydroxybutyrate and dicarboxylate/4-hydroxybutyrate cycles of CO₂ assimilation, characteristic of *Crenarchaeota* (Berg et al. 2010). 4-HBD-specific primers used in this work were specifically designed for the amplification of this gene (Table 1). This pair of primers was used for amplification of the 4-*hbd* gene from the samples of sediments of Bourlyashchy Pool. The products were found to represent the genes of 4-hydroxybutyryl-CoA dehydratase of *Pyrobaculum* sp. (Fig. S9).

No PCR products were obtained with *nifH*-, *dsrAB*- and thaumarchaeal *amoA*-specific primers from the DNA of Bourlyashchy Pool.

To assess methanogen diversity in sediments of Bourlyashchy, amplicons generated from primers targeting the *mcrA* gene were analyzed using PCR/DGGE method. As a result, four individual bands were excised, and the DNA fragments were extracted and sequenced. Due to the fact that all four sequences were very similar (more than 98.5 % identity of the deduced amino acid sequences) they were combined in one OTU (Bour_mcrA_1, accession number is KJ801908, Fig. S10) which fell into a phylogenetic cluster that comprised uncultured microorganisms only. This cluster was designated MCR-2a in accordance with the previous studies (Castro et al. 2004; Steinberg and Regan 2008, 2009). According to our phylogenetic reconstructions, this cluster constitutes one of the deepest branches on the *mcrA* tree (Fig. S10).

Discussion

Bourlyashchy Pool is the biggest thermal pool in Uzon Caldera. Active and powerful liberation of gases and extremely high temperature of water of Bourlyashchy Pool indicate its connection with the crust fracture and a significant input of hydrothermal fluid. The temperature of Bourlyashchy Pool achieving 97 °C makes it the hottest terrestrial thermal environment studied, as usually the temperature of hot springs considered “high-temperature” is in the range from 80 to 85 °C, and never exceeds 92 °C (Inskeep et al. 2013; Cole et al. 2013; Sahm et al. 2013; Huang et al. 2013; Chan et al. 2015; Vick et al. 2010; Urbeita et al. 2014). The total number of microbial cells in water and sediments of Bourlyashchy Pool, although differing from sample to sample, was very high; up to 0.7×10^6 cells per ml of water and up to 1.7×10^8 cells per ml of sediments. Such high numbers of microorganisms in sediments and water of terrestrial hot springs are not absolutely surprising as similar results were obtained by other authors for the hot springs of different geographical locations, although with lower temperatures (Huang et al. 2013; Siering et al. 2006; Belkova et al. 2007). Both bacteria and archaea play significant roles in the community; however, with the growth of temperature, the impact of the latter group increases.

Radioisotopic experiments showed that the microbial community of Bourlyashchy Pool is actively assimilating inorganic carbon. Bacteria of the order *Aquificales* are among the dominant groups both in the water and sediments of Bourlyashchy Pool. In this respect, Bourlyashchy is similar to many well-studied hot springs of Yellowstone National Park with neutral pH (Reysenbach et al. 2000; Spear et al. 2005; Inskeep et al. 2010; Takacs-Verbach et al. 2013). *Thermoproteales* dominating among archaea both in the water and in sediments are also potential lithoautotrophs possessing the dicarboxylate/4-hydroxybutyrate cycle of carbon dioxide fixation (Berg et al. 2010; Mardanov et al. 2011).

Molecular hydrogen is the most probable source of energy for lithoautotrophs in the Bourlyashchy community; its concentration in the pool water achieves 8 μM. Thiosulfate, elemental sulfur, or oxygen could serve as the electron acceptors for hydrogen-utilizing lithoautotrophs. The process of hydrogen oxidation is anaerobic or microaerobic, as the incubation of samples in 15-ml tubes supplemented with $\text{Na}^{14}\text{CO}_3$ in the presence of atmospheric air in the headspace (5 ml) did not result in the stimulation of inorganic carbon assimilation compared to anaerobic conditions (data not shown).

