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ORIGINAL PAPER

Diversity of Crenarchaeota in terrestrial hot springs in Tengchong, China

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Abstract Diversity of Crenarchaeota was investigated in eight terrestrial hot springs (pH 2.8–7.7; temperature 44–96°C) located in Tengchong, China, using 16S rRNA gene phylogenetic analysis. A total of 826 crenarchaeotal clones were sequenced and a total of 47 operational taxonomic units (OTUs) were identified. Most (93%) of the identified OTUs were closely related (89–99%) to those retrieved from hot springs and other thermal environments. Our data showed that temperature may predominate over pH in affecting crenarchaeotal diversity in Tengchong hot springs. Crenarchaeotal diversity in moderate-temperature (59–77°C) hot springs was the highest, indicating that the moderately hot-temperature springs may provide optimal conditions for speciation of Crenarchaeota.

Keywords Crenarchaeota · Diversity · Terrestrial hot springs · Tengchong

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Introduction

Archaea are divided into Euryarchaeota, Crenarchaeota, Korarchaeota and Nanoarchaeota (Dawson et al. 2006). Numerous studies have been performed to investigate the diversity of Euryarchaeota and Crenarchaeota in different environments (see review by Schleper et al. 2005; Zhang et al. 2010). The cultivated euryarchaeotes consist of diverse mesophilic and thermophilic anaerobes and halophiles, whereas the cultivated crenarchaeotal members mainly consist of hyperthermophilic sulfur-dependent thermophilic species (Huber et al. 2006) and recently retrieved ammonia-oxidizing archaeal strains (Könneke et al. 2005; de la Torre et al. 2008). The phylum Crenarchaeota represents one of the deep-branching phylogenetic lineages within prokaryotes (Woese et al. 1990; Winker and Woese 1991). Since 1990s, multiple studies using culture-independent approaches have shown the ubiquity of the crenarchaeotes in oceans (DeLong 1992; Fuhrman et al. 1992), lakes (Schleper et al. 1997), soils (Ochsenreiter et al. 2003), and other low temperature environments. Recently, mesophilic Crenarchaeota are named Thaumarchaeota based on the genome sequence of *Cenarchaeum symbiosum* which was discovered in marine environment (Brochier-Armanet et al. 2008). The majority of known species of hyperthermophilic Crenarchaeota grow optimally at temperatures ≥80°C (Stetter 1996; Boone and Castenholz 2001). Most of them are able to

grow chemolithoautotrophically on H_2, S, or H_2S, using O_2, NO_3^-, S, or Fe^{3+} as electron acceptors (Stetter 1996; Spear et al. 2005; Dawson et al. 2006), the compounds of which are widely present in hot springs.

Hot springs are a type of extreme environments widely distributed all over the world, and they are unique with respect to physical, chemical and geographical characteristics (Hugenholtz et al. 1999; Marteinsson et al. 2001; Kvist et al. 2005; Spear et al. 2005). Multiple studies have shown that hot springs harbor very diverse creanarchaeotal populations (Takai and Sako 1999; Jackson et al. 2001; Kanokratana et al. 2004; Kvist et al. 2005; Meyer-Dombard et al. 2005; Skirnisdottir et al. 2000; Spear et al. 2005; Huang et al. 2007; Costa et al. 2009; Vick et al. 2010). Recent molecular studies showed that hot springs of moderate temperature (55–70 or 65–70°C) possess the highest bacterial and archaeal diversity (Lau et al. 2006). This observation is in contrary with the general ecological principle that more extreme environments decrease diversity (Frontier 1985; Hacine et al. 2004). Is the finding of Lau and colleagues applied to other geothermal features in the world? If yes, could the temperature range be wider for hot springs having the highest crenarchaeotal diversity?

To answer the above questions, we investigated the crenarchaeotal diversity in hot springs in Tengchong, China with the use of crenarchaeotal 16S rRNA gene phylogenetic analysis. The results showed that temperature predominated over pH in affecting crenarchaeotal diversity in the investigated hot springs, and the crenarchaeotal diversity in moderate-temperature (59–77°C) hot springs was the highest.

Materials and methods

Site description and sample collection

Tengchong (in western Yunnan Province, China) is located on the northeastern edge of Tibet–Yunnan geothermal zone between the Indian and Eurasian plates, one of the most active geothermal areas in the world. Tengchong hot springs possess a variety of hydrothermal phenomena, such as hydrothermal explosions, geysers, fumaroles, boiling springs and hot springs (Kearey and Wei 1993).

