Extremophiles (2011) 15:549–563
DOI 10.1007/s00792-011-0386-z

ORIGINAL PAPER

Archaeal and bacterial diversity in hot springs on the Tibetan Plateau, China

Qiuyuan Huang · Christina Z. Dong · Raymond M. Dong · Hongchen Jiang · Shang Wang · Genhou Wang · Bin Fang · Xiaoxue Ding · Lu Niu · Xin Li · Chuanlun Zhang · Hailiang Dong

Received: 26 December 2010 / Accepted: 6 June 2011 / Published online: 22 June 2011

Abstract The diversity of archaea and bacteria was investigated in ten hot springs (elevation >4600 m above sea level) in Central and Central-Eastern Tibet using 16S rRNA gene phylogenetic analysis. The temperature and pH of these hot springs were 26–81°C and close to neutral, respectively. A total of 959 (415 and 544 for bacteria and archaea, respectively) clone sequences were obtained. Phylogenetic analysis showed that bacteria were more diverse than archaea and that these clone sequences were classified into 82 bacterial and 41 archaeal operational taxonomic units (OTUs), respectively. The retrieved bacterial clones were mainly affiliated with four known groups (i.e., *Firmicutes*, *Proteobacteria*, *Cyanobacteria*, *Chloroflexi*), which were similar to those in other neutral-pH hot springs at low elevations. In contrast, most of the archaeal clones from the Tibetan hot springs were affiliated with Thaumarchaeota, a newly proposed archaeal phylum. The dominance of Thaumarchaeota in the archaeal community of the Tibetan hot springs appears to be unique, although the exact reasons are not yet known. Statistical analysis showed that diversity indices of both archaea and bacteria were not statistically correlated with temperature, which is consistent with previous studies.

Keywords Archaea · Bacteria · Diversity · Hot springs · Thaumarchaeota · Tibet

Communicated by M. da Costa.

Q. Huang and C. Z. Dong contributed equally to this work.

Q. Huang · H. Dong
Department of Geology, Miami University, Oxford, OH 45056, USA

C. Z. Dong · R. M. Dong
Badin High School, 571 Hamilton New London Road, Hamilton, OH 45013, USA

H. Jiang (✉) · S. Wang · H. Dong (✉)
State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Beijing 100083, China
e-mail: jianghc@cugb.edu.cn

H. Dong
e-mail: dongh@muohio.edu; dongh@cugb.edu.cn

G. Wang · X. Ding · L. Niu · X. Li
School of Earth Sciences and Resources, China University of Geosciences, Beijing 100083, China

B. Fang
School of Water Resources and Environment, China University of Geosciences, Beijing 100083, China

C. Zhang
State Key Laboratory of Marine Geology, Tongji University, Shanghai 200092, China

C. Zhang
Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA

Introduction

Microbial communities in hot springs at low elevations have been extensively studied worldwide, such as those in Yellowstone National Park (Barns et al. 1994; Pace 1997; Meyer-Dombard et al. 2005; Hall et al. 2008; Mitchell 2009),

Long Valley Caldera near Mammoth Lakes, CA, USA (Vick et al. 2010), Kamchatka of Russia (Bonch-Osmolovskaya et al. 1999; Reigstad et al. 2009), Iceland (Marteinsson et al. 2001; Reigstad et al. 2009; Aguilera et al. 2010), Uttaranchal Himalaya (Kumar et al. 2004), the Tengchong area in Yunnan Province of China (Song et al. 2009, 2010; Jiang et al. 2010), Indonesia (Aditiawati et al. 2009), and Tunisia (Sayeh et al. 2010). These studies reveal that diverse microbial communities are present in terrestrial hot springs; and temperature, pH, dissolved hydrogen sulfide levels, and biogeography are important factors in controlling abundance and diversity (Ward and Castenholz 2002; Purcell et al. 2007; Whitaker et al. 2003). Among the diverse communities in hot springs, ammonia-oxidizing archaea (AOA) are a globally distributed important group (Zhang et al. 2008). Based on environmental 16S rRNA gene sequences, these archaea are placed within Crenarchaeota and some of the AOA are currently named as Thaumarchaeota (Brochier-Armanet et al. 2008). Thaumarchaeota may play a more important role in hot spring environment than previously thought; however, it is currently unknown if these organisms are widely distributed in terrestrial hot springs.

Despite these intensive studies of terrestrial thermal habitats, little is known about microbial diversity in hot springs at high elevations, especially in the Tibetan area (e.g., Lau et al. 2006). The Tibetan Plateau (>4000 m above sea level) is located in the east-central Mediterranean-Himalayas tectonic zone, and the region hosts one of the most active geothermal areas in the world and possesses many hot springs with varying environmental gradients (Hu et al. 2003). Up to now, only Lau et al. (2006, 2009) have studied microbial diversity in some hot springs in Central Tibet. Lau et al. (2006) investigated microbial community along a thermal gradient (52–83°C) of an isolated geothermal location in Central Tibet and found that the response of microbial diversity was not monotonic to thermal stress. In the other study, Lau et al. (2009) investigated bacterial diversity in five hot springs (with a temperature range of 60–65°C) in Central Tibet and found that *Proteobacteria* and phototrophic bacteria (i.e., *Chlorobi*, *Cyanobacteria*, *Chloroflexi*) were ubiquitous. However, still little is known about how bacterial and archaeal communities are distributed among different hot springs of a larger temperature range.

The objective of this study was therefore to test if microbial diversity responds to thermal stress in Tibetan hot springs of a wide temperature range. We expanded upon the previous studies by investigating archaeal and bacterial diversity in ten Tibetan hot springs over a temperature range of 26.2–81.2°C. The 16S rRNA gene phylogenetic analysis was conducted to assess any correlation between microbial diversity and environmental variables (e.g., temperature, mineralogy).

Materials and methods

Field measurements and sampling

In August 2009, a field expedition was made to the Central and Central-Eastern Tibet, and thirty hot springs were surveyed including sediment/mat color, temperature, and pH. Water pH and temperature were measured in the field using a thermometer and pH meter, respectively. Ten representative hot springs covering a range of temperatures were selected for molecular study at town Jiwa (JW) and Rongma (RM) of Nima County and town Gulu (GL) of Naqu County (Fig. 1; Table 1). These springs varied in size from 20 to 50 cm in diameter. Geographical locations and elevations of these springs were determined using a portable GPS unit (eTrex H, Garmin, US). In general temperature and pH were homogeneous, and there were no dramatic changes of sediment/mat color within a given spring. Thus, only one sediment/mat sample was collected from each spring. Microbial mats and surface sediments were collected with a hand trowel into sterile 50 mL Falcon tubes and preserved in the sucrose lysis buffer (Mitchell and Takacs-Vesbach 2008). Hand trowels were sterilized with 75% ethanol and dried after each use. A sympatric soil at Rongma was also collected for comparison with the adjacent hot spring samples. Within 1 week, the preserved samples were shipped to the laboratory in Beijing and were then stored at −80°C until further analysis.

Powder X-ray diffraction (XRD)

X-ray diffraction was performed to identify the mineralogy of the collected solids by using a Scintag X1 powder diffractometer system using CuKα radiation with a variable divergent slit and a solid-state detector (Zhang et al. 2005). For the XRD analysis, solids were air dried overnight, ground into powder, and tightly packed into the well of low-background quartz XRD slides (Gem Dugout, Inc., Pittsburgh, PA, USA). To facilitate qualitative comparisons among the samples, a similar amount of solid powder from each sample was packed into a rectangular volume of the same dimensions. The routine power was 1400 W (40 kV, 35 mA). Samples were scanned from 2 to 70° in 0.02 two-theta steps with a count time of 2 s per step. Search-match software was used to conduct mineral identification. The relative abundance of each mineral was qualitatively assigned to be one of four categories based on the relative peak intensity of characteristic peaks: very abundant, abundant, moderate, and present.

DNA extraction and PCR

Genomic DNA was extracted from ~500 mg (wet weight) of each sample using FastDNA Spin Kit for Soil (MP