The 16S rRNA genes of pine chloroplasts make up about 1 % of the total 16S rRNA gene sequences in the water of Bourlyashchy Pool, originating, most probably, from the

pine needles brought by wind from surrounding areas covered with crawling pines. The ability of hyperthermophilic crenarcheota to use organic matter of the pine needles has been shown for Yellowstone National Park isolates (Boyd et al. 2007). Thus, allochthonous organic matter is an additional energy source for the Bourlyashchy microbial community, albeit most probably not a significant one.

While the planktonic population of Bourlyashchy Pool consisted almost entirely of lithoautotrophs, in sediments the share of organotrophic archaea (*Sulfophobococcus*, *Korarchaeota*) and facultatively organotrophic *Thermoproteus* became larger. These organisms perform anaerobic degradation of organic matter produced by lithoautotrophs.

In high-temperature hot springs of Yellowstone National Park, most numerous archaeal sequences represented novel lineages of phylum and class level (Cole et al. 2013). In Bourlyashchy Pool, the most numerous group of sequences representing uncultured microorganisms was that of bacteria: the share of which were especially high in the sediments (40 %) were unclassified *Aquificae*. As the revealed genes encoding the key enzymes of reductive TCA pathway of CO_2 assimilation belonged only to cultured *Aquificae*, this could indicate that unclassified representatives of this phylum that are abundant in the sediments are organotrophs. In the water, the number of these sequences was lower; another group of uncultured microorganism sequences represented a novel order in class *Thermoprotei* and was present at approximately equal parts both in water and in the sediment.

A special feature of Bourlyashchy Pool was the presence of bacteria of the genus *Caldimicrobium*, making up a significant (52.2 %) part of the sediment bacterial population. The only known species of this genus is *Caldimicrobium rimae*, an anaerobic lithoautotrophic bacterium oxidizing molecular hydrogen and reducing elemental sulfur or thiosulfate (Miroshnichenko et al. 2009). Recent data showed that representatives of this genus can grow by anaerobic acetate oxidation via sulfur or thiosulfate reduction (Miroshnichenko, unpublished data). As a high rate of ^{14}C -acetate mineralization to CO_2 was shown for Bourlyashchy sediments, it could be attributed to the activity of *Caldimicrobium* utilizing acetate produced by organotrophs in the course of fermentation.

The temperature of Bourlyashchy Pool water and sediments (92–97 °C) is within the hyperthermophilic archaea growth temperature range; it significantly exceeds the upper temperature limits of growth of bacteria inhabiting this pool: *Caldimicrobium* (82 °C), *Sulfurihydrogenibium* (78 °C), and *Hydrogenobacter* (80 °C). This confirms the previous observation that bacteria thrive in nature at much higher temperatures than in laboratory cultures (Cole et al. 2013).

It is worth mentioning that a significant amount of mesophilic microorganisms is present in the water of Bourlyashchy Pool, while they are absent in the sediments. Most probably, they are brought to the pool with the flow of cooler water of the incoming stream and do not survive in the sediments.

Nanoarchaeota signatures were present both in the sediments and water. Notably, their relative abundance strongly correlates with the abundance of *Pyrobaculum* in these two environments. The 16S rRNA gene sequences obtained in this work with *Nanoarchaeota*-specific primers (Fig. S6) were closely related to the CU-1 sequence detected previously in Uzon Caldera (Hohn et al. 2002). As it has already been assumed for other Kamchatka sources (Stetter et al. 2005), the *Nanoarchaeota* detected in Bourlyashchy Pool could be *Pyrobaculum* symbionts.

The process of sulfate reduction recorded in Bourlyashchy is limited by temperature, as its rate increases drastically with the decrease in temperature (Table 3). We were not able to identify hyperthermophilic sulfate reducers inhabiting Bourlyashchy Pool. It should be noted that the *dsrA*-targeted primers that we used to reveal sulfate reducers could discriminate the phylum *Thermodesulfobacteria*, a very important group of thermophilic sulfate reducers. It is also noteworthy that we recently obtained experimental data on the capacity of '*Vulcanisaeta moutnovskia*' for dissimilatory sulfate reduction (Chernyh et al. 2013). It is possible that representatives of this genus participate in sulfate reduction in Bourlyashchy Pool.