The investigated hot springs in this study are located in an area of one square kilometer or so in Tengchong National Geological Park (N 24°57′, E 98°26′). Surface sediment or mat material was collected into sterile 50-mL Falcon conical tubes at each hot spring. A total of eight samples were collected from different hot springs with different characteristic of pH and temperature. All samples were stored in dry ice in the field and during transportation, and then at −80°C in the laboratory until further analysis. At each sampling location, pH and temperature were measured using a Hach® pH-meter equipped with a pH/temperature probe.

DNA extraction

Total DNA was extracted from 5 to 10 g of each sample using an UltraClean Mega DNA soil kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The precipitated DNA was gel-purified using Agarose Gel DNA Fragment Recovery Kit Ver.2.0 (TaKaRa, Dalian, China).

Construction of crenarchaeotal 16S rRNA gene clone libraries

Purified DNA was used as the template for the amplification of crenarchaeotal 16S rRNA genes by specific primers Cren7F: 5′-TTC CGG TTG ATC CYG CCG GAC C-3′ and Cren518R: 5′-GCT GGT WTT ACC GCG GCG GCT GA-3′ (Perevalova et al. 2008). PCR amplification consisted of an initial denaturation at 95°C for 10 min, followed by denaturation at 95°C for 45 s, annealing at 72°C for 2 min; 30 cycles, with the last cycle being followed by a final extension at 72°C for 20 min. Individual reagents and their concentrations were as follows: 1× PCR buffer with 1.5 mM Mg^{2+}, dNTPs (100 μM each), 0.25 μM each primer, 2.5 U of DNA polymerase (Ex-Taq) (TaKaRa, Dalian, China), and ~50 ng of total DNA. PCR products were purified using an Agarose Gel DNA Fragment Recovery Kit Ver.2.0 (TaKaRa, Dalian, China) according to the manufacturer's instructions.

Purified PCR products were ligated into pMD19-T Vector system (TaKaRa, Dalian, China) and transformed into competent *Escherichia coli* JM109 cells according to the manufacturer's instructions. Eight clone libraries were constructed, one for each of the eight hot spring samples in Tengchong. 70 to 160 positive clones per library were randomly selected and plasmid DNA was purified using DNA Fragment Purification Kit Ver.2.0 (TaKaRa, Dalian, China). Restriction fragment length polymorphism (RFLP) analysis was performed on the randomly selected clones in each clone library according to procedures described elsewhere (Song et al. 2009). Unique RFLP patterns were identified visually and representatives of each RFLP were selected for 16S rRNA gene phylogenetic analysis.

Phylogenetic analysis

Clones representing all identified RFLP types were sequenced using primer M13+ on an ABI 3100 automated sequencer. The sequences were edited using the DNASTAR program v.5.0. The potential presence of chimeric sequences was examined with Bellerophon (Huber et al. 2004).

The secondary-structures of all obtained sequences were analyzed using the Vienna RNA Package (Hofacker 2003). Potential chimeric sequences were removed. Operational taxonomic units (OTUs) were identified using DOTUR 1.53 (Schloss and Handelsman 2005). The sequences of ≥98% identity were clustered into one OTU. One sequence was selected from each OTU for phylogenetic analyses. The representative sequences of each OTU and selected closest references (GenBank:http://www.ncbi.nlm.nih.gov) were pooled and aligned using CLUSTALX1.83. Phylogenetic analysis was performed using distance-based Neighbor-Joining method with MEGA version 4.0 (Tamura et al. 2007). Bootstrap analysis was performed using 1000 pseudoreplications.

Statistical analyses

Species richness was calculated for each of the hot springs using the nonparametric estimators ACE (abundance-based coverage estimator). Coverage was calculated with the equation $C = 1 - n/N$, where n is the number of unique OTU sequences observed and N is the number of total OTUs. In addition, a rarefaction analysis of the number of unique OTUs versus the estimated OTUs was performed with the use of Rarefaction version 1.3 (http://www.uga.edu/strata/software/Software.html). The F statistical analysis was calculated for assessing the degree of differentiation between microbial communities and comparing the phylogenetic diversity of each community with the total phylogenetic diversity of the combined communities. F is defined by the equation $F_{ST} = (\theta_T - \theta_W)/\theta_T$, where θ_T is the phylogenetic diversity of the two communities combined and θ_W is the phylogenetic diversity of each community (Martin 2002; Whitaker et al. 2003; Mathur et al. 2007). Linear regression analyses were performed to evaluate the correlation between the F_{st} and temperature/pH distances using SYSTAT SigmaPlot.v10.0 package (Version 10.0).