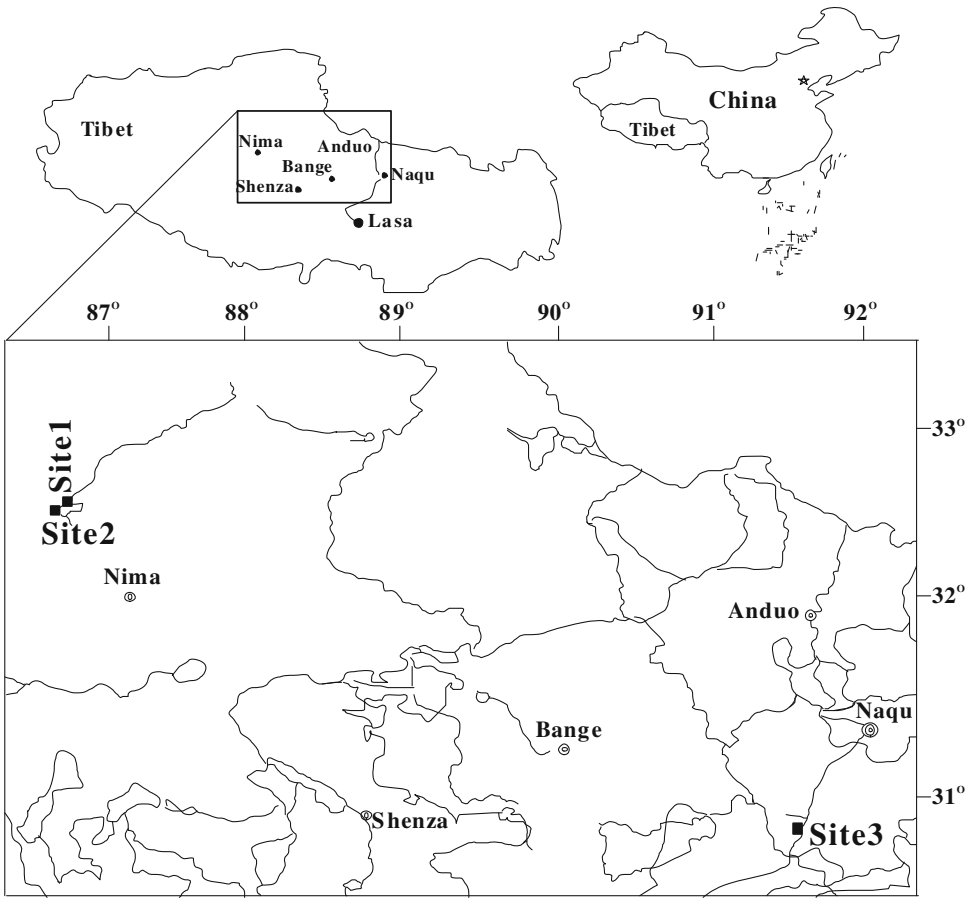


Fig. 1 A geographic map showing the sampling locations on the Tibetan Plateau, China. JW41 and JW56 were from Site 1; RM26, RM45, RM55, RM64, and RMS were from Site 2; and GL52, GL63, GL64, and GL81 were from Site 3

Table 1 Location and description of the investigated soil and ten hot springs on the Tibetan Plateau, China

Sample	GPS coordinates (N/E)		Elevation (m)	Temp (°C)	pH	Sample type	Mineralogy (wt%)						
							Calcite	Quartz	Albite	Muscovite	Illite	Biotite	Others
RMS	32°57′43.8″	86°35′49.2″	4725	NA	NA	Brown soil	UD	++	UD	++	+++	+	++
RM26	32°57′46.5″	86°35′48.1″	4719	26.2	6.8	Gray sediment	++++	++	UD	+	UD	UD	++
RM45	32°57′45.3″	86°35′49.1″	4715	45.1	7.2	Brown sediment	++++	UD	UD	UD	UD	UD	++
RM55	32°57′44.6″	86°35′48.3″	4720	55.6	7.1	Green mat	NA	NA	NA	NA	NA	NA	NA
RM64	32°57′46.3″	86°35′47.9″	4718	64.1	6.9	Calcareous sinter	++++	+	UD	UD	UD	UD	+
JW41	33°08′23.3″	86°49′53.6″	4618	41.7	6.8	Green sediment	+++	++	UD	UD	UD	UD	+
JW56	33°08′23.4″	86°49′54.4″	4630	56.0	6.5	Green sediment	+++	++	UD	UD	UD	+	++
GL52	30°52′12.9″	91°36′44.7″	4735	52.0	NA	Green mat	NA	NA	NA	NA	NA	NA	NA
GL63	30°52′35.6″	91°36′35.2″	4715	63.8	NA	Green sediment	UD	+++	+++	UD	UD	UD	++
GL64	30°52′35.4″	91°36′35.0″	4711	64.0	NA	Brown sediment	+	+++	++	+	UD	UD	+
GL81	30°52′34.1″	91°36′40.0″	4726	81.2	8.2	Black sediment	NA	NA	NA	NA	NA	NA	NA

Samples are coded as follows: *RM26* Rongma hot spring with temperature of 26.2°C, *RM45* Rongma hot spring with temperature of 45.1°C, *RM55* Rongma hot spring with temperature of 55.6°C, *RM64* Rongma hot spring with temperature of 64.1°C, *RMS* The sympatric soil near RM26, *JW41* Jiwa hot spring with temperature of 41.7°C, *JW56* Jiwa hot spring with temperature of 56°C, *GL52* Gulu hot spring with temperature of 52°C, *GL63* Gulu hot spring with temperature of 63.8°C, *GL64* Gulu hot spring with temperature of 64°C, *GL81* Gulu hot spring with temperature of 81.2°C, *NA* not available, *UD* undetectable, Mineralogy: ++++ very abundant, +++ abundant, ++ moderate, + present

Biomedical, US) according to the manufacturer's instructions. The archaeal 16S rRNA gene from the extracted DNA was amplified with archaeal forward primer Arch21F (5′-TTCYGGTTGATCCYGCCRGA-3′) and universal reverse primer Univ958R (5′-YCCGGCGTTGAMTCCA TTT-3′); while the primer set of Bac27F (5′-AGAGTT

TGATCMTGGCTCAG-3′) and Univ1492R (5′-CGGTTA CCTTGTTACGACTT-3′) was used for bacterial 16S rRNA gene PCR amplification. These primers have been used for hot spring samples (Pearson et al. 2004; Meyer-Dombard et al. 2005; Lau et al. 2009) and are effective in amplifying the 16S rRNA gene of bacteria and archaea. For PCR, a typical mixture (25 μL in volume) for both archaea and bacteria consisted of the following reagents: 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 100 μM of each deoxynucleoside triphosphate, 0.8 mM bovine serum albumin (TaKaRa, Dalian, China), 1.25 U of Taq DNA polymerase (TaKaRa, Dalian, China), and ~25 ng of total DNA. The conditions for archaeal 16S rRNA gene PCRs consisted of an initial denaturation at 95°C for 5 min, and 35 cycles of denaturing at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min. The conditions for bacterial 16S rRNA genes were as follows: an initial denaturation at 95°C for 5 min, and 35 cycles of denaturing at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min. The PCR products were purified with Agarose Gel DNA Purification Kit Ver.2.0 (TaKaRa, Dalian, China) according to the manufacturer's instructions.

Clone library construction

The purified PCR products were ligated into cloning vectors and transformed into *Escherichia coli* Trans1-T1 competent cells using pEASY-T1 cloning kit (TransGen Biotech, Beijing, China) according to the manufacturer's suggested protocol. The transformants were plated on Luria–Bertani plates containing 100 μg mL^{-1} of ampicillin, 80 μg mL^{-1} of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside). The Luria–Bertani plates were incubated at 37°C overnight. Twenty-two clone libraries (eleven each for archaea and bacteria) were constructed. Colonies were randomly selected and analyzed for the 16S rRNA gene inserts. Inserts were amplified using forward primer M13-RV (5′-CAG GAA ACA GCT ATG AC-3′) and reverse primer M13-47 (5′-GTT TTC CCA GTC ACG AC-3′). The PCR reaction system was same as described above with the following PCR conditions: 94°C for 10 min; 34 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 90 s, with a final elongation step of 72°C for 10 min. The randomly selected clones were sequenced (with primers Arch21F and Bac27F for archaea and bacteria, respectively) using the BigDye Terminator version 3.1 chemistry (Applied Biosystems, Foster City, CA, USA) with an ABI 3100 automated sequencer at Shanghai Sangon Biotech.

Phylogenetic analyses

The raw sequences were trimmed using Sequencher 4.8. The potential presence of chimeric sequences was examined with Bellerophon (Huber et al. 2004). Potential chimeric sequences were removed. The chimera-free sequences were blasted in the GenBank (http://www.ncbi.nlm.nih.gov). Operational taxonomic units (OTUs) were determined using DOTUR (Schloss and Handelsman 2005) with a 97% cutoff value. Neighbor-joining phylogenetic trees were constructed from dissimilar distance and pairwise comparisons with the Jukes–Cantor distance model using the MEGA (molecular evolutionary genetics analysis) program, version 4.1. Bootstrap value of 1000 replications was assessed in the analysis. The sequences determined in this study have been deposited in the GenBank database under accession numbers HQ287087-HQ287215.

Statistical analysis

Coverage (C) of the constructed clone libraries was calculated as follows: $C = 1 - (n_1/N)$, where n_1 is the number of phylotypes that occurred only once in the clone library and N is the total number of clones analyzed (Jiang et al. 2009). LIBSHUFF analysis for the difference between any two clone libraries was performed in the same way as described elsewhere (Jiang et al. 2008). With *zt* software (http://www.psb.ugent.be/~erbon/mantel/), the Mantel test was performed to reveal any correlation between biotic and environmental data sets according to procedures as previously described (Jiang et al. 2009).

Results

Characteristics of the sampling sites

The elevations of the ten investigated hot springs were higher than 4600 m above sea level. The temperature and pH were 26.2–81.2°C and close to neutral (6.48–8.20), respectively (Table 1).

Mineralogy of microbial mats and sediments

X-ray diffraction analysis showed that the mineral composition varied among the samples from different localities: calcite was predominant in the RM26, RM45, and RM64 samples, whereas quartz was rarely found in these samples; calcite and quartz were the two major minerals in the JW41 and JW56 samples; and quartz and albite were the two dominant minerals in GL63 and GL64 (Table 1). Other minerals included minor amounts of layer silicates such as muscovite, illite, and biotite.