The low rate of lithotrophic methanogenesis in Bourlyashchy correlates with a low number of 16S rRNA gene sequences that could be regarded as those of methanogens; they were remotely related to the genus *Methanopyrus* and were detected both in water and sediment samples. Analysis of *mcrA* genes revealed the presence of microorganisms belonging to group MCR-2a (Steinberg and Regan 2008). This group contains sequences from a limited number of mesophilic habitats: anaerobic digesters (Steinberg and Regan 2008), terrestrial subsurface environments (Fry et al. 2009; Takeuchi et al. 2011) and wetlands (Narihiro et al. 2011). However, we also detected microorganisms of the MCR-2a group in diffuse hydrothermal vent fluids (Ver Eecke et al. 2012) and in terrestrial hot springs (Merkel et al. 2015). Thus, an MCR-2a cluster may contain thermophilic methanogens.

Thermococcales are most common inhabitants of shallow-water and deep-sea submarine hydrothermal vents; only two strains were isolated from terrestrial hot springs of New Zealand (Ronimus et al. 1997; Gonzales et al. 1999). Our data show that representatives of the genus *Thermococcus* inhabit the hottest spring of Uzon Caldera with the mineralization of water being much lower than marine water. Previously, *Thermococcus* 16S rRNA genes were identified

in archaeal 16S rRNA gene libraries from Zavarzin Pool in Uzon Caldera (Burgess et al. 2012). However, we failed to cultivate *Thermococcus* from Bourlyashchy Pool on organic-rich media with elemental sulfur and salinity ranging from 0.1 to 2.5 % NaCl (w/v; data not shown). Thus, the nutrient requirements of the Kamchatkan *Thermococcus* may differ from those of other members of this genus.

In conclusion, we would like to summarize the features of terrestrial microbial communities that we found studying Bourlyashchy Pool in Uzon Caldera. Archaea and bacteria are present in more or less equal proportions, and mainly belong to cultured taxa; bacteria thrive at temperatures that exceed their temperature maximum of growth in laboratory cultures by 10–15 °C. The composition and function of water and sediment communities are different. Lithoautotrophic *Aquificales* and *Thermoproteales* are dominant in the planktonic microbial community; they also represent a significant part of the sediment population. The newly synthesized organic matter accumulates in sediments, where it is degraded anaerobically by organotrophic archaea and bacteria. The high potential rate of acetate degradation indicates the ability of the microbial community to complete the mineralization cycle, and bacteria of the phylum *Thermodesulfobacteria* may play a significant role in this step.

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References

- Auchtung T, Shyndriayeva G, Cavanaugh CM (2011) 16S rRNA phylogenetic analysis and quantification of *Korarchaeota* indigenous to the hot springs of Kamchatka, Russia. *Extremophiles* 15:105–116
- Barns SM, Fundyga RE, Jeffries MW, Pace NR (1994) Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. *Proc Natl Acad Sci USA* 91:1609–1613
- Behnke A, Engel M, Christen R, Nebel M, Klein RR, Stoeck T (2011) Depicting more accurate pictures of protistan community complexity using pyrosequencing of hypervariable SSU rRNA gene regions. *Environ Microbiol* 13:340–349
- Belkova NL, Tazaki K, Zakharova JR, Parfenova VV (2007) Activity of bacteria in water of hot springs from Southern and Central Kamchatskaya geothermal province, Kamchatka Peninsula, Russia. *Microbiol Res* 162:99–107