Nucleotide sequence accession numbers

The sequences obtained in this study were deposited in the GenBank database under accession numbers: GU075731–GU075793.

Results

Characteristics of sampling sites

The eight investigated hot springs in Tengchong possess temperature ranging from 44 to 96°C with a pH gradient of 2.8–7.7 (Table 1).

Table 1 Description of hot spring samples investigated in this study

Sample	Sample type	Temp (°C)	pH	Total dissolved solids (TOD) (mg/L)
Peal spring (PLS)	Brown sandy sediment	96	4.3	245
Great boiling pot A (GBA)	Ashen geyserite	84	6.6	2070
Wuming spring (WMS)	Gray sediment	77	7.7	ND
Huangguanqing spring (HGS)	Sandy sediment	74	2.8	ND
Bridge spring A (BSA)	Black mat	59	7.5	656
Shuirebaozhaqu (SRQ)	Green sediment	45	7.5	ND
Great boiling pot F (GBF)	Red sediment	44	3.4	ND
Bridge spring B (BSB)	Green mat	44	7.5	650

ND not determined

Table 2 Diversity indices of crenarchaeotal 16S rRNA gene clone libraries retrieved from eight hot springs in Tengchong, Yuanan, China

Community	No. clones	No. OTUs	Richness (ACE)	C coverage (%)	Avg. BLAST identity for all phylotypes (%)[a]	Shannons index (Chao and Shen)	Simpson index (MLE)
PLS	75	4	4.0 (4.0, 4.0)	100	94	1.03 (0.86, 1.19)	0.433 (0.208, 0.658)
GBA	67	6	6.0 (6.0, 6.0)	100	95.5	1.44 (1.27, 1.61)	0.287 (0.146, 0.428)
WMS	148	15	15.5 (15.0, 20.5)	99.3	93.9	2.41 (2.23, 2.58)	0.116 (0.085, 0.148)
HGQ	154	10	10.0 (10.0,10.0)	100	94.6	2.11 (2.02, 2.20)	0.137 (0.107, 0.166)
BSA	149	11	11.5 (11.0, 17.5)	99.3	94.1	2.09 (1.87,2.31)	0.149 (0.101, 0.197)
SRQ	61	5	5.0 (5.0, 5.0)	100	96.2	1.34 (1.18, 1.50)	0.306 (0.180, 0.431)
BSB	84	6	6.0 (6.0, 6.0)	100	95.3	1.51 (1.36, 1.66)	0.266 (0.188, 0.345)
GBF	88	7	7.0 (7.0, 7.0)	100	97	1.64 (1.49, 1.80)	0.241 (0.170, 0.311)

[a] Average percentage similarity among all phylotypes from a given location with closest matches in the NCBI GenBank database

Table 3 Clones from investigated hot springs and closest relatives determined by DOTUR program and BLAST