Table 2 Ecological estimates and major group affiliation of the bacterial 16S rRNA gene clone sequences retrieved from the soil and ten hot springs on the Tibetan Plateau

Community	RMS	RM26	RM45	RM55	RM64	JW41	JW56	GL52	GL63	GL64	GL81
No. of clones	24	49	31	24	50	47	21	48	39	45	37
Coverage (%)	64	77.6	100	79.2	90	97.9	85.7	95.8	100	91.1	100
No. of observed OTUs	11	16	2	10	12	5	5	6	4	10	2
Chaol	25 (15.5, 104)	43.5 (22.7, 129.5)	2 (2, 2)	20 (11.9, 62.2)	14.5 (12.4, 29)	5 (5, 5)	6.5 (5.1, 20.1)	6.5 (6.0, 14.3)	4 (4, 4)	16 (11, 47.9)	2 (2, 2)
Shannon (H')	2.18 (1.78, 2.59)	2.17 (1.84, 2.5)	0 (−0.03, 0.03)	2.1 (1.81, 2.40)	1.88 (1.57, 2.19)	1.43 (1.3, 1.6)	0.87 (0.38, 1.35)	0.87 (0.54, 1.20)	0.8 (0.49, 1.11)	1.94 (1.71, 2.18)	0.66 (0.57, 0.76)
Major group affiliation	Number of clones (Relative percentage in each clone library)										
Acidobacteria	2 (8.3%)				9 (18.0%)					1 (2.2%)	
Bacteroidetes		1 (2.0%)			12 (24.0%)			3 (6.3%)	2 (5.1%)		
Proteobacteria	9 (37.5%)	8 (16.3%)		19 (79.2%)	6 (12.0%)	10 (21.3%)	2 (9.5%)	38 (79.2%)		8 (17.8%)	37 (100%)
Nitrospirae										1 (2.2%)	
Firmicutes	13 (54.2%)	38 (77.6%)	29 (93.5%)	5 (20.8%)	21 (42.0%)	22 (46.8%)	3 (14.3%)		3 (7.7%)	20 (44.4%)	
Aquificae										3 (6.7%)	
Cyanobacteria and Chloroflexi		1 (2.0%)			2 (4.0%)	15 (31.9%)	16 (76.2%)	7 (14.6%)	34 (87.2%)	11 (24.4%)	
Planctomycetes			2 (6.5%)								
Thermodesulfobacteria										1 (2.2%)	
Unclassified Bacterium		1 (2.0%)									

Bacterial 16S rRNA gene phylogenetic analysis

Four hundred and fifteen bacterial clone sequences were obtained, and they could be classified into the following groups: *Firmicutes*, *Proteobacteria*, *Cyanobacteria*, *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, *Nitrospirae*, *Planctomycetes*, *Thermodesulfobacteria*, *Aquificae*, and unclassified bacteria (Table 2; Fig. 2). Among these groups, *Firmicutes*, *Proteobacteria*, *Chloroflexi* and *Cyanobacteria* were the major components in the bacterial 16S rRNA gene clone libraries, and they accounted for 91% of all bacterial 16S rRNA gene clone sequences (Table 2).

Cyanobacteria and Chloroflexi

Eighty-six clone sequences (20.7%: 86 out of 415) were affiliated with *Cyanobacteria* and *Chloroflexi* (Fig. 2a; Table 2), and these sequences were derived from hot springs with relatively low temperatures (<64°C). Most of these sequences were closely related to clones retrieved from hot spring environments, such as those in Tibet (Lau et al. 2009), Yellowstone National Park (Allewalt et al. 2006), Thailand (Portillo et al. 2009), and Bulgarian (Tomova et al. 2010). Among the *Cyanobacteria*, three clone sequences from JW56 were related to *Synechococcus* sp. TS-91 isolated from Octopus Spring (49–70°C) in Yellowstone National Park (Allewalt et al. 2006). In the *Chloroflexi* group, four clone sequences were closely related to sequences retrieved from Bor Khlueng Hot Spring (50–57°C) in Thailand (Kanokratana et al. 2004), and 41 clone sequences from GL63 and GL64 (30 and 11, respectively) were related (94%) to a *Chloroflexi* bacterium (FM164953) isolated from a geothermal spring (79°C) in Bulgaria (Tomova et al. 2010).

Proteobacteria

One hundred and thirty-seven sequences (33.0%: 137 out of 415) were affiliated with *Proteobacteria*, and these sequences could be classified into subgroups: *Alpha-*, *Beta-*, *Gamma-*, and *Deltaproteobacteria* (Fig. 2b; Table 2). The clone sequences affiliated with *Gammaproteobacteria* were predominant (76.6%: 105 out of 137), and most of them were closely (98–100%) related to cultured *Gammaproteobacteria* and clones retrieved from low-temperature habitats, such as ice, soils, and sediments. One clone sequence from GL52 was closely related (99%) to *Thiofaba tepidiphila*, a novel obligately chemolithoautotrophic, sulfur-oxidizing bacterium of the *Gammaproteobacteria* isolated from a hot spring (45°C and pH 7.0) in Fukushima prefecture, Japan (Mori and Suzuki 2008).

Fig. 2 **a** Neighbor-joining tree (partial sequences, ~700 bp) showing the phylogenetic relationships of bacterial 16S rRNA gene sequences cloned from the hot spring samples on the Tibetan Plateau to closely related sequences from the GenBank database. One representative clone type within each OTU is shown, and the number of clones is shown at the end (after the GenBank accession number). The number of clones is omitted if there is only one clone within a given OTU. Clone sequences from this study are coded as follows for the example of RM64-B001 (HQ287172) 9: RM64, sample name; B, bacterium; 001, number of clone type; HQ287172, GenBank accession number; 9, number of clone sequences. *Scale bar* indicates Jukes-Cantor distances. *Aquifex pyrophilus* is used as an outer group, and a single tree showing all bacterial sequences is created. **b**, **c** Subtrees for *Proteobacteria* and *Firmicutes*

Firmicutes

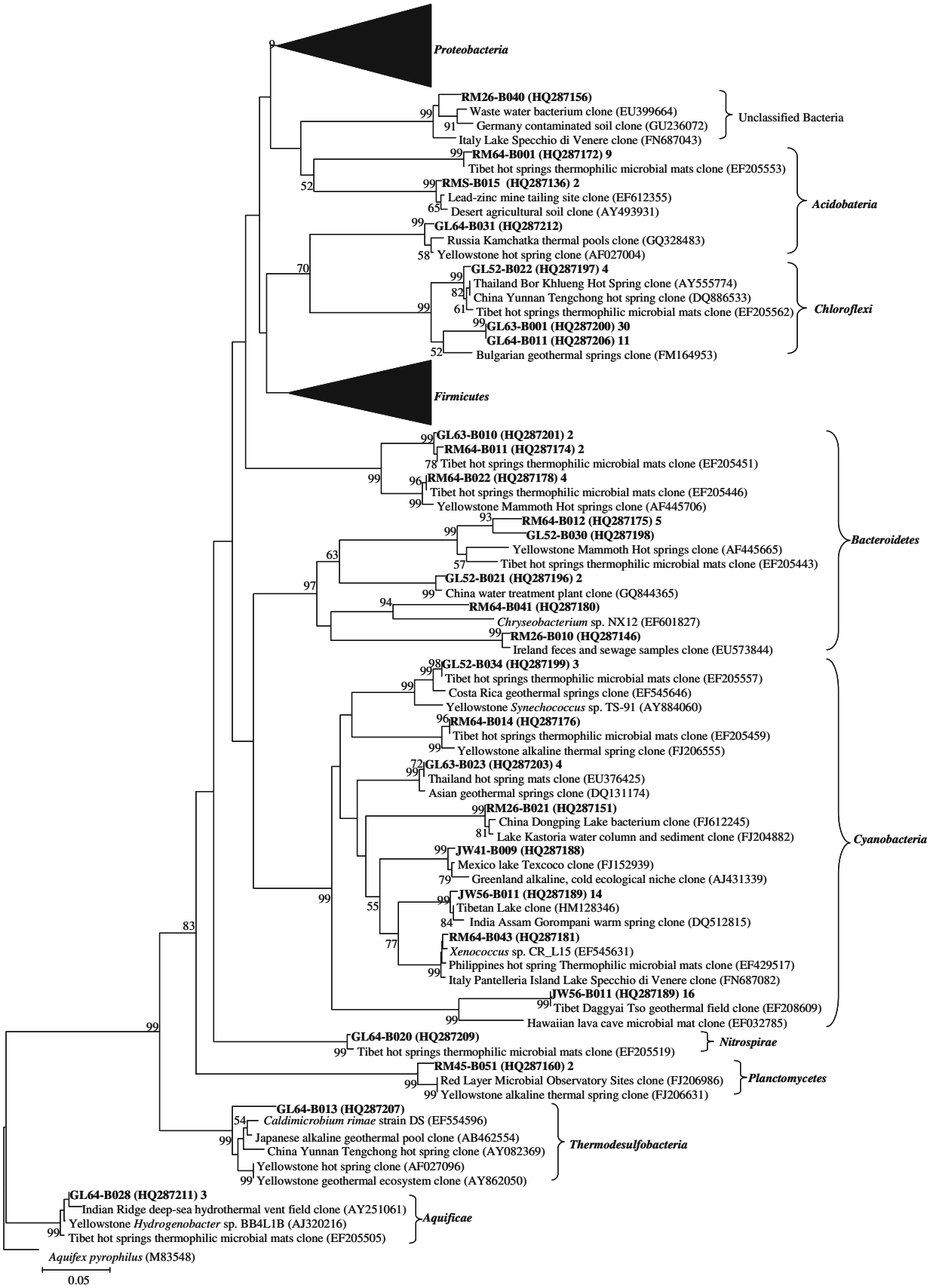
One hundred and fifty-four clone sequences (37.1%: 154 out of 415) were affiliated with *Firmicutes*. The *Firmicutes* sequences can be classified into two subgroups: *Clostridia* and *Bacillales* (Fig. 2c; Table 2), among which *Clostridia* sequences were predominant (93%: 140 out of 154). The *Clostridia* sequences were closely related (92–99%) to clones retrieved from low- or high-temperature environments. Nine sequences from GL64 were related (93%) to bacterium clone TMP-B3 (EU544532), which was obtained from a high-temperature mud pool in the Taupo Volcanic Zone, New Zealand (GenBank description). The majority of *Bacillales* sequences (88.9%: 8 out of 9) were obtained from the soil sample (RMS) and they were closely related (99%) to isolates or clones recovered from soda lakes (Joshi et al. 2008; Wu et al. 2010).