- Berg IA, Ramos-Vera WH, Petri A, Huber H, Fuchs G (2010) Study of the distribution of autotrophic CO₂ fixation cycles in Crenarchaeota. *Microbiology* 156:256–269
- Bonch-Osmolovskaya EA, Sokolova TG, Kostrikina NA, Zavarzin GA (1990) *Desulfurella acetivorans* gen. nov., sp. nov., a new thermophilic sulfur-reducing bacterium. *Arch Microbiol* 153:151–155
- Bonch-Osmolovskaya EA, Miroshnichenko ML, Kostrikina NA, Chernyh NA, Zavarzin GA (1991) *Thermoproteus uzoniensis* sp. nov., a new extremely thermophilic archaeobacterium from Kamchatka continental hot springs. *Arch Microbiol* 154:556–559
- Bonch-Osmolovskaya EA, Miroshnichenko ML, Slobodkin AI, Sokolova TG, Karpov GA, Kostrikina NA et al (1999) Biodiversity of anaerobic prokaryotes in terrestrial hot springs of Kamchatka. *Microbiol (Mikrobiologiya)* 68:343–351
- Boyd ES, Jackson RA, Encarnacion G, Zahn JA, Beard T, Leavitt WD, Zhang CL, Pearson A, Geesey GG (2007) Isolation, characterization and ecology of sulfur-respiring *Crenarchaea* inhabiting acid-sulfate-chloride containing geothermal springs in Yellowstone National Park. *Appl Environ Microbiol* 73:6669–6677
- Brock TD (1978) *Thermophilic microorganisms and life at high temperatures*. Springer, New York
- Burgess EA, Unrine JM, Mills GL, Romanek CS, Wiegel J (2012) Comparative geochemical and microbiological characterization of two thermal pools in the Uzon Caldera, Kamchatka, Russia. *Microb Ecol* 63:471–489
- Campbell BJ, Stein JL, Cary SC (2003) Evidence of chemolithoautotrophy in the bacterial community associated with *Alvinella pompejana*, a hydrothermal vent polychaete. *Appl Environ Microbiol* 69:5070–5078
- Casamayor EO, Schäfer H, Bañeras L, Pedrós-Alió C, Muyzer G (2000) Identification of spatio-temporal differences between microbial assemblages from two neighboring sulfurous lakes: comparison by microscopy and denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 66(499):508
- Casanueva A, Galada N, Baker GC, Grant WD, Heaphy S, Jones B et al (2008) Nanoarchaeal 16S rRNA gene sequences are widely dispersed in hyperthermophilic and mesophilic halophilic environments. *Extremophiles* 12:651–656
- Castro H, Ogram A, Reddy KR (2004) Phylogenetic characterization of methanogenic assemblages in eutrophic and oligotrophic areas of the Florida Everglades. *Appl Environ Microbiol* 70:6559–6568
- Chan SC, Chan K-G, Tay Y-L, Chua Y-H, Goh KM (2015) Diversity of thermophiles in a Malaysian hot spring determined using 16S rRNA and shotgun metagenome sequencing. *Frontiers Microbiol* 6:177
- Chernyh N, Frolov E, Miroshnichenko M, Kovaleva O, Prokofieva M, Lebedinsky A, Bonch-Osmolovskaya E (2013) Dissimilatory sulfate reduction in the crenarchaeote “*Vulcanisaeta moutnovskia*” grown on various substrates. Abstracts of Thermophiles 12th International Meeting at the University of Regensburg
- Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ et al (2009) The ribosomal database project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* 37:141–145
- Cole JK, Peacock JP, Dodsworth JA, Williams AJ, Thompson DB, Dong H, Hedlund BP (2013) Sediment microbial communities in Great Boiling Spring are controlled by temperature and distinct from water communities. *ISME J* 7:718–729
- Eme L, Reigstad LJ, Spang A, Lanzén A, Weinmaier T, Rattei T et al (2013) Metagenomics of Kamchatkan hot spring filaments reveal two new major (hyper)thermophilic lineages related to Thaumarchaeota. *Res Microbiol* 164:425–438