Around 9.2% (38 out of 415) of the total bacterial clone sequences were affiliated with some minor groups, such as *Acidobacteria*, *Bacteroidetes*, *Nitrospirae*, *Planctomycetes*, *Thermodesulfobacteria*, *Aquificae*, and unclassified bacteria (Fig. 2a; Table 2). Most of these sequences were closely (98–99%) related to clones retrieved from geothermal features, such as Tibetan hot springs (Lau et al. 2009), Yellowstone hot springs (Hugenholtz et al. 1998; Boomer et al. 2009), and Japanese alkaline geothermal pool (Kimura et al. 2010).

Archaeal 16S rRNA gene phylogenetic analysis

Five hundred and forty-four archaeal 16S rRNA gene clone sequences were obtained and the numbers of clones represented 87.2–100% coverage for each clone library (Table 3; Fig. 3). These archaeal clone sequences were affiliated with Euryarchaeota, Thaumarchaeota (Brochier-Armanet et al. 2008; Spang et al. 2010), and Crenarchaeota (Fig. 3).

The dominant group was Thaumarchaeota, which accounted for around 75% (407 out of 544) of all archaeal clone sequences retrieved in this study. These Thaumarchaeotal sequences were grouped with two previously


Proteobacteria
RM26-B040 (HQ287156)
Waste water bacterium clone (EU399664)
Germany contaminated soil clone (GU236072)
Italy Lake Specchio di Venere clone (FN687043)
Unclassified Bacteria
RM64-B001 (HQ287172) 9
Tibet hot springs thermophilic microbial mats clone (EF205553)
RMS-B015 (HQ287136) 2
Lead-zinc mine tailing site clone (EF612355)
Desert agricultural soil clone (AY493931)
GL64-B031 (HQ287212)
Russia Kamchatka thermal pools clone (GQ328483)
Yellowstone hot spring clone (AF027004)
Acidobateria
GL52-B022 (HQ287197) 4
Thailand Bor Khlueng Hot Spring clone (AY555774)
China Yunnan Tengchong hot spring clone (DQ886533)
Tibet hot springs thermophilic microbial mats clone (EF205562)
GL63-B001 (HQ287200) 30
GL64-B011 (HQ287206) 11
Bulgarian geothermal springs clone (FM164953)
Chloroflexi
Firmicutes
GL63-B010 (HQ287201) 2
RM64-B011 (HQ287174) 2
Tibet hot springs thermophilic microbial mats clone (EF205451)
RM64-B022 (HQ287178) 4
Tibet hot springs thermophilic microbial mats clone (EF205446)
Yellowstone Mammoth Hot springs clone (AF445706)
RM64-B012 (HQ287175) 5
GL52-B030 (HQ287198)
Yellowstone Mammoth Hot springs clone (AF445665)
Tibet hot springs thermophilic microbial mats clone (EF205443)
GL52-B021 (HQ287196) 2
China water treatment plant clone (GQ844365)
RM64-B041 (HQ287180)
Chryseobacterium sp. NX12 (EF601827)
RM26-B010 (HQ287146)
Ireland feces and sewage samples clone (EU573844)
Bacteroidetes
GL52-B034 (HQ287199) 3
Tibet hot springs thermophilic microbial mats clone (EF205557)
Costa Rica geothermal springs clone (EF545646)
Yellowstone Synechococcus sp. TS-91 (AY884060)
RM64-B014 (HQ287176)
Tibet hot springs thermophilic microbial mats clone (EF205459)
Yellowstone alkaline thermal spring clone (FJ206555)
GL63-B023 (HQ287203) 4
Thailand hot spring mats clone (EU376425)
Asian geothermal springs clone (DQ131174)
RM26-B021 (HQ287151)
China Dongping Lake bacterium clone (FJ612245)
Lake Kastoria water column and sediment clone (FJ204882)
JW41-B009 (HQ287188)
Mexico lake Texcoco clone (FJ152939)
Greenland alkaline, cold ecological niche clone (AJ431339)
JW56-B011 (HQ287189) 14
Tibetan Lake clone (HM128346)
India Assam Gorompani warm spring clone (DQ512815)
RM64-B043 (HQ287181)
Xenococcus sp. CR_L15 (EF545631)
Philippines hot spring Thermophilic microbial mats clone (EF429517)
Italy Pantelleria Island Lake Specchio di Venere clone (FN687082)
JW56-B011 (HQ287189) 16
Tibet Daggyai Tso geothermal field clone (EF208609)
Hawaiian lava cave microbial mat clone (EF032785)
Cyanobacteria
GL64-B020 (HQ287209)
Tibet hot springs thermophilic microbial mats clone (EF205519)
Nitrospirae
RM45-B051 (HQ287160) 2
Red Layer Microbial Observatory Sites clone (FJ206986)
Yellowstone alkaline thermal spring clone (FJ206631)
Planctomycetes
GL64-B013 (HQ287207)
Caldimicrobium rimae strain DS (EF554596)
Japanese alkaline geothermal pool clone (AB462554)
China Yunnan Tengchong hot spring clone (AY082369)
Yellowstone hot spring clone (AF027096)
Yellowstone geothermal ecosystem clone (AY862050)
Thermodesulfobacteria
GL64-B028 (HQ287211) 3
Indian Ridge deep-sea hydrothermal vent field clone (AY251061)
Yellowstone Hydrogenobacter sp. BB4L1B (AJ320216)
Tibet hot springs thermophilic microbial mats clone (EF205505)
Aquificae
Aquifex pyrophilus (M83548)
0.05