- Fry JC, Horsfield B, Sykes R, Cragg BA, Heywood C, Kim GT et al (2009) Prokaryotic populations and activities in an interbedded coal deposit, including a previously deeply buried section (1.6–2.3 km) above 150 Ma basement rock. *Geomicrobiol J* 26:163–178
- Gonzales JM, Sheckells D, Viebahn M, Krupatkina D, Borges KM, Robb FT (1999) *Thermococcus waiotapuensis* sp. nov., an extremely thermophilic archaeon isolated from a freshwater hot spring. *Arch Microbiol* 172:95–101
- Gorlenko VM, Bonch-Osmolovskaya EA, Kompantseva EI, Starynin DA (1987) Differentiation of microbial communities in connection with a change in the physicochemical conditions in Thermophile spring. *Microbiol (Mikrobiologiya)* 56:250–257
- Gumerov VM, Mardanov AV, Beletsky AV, Bonch-Osmolovskaya EA, Ravin NV (2011) Molecular analysis of microbial diversity in the Zavarzin spring, Uzon Caldera Kamchatka. *Mikrobiol (Mikrobiologiya)* 80:258–265
- Hedlund BP, Cole JK, Williams AJ, Hou W, Zhou E, Li W, Dong H (2012) A review of the microbiology of the Rehai geothermal field in Tengchong, Yunnan Province, China. *Geosci Front* 3:273–288
- Hohn MJ, Hedlund BP, Huber H (2002) Detection of 16S rDNA sequences representing the novel phylum “Nanoarchaeota”: indication for a wide distribution in high temperature biotopes. *Syst Appl Microbiol* 25:551–554
- Huang Q, Jiang H, Briggs BR, Wang S, Hou W, Li G, Wu G, Solis R, Arcilla CA, Abrajano T, Dong H (2013) Archaeal and bacterial diversity in acidic to circumneutral hot springs in the Philippines. *FEMS Microbiol Ecol* 85:452–464
- Huber JA, Mark Welch DB, Morrison HG, Huse SM, Neal PR, Butterfield DA, Sogin ML (2007) Microbial population structures in the deep marine biosphere. *Science* 318:97–100
- Hugenholtz P, Pitulle C, Hershberger KL, Pace NR (1998) Novel division level bacterial diversity in a Yellowstone hot spring. *J Bacteriol* 180:366–376
- Hügler M, Gärtner A, Imhoff JF (2011) Functional genes as markers for sulfur cycling and CO₂ fixation in microbial communities of hydrothermal vents of the Logatchev field. *FEMS Microbiol Ecol* 73:526–537
- Inskeep WP, Rusch DB, Zackary JJ, Herrgard MJ, Kozubal MA, Richardson TH et al (2010) Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. *PLoS ONE* 5:1–15
- Inskeep WP, Jay ZJ, Tringe SG, Herrgard MJ, Rusch DB et al (2013) The YNP metagenome project: environmental parameters responsible for microbial distribution in the Yellowstone geothermal ecosystem. *Front Microbiol* 4:67
- Itoh T, Iino T (2013) Phylogeny and biological features of thermophiles. In: Satyanarayana T, Kawarabayasi Y, Littlechild J (eds) *Thermophilic microbes in environmental and industrial biotechnology: biotechnology of thermophiles*. Springer, Dordrecht, pp 249–270
- Kublanov IV, Perevalova AA, Slobodkina GB, Lebedinsky AV, Bidzhieva SKh et al (2009) Biodiversity of thermophilic prokaryotes with hydrolytic activities in hot springs of Uzon Caldera, Kamchatka. *Appl Environ Microbiol* 75:286–291
- Lau MC, Aitchison JC, Pointing SB (2009) Bacterial community composition in thermophilic microbial mats from five hot springs in Central Tibet. *Extremophiles* 13:139–149
- Leininger S, Urlich T, Schloter M, Schwark L, Qi J, Nicol GW et al (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442:806–809
- Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadukumar et al (2004) ARB: a software environment for sequence data. *Nucleic Acids Res* 32:1363–1371
- Luton PE, Wayne JM, Sharp RJ, Riley PW (2002) The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology* 148:3521–3530