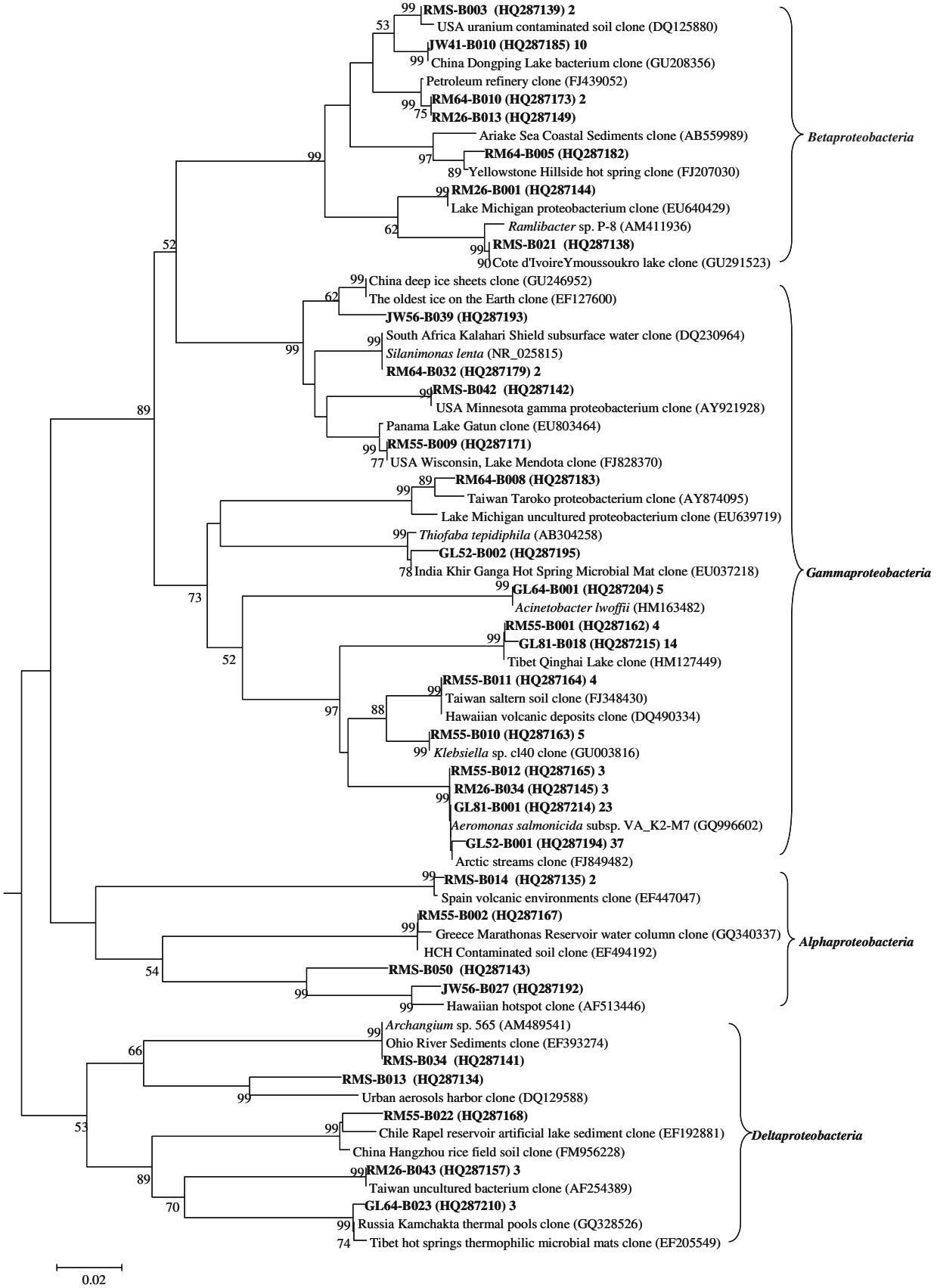


Fig. 2 continued

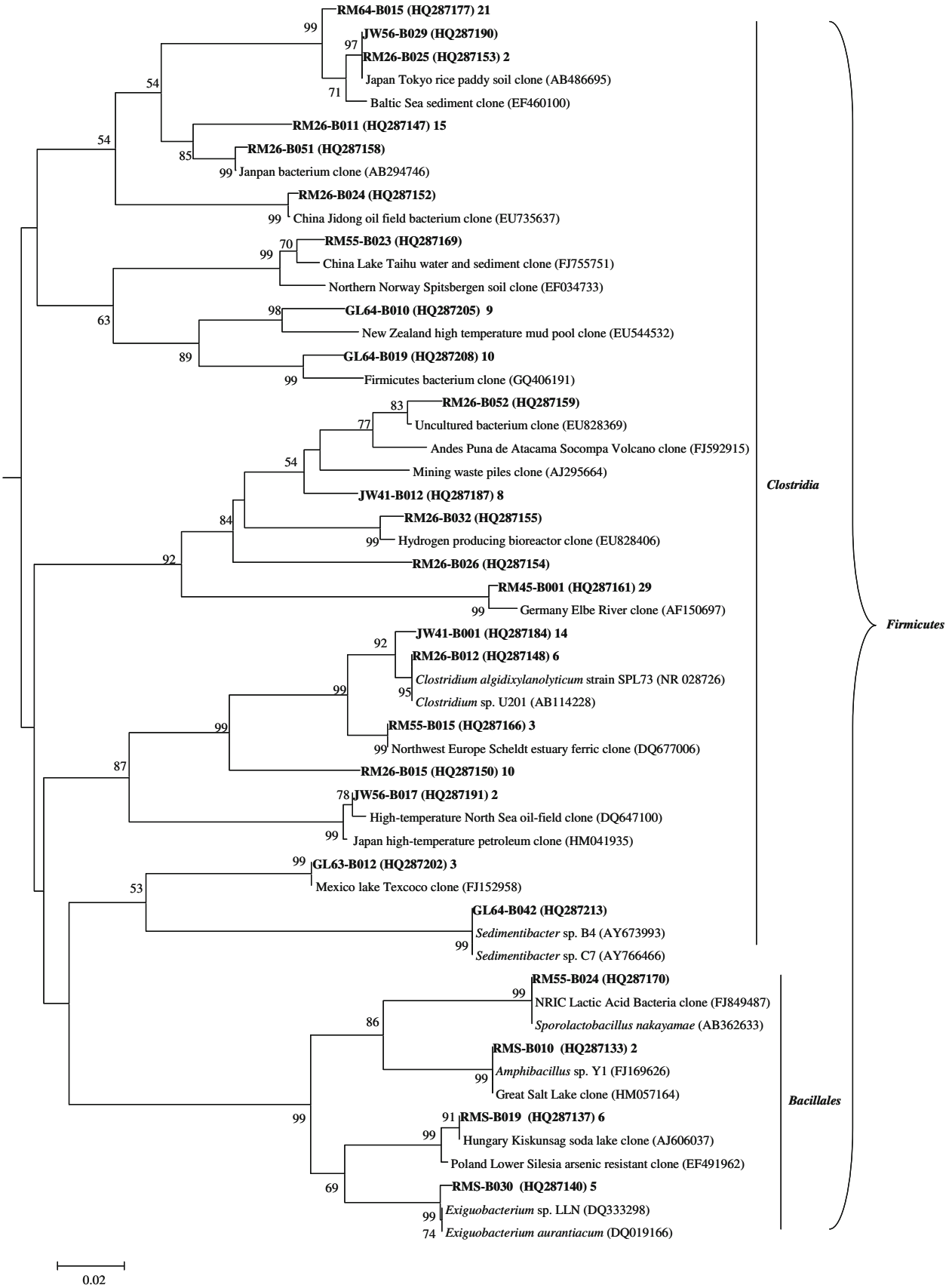


Fig. 2 continued

Table 3 Ecological estimates and major group affiliation of the archaeal 16S rRNA gene clone sequences retrieved from the soil and ten hot springs on the Tibetan Plateau

Community	RMS	RM26	RM45	RM55	RM64	JW41	JW56	GL52	GL63	GL64	GL81
No. of clones	55	49	47	56	45	48	55	46	47	49	47
Coverage (%)	96.4	98	100	100	88.9	100	100	93.5	87.2	98	100
No. of observed OTUs	6	5	2	1	8	1	1	3	8	4	2
Chao	7.5 (6.1, 22.1)	5 (5, 5)	2 (2, 2)	1 (1, 1)	13 (8.8, 40.1)	1 (1, 1)	1 (1, 1)	3 (3, 3)	13 (8.8, 40.1)	4 (4, 4)	2 (2, 2)
Shannon (H')	0.62 (0.3, 0.9)	0.76 (0.5, 1.1)	0.1 (0, 0.3)	0 (0, 0.3)	0.99 (0.6, 1.4)	0 (0, 0.03)	0 (0, 0.03)	0.28 (0.04, 0.52)	1.27 (1.0, 1.6)	0.65 (0.4, 1.9)	0.46 (0.3, 0.6)
Major group affiliation	Number of clones (Relative percentage in each clone library)										
Thaumarchaeota		5 (10.2%)	47 (100%)	56 (100%)	34 (75.6%)	48 (100%)	55 (100%)	43 (93.5%)	22 (46.8%)	3 (6.1%)	39 (83.0%)
Euryarchaeota											
MKCS-J		2 (4.1%)			1 (2.2%)				2 (4.3%)		
Halobacteriales					4 (8.9%)						
Methanomicrobiales	55 (100%)	41 (83.7%)						1 (2.1%)	2 (4.3%)	41 (83.7%)	
Crenarchaeota											
Uncultured Crenarchaeota		1 (2.0%)			6 (13.3%)			2 (4.3%)	18 (38.3%)	5 (10.2%)	3 (6.4%)
Desulfurococcales									3 (6.4%)		5 (10.6%)

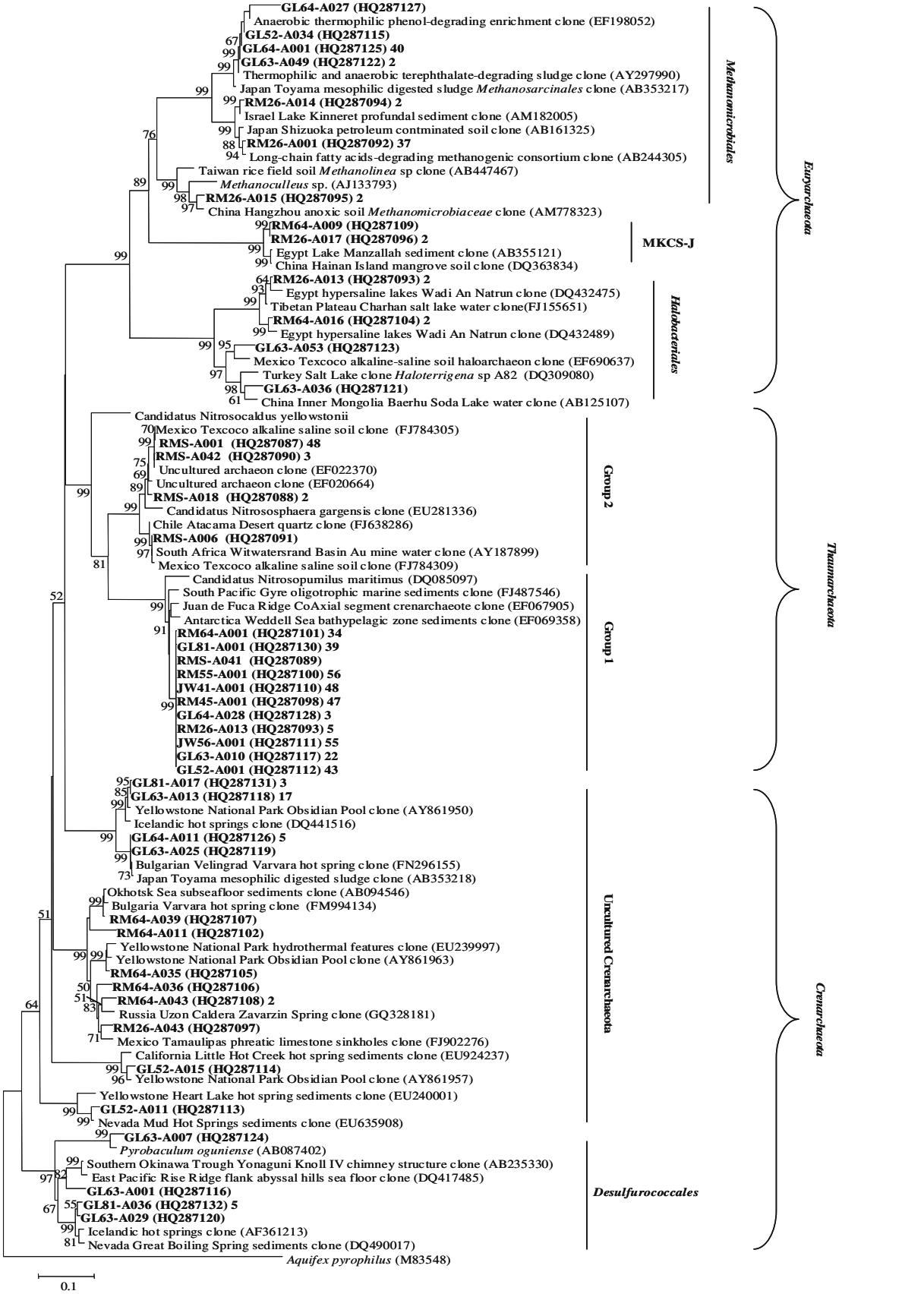
Fig. 3 Neighbor-joining tree (partial sequences, ~700 bp) showing the phylogenetic relationships of archaeal 16S rRNA gene sequences cloned from hot spring samples on the Tibetan Plateau to closely related sequences from the GenBank database. The same algorithms as those for the bacterial tree were used

isolated ammonia-oxidizing archaea (Fig. 3): Candidatus *Nitrosopumilus maritimus* (Konneke et al. 2005) and Candidatus *Nitrososphaera gargensis* (Hatzenpichler et al. 2008). These sequences can be divided into two subgroups: Group 1 and Group 2. Group 1 included all the clone sequences retrieved from hot springs in this study, and Group 2 included the sequences from the soil sample (RMS) (Fig. 3; Table 3).

The euryarchaeotal sequences included three subgroups: *Methanomicrobiales*, MKCS-J (Yan et al. 2006), and *Halobacteriales*; while the crenarchaeotal sequences consisted of *Thermoproteales*, *Desulfurococcales* and uncultured *Crenarchaeota* (Fig. 3; Table 3). *Methanomicrobiales* accounted for 17% (85 out of 489) of all archaeal clone sequences (Table 3). In the *Methanomicrobiales* group, 44 clone sequences (1, 2, and 41 from GL52, GL63, and GL64, respectively) were closely related (97–99%) to archaeal clones (EF198052 and AY297990) retrieved from thermal origins (Chen et al. 2004, 2008). Thirty-five clone sequences were closely related (95–99%) to uncultured crenarchaeal clones retrieved from hot spring environments, such as Obsidian Pool in Yellowstone National Park (Spear et al. 2005), hot springs in Bulgarian (Tomova et al. 2010), Iceland, and Russia (Reigstad et al. 2008). *Desulfurococcales* contained seven clone sequences from GL63 and GL81 (Table 3; Fig. 3) and were closely related (95–98%) to clones originated from such hot springs as Iceland hot springs and Nevada Great Boiling Spring (Marteinsson et al. 2001; Costa et al. 2009).

Statistical analysis

At the 97% OTU cutoff value, bacteria were more diverse than archaea (Table 1). The LIBSHUFF analysis showed that the Rongma soil (RMS) was statistically ($P < 0.05$) different from the hot spring samples with respect to bacterial and archaeal communities (Fig. 4). There appeared to be some site-specific grouping. For example, the bacterial sequences from Gulu appeared to be clustered together, and all archaeal sequences from Rongma formed a distinct group which was different from clusters of the Gulu sequences (Fig. 4). The Mantel test showed that the temperature was not statistically correlated with the microbial diversity at either the OTU ($r = 0.241$ and $P = 0.093$ for bacteria, and $r = -0.046$ and $P = 0.446$ for archaea) or the major group levels ($r = 0.016$ and $P = 0.378$ for bacteria, and $r = 0.226$ and $P = 0.119$ for archaea).


GL64-A027 (HQ287127)
Anaerobic thermophilic phenol-degrading enrichment clone (EF198052)
GL52-A034 (HQ287115)
GL64-A001 (HQ287125) 40
GL63-A049 (HQ287122) 2
Thermophilic and anaerobic terephthalate-degrading sludge clone (AY297990)
Japan Toyama mesophilic digested sludge Methanosarcinales clone (AB353217)
RM26-A014 (HQ287094) 2
Israel Lake Kinneret profundal sediment clone (AM182005)
Japan Shizuoka petroleum contminated soil clone (AB161325)
RM26-A001 (HQ287092) 37
Long-chain fatty acids-degrading methanogenic consortium clone (AB244305)
Taiwan rice field soil Methanolinea sp clone (AB447467)
Methanoculleus sp. (AJ133793)
RM26-A015 (HQ287095) 2
China Hangzhou anoxic soil Methanomicrobiaceae clone (AM778323)
Methanomicrobiales
RM64-A009 (HQ287109)
RM26-A017 (HQ287096) 2
Egypt Lake Manzallah sediment clone (AB355121)
China Hainan Island mangrove soil clone (DQ363834)
MKCS-J
RM26-A013 (HQ287093) 2
Egypt hypersaline lakes Wadi An Natrun clone (DQ432475)
Tibetan Plateau Charhan salt lake water clone(FJ155651)
RM64-A016 (HQ287104) 2
Egypt hypersaline lakes Wadi An Natrun clone (DQ432489)
GL63-A053 (HQ287123)
Mexico Texcoco alkaline-saline soil haloarchaeon clone (EF690637)
Turkey Salt Lake clone Haloterrigena sp A82 (DQ309080)
GL63-A036 (HQ287121)
China Inner Mongolia Baerhu Soda Lake water clone (AB125107)
Halobacteriales
Euryarchaeota
Candidatus Nitrosocaldus yellowstonii
Mexico Texcoco alkaline saline soil clone (FJ784305)
RMS-A001 (HQ287087) 48
RMS-A042 (HQ287090) 3
Uncultured archaeon clone (EF022370)
Uncultured archaeon clone (EF020664)
RMS-A018 (HQ287088) 2
Candidatus Nitrososphaera gargensis clone (EU281336)
Chile Atacama Desert quartz clone (FJ638286)
RMS-A006 (HQ287091)
South Africa Witwatersrand Basin Au mine water clone (AY187899)
Mexico Texcoco alkaline saline soil clone (FJ784309)
Group 2
Candidatus Nitrosopumilus maritimus (DQ085097)
South Pacific Gyre oligotrophic marine sediments clone (FJ487546)
Juan de Fuca Ridge CoAxial segment crenarchaeote clone (EF067905)
Antarctica Weddell Sea bathypelagic zone sediments clone (EF069358)
RM64-A001 (HQ287101) 34
GL81-A001 (HQ287130) 39
RMS-A041 (HQ287089)
RM55-A001 (HQ287100) 56
JW41-A001 (HQ287110) 48
RM45-A001 (HQ287098) 47
GL64-A028 (HQ287128) 3
RM26-A013 (HQ287093) 5
JW56-A001 (HQ287111) 55
GL63-A010 (HQ287117) 22
GL52-A001 (HQ287112) 43
Group 1
Thaumarchaeota
GL81-A017 (HQ287131) 3
GL63-A013 (HQ287118) 17
Yellowstone National Park Obsidian Pool clone (AY861950)
Icelandic hot springs clone (DQ441516)
GL64-A011 (HQ287126) 5
GL63-A025 (HQ287119)
Bulgarian Velingrad Varvara hot spring clone (FN296155)
Japan Toyama mesophilic digested sludge clone (AB353218)
Okhotsk Sea subseafloor sediments clone (AB094546)
Bulgaria Varvara hot spring clone (FM994134)
RM64-A039 (HQ287107)
RM64-A011 (HQ287102)
Yellowstone National Park hydrothermal features clone (EU239997)
Yellowstone National Park Obsidian Pool clone (AY861963)
RM64-A035 (HQ287105)
RM64-A036 (HQ287106)
RM64-A043 (HQ287108) 2
Russia Uzon Caldera Zavarzin Spring clone (GQ328181)
RM26-A043 (HQ287097)
Mexico Tamaulipas phreatic limestone sinkholes clone (FJ902276)
California Little Hot Creek hot spring sediments clone (EU924237)
GL52-A015 (HQ287114)
Yellowstone National Park Obsidian Pool clone (AY861957)
Uncultured Crenarchaeota
Yellowstone Heart Lake hot spring sediments clone (EU240001)
GL52-A011 (HQ287113)
Nevada Mud Hot Springs sediments clone (EU635908)
GL63-A007 (HQ287124)
Pyrobaculum oguniense (AB087402)
Southern Okinawa Trough Yonaguni Knoll IV chimney structure clone (AB235330)
East Pacific Rise Ridge flank abyssal hills sea floor clone (DQ417485)
GL63-A001 (HQ287116)
GL81-A036 (HQ287132) 5
GL63-A029 (HQ287120)
Icelandic hot springs clone (AF361213)
Nevada Great Boiling Spring sediments clone (DQ490017)
Desulfurococcales
Crenarchaeota
Aquifex pyrophilus (M83548)
0.1

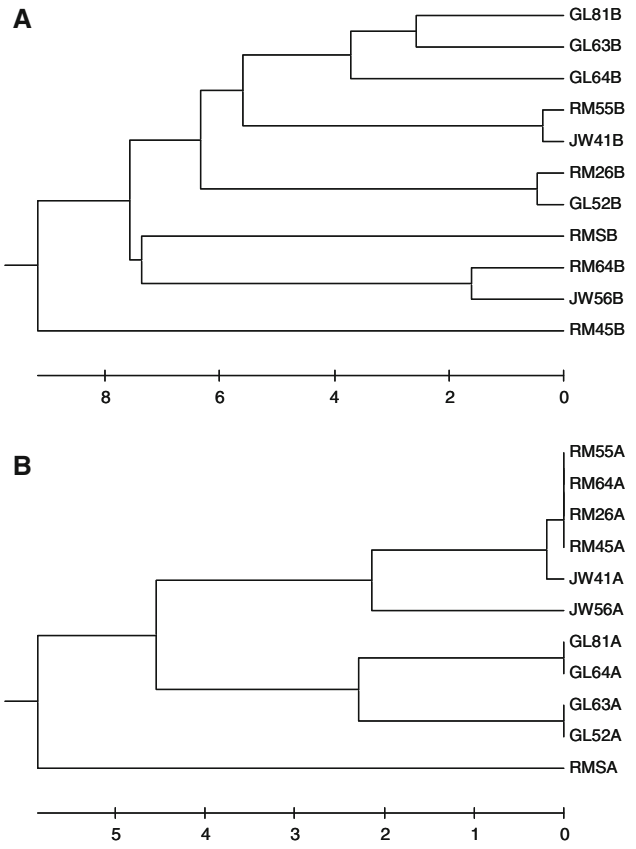


Fig. 4 **a** Clustering of the different bacterial 16S rRNA gene clone libraries based on ΔCxy values obtained from the LIBSHUFF analysis. Unweighted-pair group method with average linkages in MEGA4.1 was used to construct the tree. The parameter ΔCxy in the LIBSHUFF represents the difference in coverage of two clone libraries (the larger ΔCxy, the greater dissimilarity between the given clone libraries). Clone libraries are coded as follows for the example of GL81B/A: GL, sample site, Gulu; 81, temperature in Celsius; B, bacterium; A, archaea. **b** The UniFrac metric tree showing the difference of archaeal 16S rRNA gene clone libraries from ten Tibetan hot springs. The software for the analysis was available at http://whitman.myweb.uga.edu/libshuff.html