- Mardanov AV, Gumerov VM, Beletsky AV, Perevalova AA, Karpov GA, Bonch-Osmolovskaya EA et al (2011a) Uncultured archaea dominate in the thermal groundwater of Uzon Caldera, Kamchatka. *Extremophiles* 15:365–372
- Mardanov AV, Gumerov VM, Beletsky AV, Prokofeva MI, Bonch-Osmolovskaya EA, Ravin NV et al (2011b) Complete genome sequence of the thermoacidophilic crenarchaeon *Thermoproteus uzoniensis* 768–20. *J Bacteriol* 193:3156–3157
- Mehta MP, Butterfield DA, Baross JA (2003) Phylogenetic diversity of nitrogenase (*nifH*) genes in deep-sea and hydrothermal vent environments of the Juan de Fuca Ridge. *Appl Environ Microbiol* 69:960–970
- Merkel AY, Podosokorskaya OA, Chernyh NA, Bonch-Osmolovskaya EA (2015) Distribution, diversity and numbers of methanogenic archaea in terrestrial hot springs of Kamchatka and Saint-Miguel Island. *Microbiol (Mikrobiologiya)* 84:1–8
- Miroshnichenko ML, Kostrikina NA, Rainey FA, Hippe H, Bonch-Osmolovskaya EA (1998) *Desulfurella kamchatkensis* sp. nov. and *Desulfurella propionica* sp. nov., new thermophilic sulfur-reducing bacteria from Kamchatka hot vents. *Int J System Bacteriol* 48:475–479
- Miroshnichenko ML, Hippe H, Stackebrandt E, Kostrikina NA, Chernyh NA, Jeanthon C, Nazina TN, Belyaev SS, Bonch-Osmolovskaya EA (2001) Isolation and characterization of *Thermococcus sibiricus* sp. nov. from a Western Siberia high-temperature oil reservoir. *Extremophiles* 5:85–91
- Miroshnichenko ML, Tourova TP, Kolganova TP, Kostrikina NA, Bonch-Osmolovskaya EA (2008) *Ammonifex thiophilus* sp. nov., a hyperthermophilic anaerobic bacterium from a Kamchatka hot spring. *Int J Syst Evol Microbiol* 58:2935–2938
- Miroshnichenko ML, Lebedinsky AV, Chernyh NA, Tourova TP, Kolganova TV, Spring S, Bonch-Osmolovskaya EA (2009) *Caldimicrobium rimae* gen. nov., sp. nov., a novel extremely thermophilic facultatively lithoautotrophic anaerobic bacterium from the Uzon Caldera, Kamchatka. *Int J Syst Evol Microbiol* 59:1040–1044
- Muyzer G, Dewaal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16 s rRNA. *Appl Environ Microbiol* 59:695–700
- Nakagawa T, Fukui M (2002) Phylogenetic characterization of microbial mats and streamers from a Japanese alkaline hot spring with a thermal gradient. *J Gen Appl Microbiol* 48:211–222
- Narihiro T, Hori T, Nagata O, Hoshino T, Yumoto I, Kamagata Y (2011) The impact of aridification and vegetation type on changes in the community structure of methane-cycling microorganisms in Japanese wetland soils. *Biosci Biotechnol Biochem* 75:1727–1734
- Perevalova AA, Kolganova TV, Birkeland N-K, Schleper C, Bonch-Osmolovskaya EA, Lebedinsky AV (2008) Distribution of *Crenarchaeota* representatives in terrestrial hot springs of Russia and Iceland. *Appl Environ Microbiol* 74:7620–7628
- Pernthaler J, Glöckner FO, Schönhuber W, Amann R (2001) Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. *Methods Microbiol* 30:207–226
- Pimenov NV, Bonch-Osmolovskaya EA (2006) In situ activity studies in thermal environments. In: Rainey FA, Oren A (eds) *Methods in microbiology*. *Extremophiles*, vol 35. Elsevier, Academic Press, Amsterdam, Boston, Heidelberg, pp 29–54
- Podosokorskaya OA, Kadnikov VV, Gavrilov SN, Mardanov AV, Merkel AY, Karnachuk OV, Ravin NV, Bonch-Osmolovskaya EA, Kublanov IV (2013) Characterization of *Melioribacter roseus* gen. nov., sp. nov., a novel facultatively anaerobic thermophilic cellulolytic bacterium from the class *Ignavibacteria*, and a proposal of a novel bacterial phylum *Ignavibacteria*. *Environ Microbiol* 15:1759–1771