Discussion

Microbial diversity in Tibetan hot springs

The phylogenetic analysis showed that the bacterial communities were highly diverse and the predominant groups (*Proteobacteria*, *Firmicutes*, *Chloroflexi*, and *Cyanobacteria*) were similar to those in hot springs from the Central Tibet (Lau et al. 2006, 2009) and from low-elevation and neutral-pH environments (Meyer-Dombard et al. 2005; Spear et al. 2005; Stout et al. 2009; Sayeh et al. 2010). This observation suggested that elevation was not a major factor influencing the bacterial distribution in global geothermal features.

The archaeal communities were less diverse than bacterial (as indicated by number of OTU and diversity indices in Tables 2 and 3) but more so than the archaeal community observed in hot springs of Central Tibet observed in a previous study (Lau et al. 2006). One possible reason may be ascribed to the different techniques employed in the two studies: Lau and colleagues used the technique of 16S rDNA denaturing gradient gel electrophoresis (DGGE); instead, we used 16S rDNA cloning technique. These two techniques have different resolutions when used in characterizing microbial communities (Kisand and Wikner 2003).

The archaeal communities in many Tibetan hot springs studied here were dominated with Thaumarchaeota, showing the uniqueness of Tibetan hot springs relative to other springs. This result is different from those of Lau et al. (2006) who did not observe any thaumarchaeotal sequences in hot springs from Tibet. There are several possible reasons for such inconsistency. First, Thaumarchaeota is a newly proposed phylum (Spang et al. 2010) and it was previously considered as part of Crenarchaeota. Thus, it is conceivable that some of crenarchaeotal sequences reported in the Lau et al. study (2006) may have belonged to Thaumarchaeota. However, a close examination revealed that none of the organisms reported by Lau et al. (2006) belonged to Thaumarchaeota. Second, the sampling sites in the Lau et al. (2006) study are different from those in this study, although they are all in Central Tibet. It is possible that archaeal community may be different between different sites. Indeed, our data (Table 3) showed site-to-site variations in archaeal community structure. Third, the inconsistency between our results and those of Lau et al. (2006) may have been caused by different primer sets used. Whereas archaeal primers Arch21F/Univ958R were used in this study, the Archaea 344F/905R primers were used in the Lau et al. (2006) study. Although both sets of primers have been used for hot spring samples (Pearson et al. 2004; Meyer-Dombard et al. 2005), it is not clear which set is superior in terms of covering archaeal diversity in Tibetan hot springs. In addition, the dominance of the Thaumarchaeotal sequences in our samples might conceivably be caused by PCR-cloning bias. However, the Thaumarchaeotal sequences formed sample-specific grouping: all hot spring sequences were clustered within Group 1, and those from the soil sample (RMS) were grouped within Group 2. These results suggested that the dominance of the Thaumarchaeotal sequences in the Tibetan hot springs studied in this research should be a true reflection of the archaeal community composition.

To date, the dominance of the Thaumarchaeota sequences in Tibetan hot spring springs has never been observed. A previous study reported that the Thaumarchaeota phylum only contains ammonia oxidizers *Nitrosopumilus maritimus*, *Nitrososphaera gargensis*, and *Cenarchaeum*

symbiosum and clone sequences of putative ammonia-oxidizing isolates (Spang et al. 2010). Thus, the dominance of the Thaumarchaeota suggested that archaeal ammonia oxidation may be a key element-cycling process in the Tibetan hot springs. However, future work is necessary to confirm the functional traits of the Thaumarchaeota.

Response of microbial diversity to temperature in the Tibetan hot springs

Previous studies have shown that diverse microbial communities can inhabit global hot springs of a wide temperature range (from ambient to boiling) (Barns et al. 1994; Pace 1997; Bonch-Osmolovskaya et al. 1999; Marteinsson et al. 2001; Kumar et al. 2004; Meyer-Dombard et al. 2005; Lau et al. 2006, 2009; Hall et al. 2008; Aditiawati et al. 2009; Mitchell 2009; Reigstad et al. 2009; Song et al. 2009, 2010; Aguilera et al. 2010; Jiang et al. 2010; Sayeh et al. 2010; Vick et al. 2010) and that microbial diversity did not show a monotonic response to thermal stress (Lau et al. 2006; Mitchell 2009). Statistical analysis in this study also confirmed that there was no monotonic relationship between microbial diversity and temperature in Tibetan hot springs. Moreover, certain clone sequences from the Tibetan hot springs were even related to those of low-temperature origin (e.g., soil, lacustrine/marine sediments) (Figs. 2, 3). This was especially true for the spring of the highest temperature, i.e., GL81 (81°C), where its microbial community was dominated by apparently mesophilic Thaumarchaeota and gammaproteobacteria. This inconsistency has been observed in an actinobacterial study in hot springs (Song et al. 2009), and it could be ascribed to two reasons: One explanation may be due to potential contamination from sympatric soils. However, the LIBSHUFF analysis showed that the microbial communities in the investigated hot springs were distinctly different ($P < 0.05$) from that in the sympatric soil sample (Fig. 4). This distinct separation suggested that the bacterial and archaeal 16S rRNA gene clone sequences of the hot springs were compositionally different from those retrieved in the sympatric soil, and thus potential contamination from surrounding soil can be ruled out. The other possible reason may be that microorganisms with a significant level of 16S rRNA gene sequences similarity may have distinct physiological properties (Jaspers and Overmann 2004). The calculated G + C contents of the partial archaeal 16S rRNA gene clone sequences for all samples showed an approximate positive correlation with the measured temperatures of the springs from which the mat or sediment samples were collected (graph not shown), as consistent with a previous report (Kimura et al. 2006). This approximate correlation suggests that the thermophilic nature of clones from the high-temperature spring (GL81) cannot be completely excluded. Apparent "mesophilic" Thaumarchaeota and gammaproteobacteria might actually contain some thermophilic members.

Among other factors that may be important in controlling microbial distribution, mineralogy and site isolation appear to have some influence. There were some important differences in mineralogy among the sites. For example, spring mats or sediments from Rongma were dominated by carbonates (calcite), but those from Gulu were mostly composed of quartz and feldspars (Table 1). Whereas it was possible that the distinct differences in microbial composition between these two sites (Fig. 4) may be related to mineralogy, it is equally possible that this site-specific difference may also be due to the distance effect. Definitive resolution of these various controlling factors must await future investigations, where a much larger set of both geochemical and molecular data becomes available.

Conclusions

The bacterial communities in the studied Tibetan hot springs were predominated by *Firmicutes*, *Proteobacteria*, *Cyanobacteria*, and *Chloroflexi*, which was similar to other hot springs reported in literature. In comparison, archaea were less diverse than bacteria. The archaeal communities of the studied Tibetan hot springs mainly consisted of Thaumarchaeota clone sequences, which have never been reported to be a dominant group in any other hot springs worldwide. Statistical analysis confirmed that temperature was not significantly correlated with the microbial diversity, suggesting that other factors, such as mineralogy and distance, may be important in controlling microbial distribution in the Tibetan hot springs.

Acknowledgments This research was supported by grants from the National Science Foundation of the United States (OISE 0968421) and China (40972211, 41030211, and 41002123) and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry, the Fundamental Research Funds for the Central Universities (2010ZY16 and 2011YXL03). Three anonymous reviewers are acknowledged for their constructive comments which greatly improved the quality of this manuscript.

References

Aditiawati P, Yohandini H, Madayanti F, Akhmaloka (2009) Microbial diversity of acidic hot spring (kawah hujan B) in geothermal field of kamojang area, west Java-Indonesia. Open Microbiol J 3:58–66

Aguilera A, Souza-Egipsy V, Gonzalez-Toril E, Rendueles O, Amils R (2010) Eukaryotic microbial diversity of phototrophic microbial mats in two Icelandic geothermal hot springs. Int Microbiol 13:21–32

Allewalt JP, Bateson MM, Revsbech NP, Slack K, Ward DM (2006) Effect of temperature and light on growth of and photosynthesis by *Synechococcus* isolates typical of those predominating in the

Octopus Spring microbial mat community of Yellowstone National Park. Appl Environ Microbiol 72:544–550
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
Bonch-Osmolovskaya EA, Miroshnichenko ML, Slobodkin AI, Sokolova TG, Karpov GA, Kostrikina NA, Zavarzina DG, Prokof'eva MI, Rusanov II, Pimenov NV (1999) Biodiversity of anaerobic lithotrophic prokaryotes in terrestrial hot springs of Kamchatka. Microbiology 68:343–351
Boomer SM, Noll KL, Geesey GG, Dutton BE (2009) Formation of multilayered photosynthetic biofilms in an alkaline thermal spring in Yellowstone National Park, Wyoming. Appl Environ Microbiol 75:2464–2475
Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P (2008) Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nat Rev Microbiol 6:245–252
Chen C-L, Macarie H, Ramirez I, Olmos A, Ong SL, Monroy O, Liu W-T (2004) Microbial community structure in a thermophilic anaerobic hybrid reactor degrading terephthalate. Microbiology 150:3429–3440
Chen CL, Wu JH, Liu WT (2008) Identification of important microbial populations in the mesophilic and thermophilic phenol-degrading methanogenic consortia. Water Res 42:1963–1976
Costa KC, Navarro JB, Shock EL, Zhang CL, Soukup D, Hedlund BP (2009) Microbiology and geochemistry of great boiling and mud hot springs in the United States Great Basin. Extremophiles 13:447–459
Hall JR, Mitchell KR, Jackson-Weaver O, Kooser AS, Cron BR, Crossey LJ, Takacs-Vesbach CD (2008) Molecular characterization of the diversity and distribution of a thermal spring microbial community by using rRNA and metabolic genes. Appl Environ Microbiol 74:4910–4922
Hatzenpichler R, Lebedeva EV, Spieck E, Stoecker K, Richter A, Daims H, Wagner M (2008) A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. Proc Natl Acad Sci USA 105:2134–2139
Hu X, Sun J, Yao Z, Rong F (2003) Research on the influence of geothermal activities and exploitation to geological environment in Tibet (in Chinese). J Mt Res 21:45–48
Huber T, Faulkner G, Hugenholtz P (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20:2317–2319
Hugenholtz P, Pitulle C, Hershberger KL, Pace NR (1998) Novel division level bacterial diversity in a Yellowstone hot spring. J Bacteriol 180:366–376
Jaspers E, Overmann J (2004) Ecological significance of microdiversity: identical 16S rRNA gene sequences can be found in Bacteria with highly divergent genomes and ecophysiologies. Appl Environ Microbiol 70:4831–4839
Jiang H, Dong H, Yu B, Ye Q, Shen J, Rowe H, Zhang C (2008) Dominance of putative marine benthic archaea in Qinghai Lake, northwestern China. Environ Microbiol 10:2355–2367
Jiang H, Dong H, Deng S, Yu B, Huang Q, Wu Q (2009) Response of archaeal community structure to environmental changes in lakes on the Tibetan Plateau, northwestern China. Geomicrobiol J 26:289–297
Jiang H, Huang Q, Dong H, Wang P, Wang F, Li W, Zhang C (2010) RNA-based investigation of ammonia-oxidizing archaea in hot springs of Yunnan Province, China. Appl Environ Microbiol 76:4538–4541
Joshi AA, Kanekar PP, Kelkar AS, Shouche YS, Vani AA, Borgave SB, Sarnaik SS (2008) Cultivable bacterial diversity of alkaline Lonar Lake, India. Microb Ecol 55:163–172
Kanokratana P, Chanapan S, Pootanakit K, Eurwilaichitr L (2004) Diversity and abundance of Bacteria and Archaea in the Bor Khlueng Hot Spring in Thailand. J Basic Microbiol 44:430–444
Kimura H, Sugihara M, Kato K, Hanada S (2006) Selective phylogenetic analysis targeted at 16S rRNA genes of thermophiles and hyperthermophiles in deep-subsurface geothermal environments. Appl Environ Microbiol 72:21–27
Kimura H, Mori K, Nashimoto H, Hattori S, Yamada K, Koba K, Yoshida N, Kato K (2010) Biomass production and energy source of thermophiles in a Japanese alkaline geothermal pool. Environ Microbiol 12:480–489
Kisand V, Wikner J (2003) Limited resolution of 16S rDNA DGGE caused by melting properties and closely related DNA sequences. J Microbiol Methods 54:183–191
Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546
Kumar B, Trivedi P, Mishra AK, Pandey A, Palni LMS (2004) Microbial diversity of soil from two hot springs in Uttaranchal Himalaya. Microbiol Res 159:141–146
Lau MCY, Jing H, Aitchison JC, Pointing SB (2006) Highly diverse community structure in a remote central Tibetan geothermal spring does not display monotonic variation to thermal stress. FEMS Microbiol Ecol 57:80–91
Lau MCY, Aitchison JC, Pointing SB (2009) Bacterial community composition in thermophilic microbial mats from five hot springs in central Tibet. Extremophiles 13:139–149
Marteinsson VT, Hauksdottir S, Hobel CFV, Kristmannsdottir H, Hreggvidsson GO, Kristjansson JK (2001) Phylogenetic diversity analysis of subterranean hot springs in Iceland. Appl Environ Microbiol 67:4242–4248
Meyer-Dombard DR, Shock EL, Amend JP (2005) Archaeal and bacterial communities in geochemically diverse hot springs of Yellowstone National Park, USA. Geobiology 3:211–227
Mitchell KR (2009) Controls on microbial community structure in thermal environments: exploring bacterial diversity and the relative influence of geochemistry and geography. Dissertation, University of New Mexico. http://hdl.handle.net/1928/9315
Mitchell KR, Takacs-Vesbach CD (2008) A comparison of methods for total community DNA preservation and extraction from various thermal environments. J Ind Microbiol Biotechnol 35:1139–1147
Mori K, Suzuki K-i (2008) *Thiofaba tepidiphila* gen. nov., sp. nov., a novel obligately chemolithoautotrophic, sulfur-oxidizing bacterium of the Gammaproteobacteria isolated from a hot spring. Int J Syst Evol Microbiol 58:1885–1891
Pace NR (1997) A Molecular View of Science 276:734–740
Pearson A, Huang Z, Ingalls AE, Romanek CS, Wiegel J, Freeman KH, Smittenberg RH, Zhang CL (2004) Nonmarine crenarchaeol in Nevada hot springs. Appl Environ Microbiol 70:5229–5237
Portillo MC, Sririn V, Kanoksilapatham W, Gonzalez JM (2009) Differential microbial communities in hot spring mats from Western Thailand. Extremophiles 13:321–331
Purcell D, Sompong U, Lau CY, Barraclough TG, Peerapornpisal Y, Pointing SB (2007) The effects of temperature, pH and sulphide on the community structure of hyperthermophilic streamers in hot springs of northern Thailand. FEMS Microbiol Ecol 60(3):456–466
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 (2009) Diversity and abundance of Korarchaeota in terrestrial hot springs of Iceland and Kamchatka. ISME J 4:346–356
Sayeh R, Birrien JL, Alain K, Barbier G, Hamdi M, Prieur D (2010) Microbial diversity in Tunisian geothermal springs as detected by molecular and culture-based approaches. Extremophiles 14:501–514

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71:1501–1506

Song Z, Jiang H, Zhi X, Zhang C, Dong H, Li W (2009) Actinobacterial diversity in hot springs in Tengchong (China), Kamchatka (Russia), and Nevada (USA). Geomicrobiol J 26:256–263

Song Z-Q, Chen J-Q, Jiang H-C, Zhou E-M, Tang S-K, Zhi X-Y, Zhang L-X, Zhang C-LL, Li W-J (2010) Diversity of Crenarchaeota in terrestrial hot springs in Tengchong, China. Extremophiles 14:287–296

Spang A, Hatzenpichler R, Brochier-Armanet C, Rattei T, Tischler P, Spieck E, Streit W, Stahl DA, Wagner M, Schleper C (2010) Distinct gene set in two different lineages of ammonia-oxidizing archaea supports the phylum *Thaumarchaeota*. Trends Microbiol 18:331–340

Spear JR, Walker JJ, McCollom TM (2005) Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. Proc Natl Acad Sci USA 102:2555–2560

Stout LM, Blake RE, Greenwood JP, Martini AM, Rose EC (2009) Microbial diversity of boron-rich volcanic hot springs of St. Lucia, Lesser Antilles. FEMS Microbiol Ecol 70:402–412

Tomova I, Stoilova-Disheva M, Lyutskanova D, Pascual J, Petrov P, Kambourova M (2010) Phylogenetic analysis of the bacterial community in a geothermal spring, Rupi Basin, Bulgaria. World J Microbiol Biotechnol 26:2019–2028

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

Ward D, Castenholz R (2002) Cyanobacteria in geothermal habitats. In: Whitton B, Potts M (eds) The ecology of Cyanobacteria. Springer, Netherlands, pp 37–59

Whitaker RJ, Grogan DW, Taylor JW (2003) Geographic barriers isolate endemic populations of hyperthermophilic Archaea. Science 301(5635):976–978

Wu X-Y, Zheng G, Zhang W–W, Xu X-W, Wu M, Zhu X-F (2010) *Amphibacillus jilinensis* sp. nov., a facultatively anaerobic, alkaliphilic bacillus from a soda lake. Int J Syst Evol Microbiol 60:2540–2543

Yan B, Hong K, Yu ZM (2006) Archaeal communities in mangrove soil characterized by 16S rRNAgene clones. J Microbiol 44:566–571

Zhang G, Dong H, Xu Z, Zhao D, Zhang C (2005) Microbial diversity in ultra-high-pressure rocks and fluids from the Chinese continental scientific drilling project in China. Appl Environ Microbiol 71:3213–3227

Zhang CL, Ye Q, Huang Z, Li W, Chen J, Song Z, Zhao W, Bagwell C, Inskeep WP, Ross C, Gao L, Wiegel J, Romanek CS, Shock EL, Hedlund BP (2008) Global occurrence of archaeal amoA genes in terrestrial hot springs. Appl Environ Microbiol 74:6417–6426