- Purcell DU, Sompomg U, Yim LC, Barraclough TG, Peerapornisal Y, Pointing SB (2007) The effects of temperature, pH and sulfide on the community structure of hyperthermophilic streamers in hot springs of northern Thailand. *FEMS Microbiol Ecol* 60:456–466
- Quince C, Lanzen A, Davenport RJ, Turnbaugh PJ (2011) Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* 28:12–38
- Reigstad LJ, Richter A, Daims H, Urich T, Schwark L, Schleper C (2008) Nitrification in terrestrial hot springs of Iceland and Kamchatka. *FEMS Microbiol Ecol* 64:167–174
- Reigstad LJ, Jorgensen SL, Schleper C (2010) Diversity and abundance of *Korarchaeota* in terrestrial hot springs of Iceland and Kamchatka. *ISME J* 4:346–356
- Reysenbach A-L, Wickham GS, Pace NR (1994) Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. *Appl Environ Microbiol* 60:2113–2119
- Reysenbach A-L, Ehringer M, Hershberger K (2000) Microbial diversity at 83°C in Calcite Spring, Yellowstone National Park: the same environment where the *Aquificales* and “*Korarchaeota*” coexist. *Extremophiles* 4:61–67
- Ronimus RS, Reysenbach AL, Musgrave DR, Morgan HW (1997) The phylogenetic position of the *Thermococcus* isolate AN1 based on 16S rRNA gene sequence analysis: a proposal that AN1 represents a new species. *Thermococcus zilligii* sp. nov. *Arch Microbiol* 168:245–248
- Rozanov AS, Bryanskaya AV, Malup TK, Meshcheryakova IA, Lazareva EV, Taran OP, Ivanisenko TV, Ivanisenko VA, Zhmodik SM, Kolchanov NA, Peltek SE (2014) Molecular analysis of the benthos microbial community in Zavarzin thermal spring (Uzon Caldera, Kamchatka, Russia). *BMC Genom* 15:2–15
- Sahm K, John P, Nacke H, Wemheuer B, Grote R, Daniel R, Antranikian G (2013) High abundance of heterotrophic prokaryotes in hydrothermal springs of the Azores as revealed by a network of 16S rRNA gene-based methods. *Extremophiles* 17:649–662
- Siering PL, Clarke JN, Wilson MS (2006) Geochemical and biological diversity of active hot springs in Lassen Volcanic National Park. *Geomicrobiol J* 23:129–141
- Skirmisdottir S, Hreggvidsson GO, Hjörleifsdottir S, Marteinnsson VT, Petursdottir SK, Holst O, Kristjansson JK (2000) Influence of sulfide and temperature on species composition community structure of hot spring microbial mats. *Appl Environ Microbiol* 66:2835–2841
- Slobodkina GB, Chernyh NA, Slobodkin AI, Subbotina IV, Bonch-Osmolovskaya EA, Lebedinsky AV (2004) PCR-based detection of hyperthermophilic *Archaea* of the family *Thermococcaceae*. *Appl Environ Microbiol* 70:5701–5703
- Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored “rare biosphere”. *Proc Natl Acad Sci USA* 103:12115–12120
- Spear JR, Walker JJ, McCollom TM, Pace N (2005) Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. *Proc Natl Acad Sci USA* 102:2555–2560
- Stahl DA, Amann R (1991) Development and application of nucleic acid probes. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. Wiley and Sons, New York, pp 205–248
- Steinberg LM, Regan JM (2008) Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. *Appl Environ Microbiol* 74:6663–6671
- Steinberg LM, Regan JM (2009) *mcrA*-targeted real-time quantitative PCR method to examine methanogen communities. *Appl Environ Microbiol* 75:4435–4442

- Stetter KO (1996) Hyperthermophilic prokaryotes. *FEMS Microbiol Rev* 18:149–158
- Stetter KO, Hohn MJ, Huber H, Rachel R, Mathur E, Hedlund B et al. (2005) A novel kingdom of parasitic *Archaea*. In “Geothermal Biology and Geochemistry in Yellowstone National Park”. Proceeding of the Thermal Biology Institute Workshop, Yellowstone National Park, WY, October 2003, eds W. P. Inskeep and T. R. McDermott (Bozeman, MT: Montana State University Publications), pp 249–259
- Takacs CD, Ehringer M, Favre R, Cermola M, Eggetsson G, Palsdotir A, Reysenbach A (2001) Phylogenetic characterization of the blue filamentous community from Icelandic hot spring. *FEMS Microbiol Ecol* 35:123–128
- Takacs-Verbach C, Inskeep WP, Jay ZJ, Herrgard MJ, Rusch DB, Tringle SG et al (2013) Metagenome sequence analysis of filamentous microbial communities obtained from geochemically distinct geothermal channels reveals specialization of three Aquificales lineages. *Frontiers Microbiol.* 4:84
- Takeuchi M, Yoshioka H, Seo Y, Tanabe S, Tamaki H, Kamagata Y, Takahashi HA, Igari S, Mayumi D, Sakata S (2011) A distinct freshwater-adapted subgroup of ANME-1 dominates active archaeal communities in terrestrial sub-surfaces in Japan. *Environ Microbiol* 13:3206–3218
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molec Biol Evol* 30:2725–2729
- Urbeita MS, Gozales Toril E, Giaveno MA, Aquilera Bazan A, Donati ER (2014) Archaeal and bacterial diversity in five different hydrothermal ponds in the Copahue region of Argentina. *Syst Appl Microbiol* 37:429–441
- Ver Eecke HC, Butterfield DA, Huber JA, Lilley MD, Olson EJ, Roe KK et al (2012) Hydrogen-limited growth of hyperthermophilic methanogens at deep-sea hydrothermal vents. *Proc Natl Acad Sci USA* 109:13674–13679
- Vick TJ, Dodsworth JA, Costa KC, Shock EL, Hedlund BP (2010) Microbiology and geochemistry of Little Hot Creek, a hot spring environment in the Long Valley Caldera. *Geobiology* 8:140–154
- Wagner M, Roger AJ, Flax JL, Brusseau GA, Stahl DA (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J Bacteriol* 180:2975–2982
- Wagner ID, Varghese LB, Hemme CL, Wiegel J (2013) Multilocus sequence analysis of *Thermoanaerobacter* isolates reveals recombining, but differentiated, populations from geothermal springs of the Uzon Caldera, Kamchatka, Russia. *Front Microbiol* 21:4–169
- Walters WA, Caporaso JG, Lauber CL, Berg-Lyons D, Fierer N, Knight R (2011) PrimerProspector: *de novo* design and taxonomic analysis of barcoded PCR primers. *Bioinformatics* 27:2–4
- Wemheuer B, Taube R, Akyol P, Wemheuer F, Daniel R (2013) Microbial diversity and biochemical potential encoded by thermal spring metagenomes derived from the Kamchatka Peninsula. *Archaea* 2013:1–13
- Wiegel J (1992) The obligately anaerobic thermophilic bacteria. In: Kristjansson JK (ed) *Thermophilic bacteria*. CRC Press, Boca Raton, pp 105–184
- Yamamoto H, Hiraishi A, Kato K, Chiura HX, Maki Y, Shimizu A (1998) Phylogenetic evidence for the existence of novel thermophilic bacteria in hot spring sulfur-turf microbial mats in Japan. *Appl Environ Microbiol* 64:1680–1687
- Zhao W, Romanek CS, Mills G, Wiegel J, Zhang CL (2005) Geochemistry and microbiology of hot springs in Kamchatka, Russia. *Geological J China Univ* 11:217–223