OTU ID	PLS	GBA	WMS	HGS	BSA	SRQ	GBF	BSB	Closest relative (% 16Sr RNA gene identity)	GenBank accession no.
OTU 1	PLS9 (4)								Hot spring clone Hverd026A (93)	DQ441483
OTU 2	PLS21(9)								Hot spring clone Hverd026A (93)	DQ441483
OTU 3	PLS51 (28)								Hot spring clone Hverd026A (93)	DQ441483
OTU 4		GBA6 (3)							Hot spring HL1env.11(93)	EU239999
OTU 5			WMS1(4)						Hot spring clone SSW_L4_E05 (96)	EU635928
OTU 6			WMS5 (1)						Hot spring clone SSW_L5_H08 (94)	EU635922
OTU 7			WMS51 (2)						Hot spring clone YNP_BP_A49 (91)	DQ243731
OTU 8			WMS15 (7)						Hot spring clone YNP_ObP_A5 (97)	DQ243757
OTU 9			WMS60 (1)						Epithermal gold mine clone HAuD-LA30 (98)	AB113632
OTU 10			WMS38 (5)						Hot spring clone LHC4_L2_G04 (92)	EU635907
OTU 11			WMS11(18)						Hot spring clone YNP_BP_A60 (91)	DQ243732
OTU 12			WMS27 (5)						Hydrothermal fluids clone pYK04-13(91)	AB235330
OTU 13			WMS10(20)						Hydrothermal Archaea clone a87Y32 (91)	DQ417485
OTU 14			WMS64 (1)						Hot spring clone YNP_BP_A22 (89)	DQ228585
OTU 15			WMS3 (5)						Hot spring clone Hverd014N (94)	DQ441506
OTU 16				HGS14 (2)					Hot spring clone Hverd050N (94)	DQ441516
OTU 17				HGS17 (14)					Hot spring clone pJP 41 (96)	L25301
OTU 18				HGS29 (8)					Hydrothermal archaeota clone a87Y32 (92)	DQ417485
OTU 19				HGS61 (6)					Hydrothermal archaeota clone a87Y32 (90)	DQ417485
OTU 20				HGS5 (3)					Sulfide vent clone 4136-1-92 (93)	EU427996
OTU 21					BSA3 (2)				Geothermal water clone 10-H-08 (95)	AB201309
OTU 22					BSA8 (11)				Geothermal water clone 10-H-08 (96)	AB201309
OTU 23					BSA56 (22)				Epithermal gold mine clone HAuD-la42 (97)	AB113634
OTU 24					BSA29 (19)				Mangrove clone MKCSB-A3(90)	DQ363754
OTU 25					BSA65 (9)				Hydrothermal fluid clone (94)	AB301869
OTU 26					BSA63 (1)				*Sulfurous* freshwater clone 2C3 (89)	AJ937875
OTU 27					BSA76 (6)				Rhizosphere archaeota clone (92)	EF021134
OTU 28					BSA30 (18)				Rhizosphere archaeota clone (94)	EF021161
OTU 29						SRQ68 (3)			Lake sediment clone LCDARCH142 (98)	EU247289
OTU 30						SRQ46 (39)			Hot spring DGGE band BL1017 (90)	EU586818
OTU 31						SRQ62 (16)			Soil clone ArcC-s_cB07 (97)	EU307056
OTU 32							GBF17 (26)		Thermal soil clone YNPFFA108 (91)	AB072728
OTU 33							GBF42 (7)		*Metallosphaera* sp. J1 (90)	AF167083
OTU 34							GBF2 (9)		Epithermal gold mine clone (97)	AB072728
OTU 35							GBF9 (13)		Thermal soil clone YNPFFA108 (97)	AF391993

Table 3 continued

OTU ID	PLS	GBA	WMS	HGS	BSA	SRQ	GBF	BSB	Closest relative (% 16Sr RNA gene identity)	GenBank accession no.
OTU 36								BSB127(11)	Petroleum contaminated soil (99)	AB161339
OTU 37								BSB143(32)	*Vulcanisaeta distributa* (98)	AB063641
OTU 38	PLS4 (59)	GBA2 (30)							*Sulfolobus* sp. DGG22 (97 to 98)	FJ489516
OTU 39		GBA34 (39)		HGS56 (7)					Thermophilic reactor clone (96)	DQ383349
OTU 40			WMS54 (1)	HGS15 (20)					Hot spring clone YNP_ObP_A5 (97 to 98)	DQ2437572
OTU 41			WMS16 (9)	HGS27 (16)					Hot spring clone Hverd014N (97 to 98)	DQ441506
OTU 42					BSA1 (3)			BSB44 (8)	Soil clone ArcC-u_cE05 (94)	EU307069
OTU 43						SRQ14 (7)	GBF25(3)		Rhizosphere archaeota clone (98 to 99)	EF020745
OTU 44		GBA8 (3)	WMS12 (9)	HGS13 (11)					Sulfide vent clone 4136-1-92 (93)	EU427996
OTU 45					BSA36 (2)		GBF55 (38)	BSB125(37)	Epithermal gold mine clone (97 to 98)	AB072728
OTU 46		GBA7 (4)				SRQ20 (34)	GBF60 (5)	BSB6 (4)	Hot spring clone pJP 41 (97 to 98)	L25301
OTU 47		GBA1 (21)	WMS13 (9)	HGS23 (17)	BSA51 (7)			BSB139 (8)	Hot spring clone SSE_L4_H05 (97)	Eu635920

The number in the parentheses means the number of percentage of the total clone library

Diversity and phylogenetic analysis of Crenarchaeota

A total of eight crenarchaeotal 16S rRNA gene clone libraries (one for each hot spring sample) were constructed, and a total of 826 clones were randomly picked and subjected to RFLP analysis (Table 2). The coverage calculation (Table 2) and rarefaction analysis (data not shown) indicated that the analyzed clones almost covered the diversity of crenarchaeotal populations in every hot spring. One clone was selected from each RFLP type in each clone library, and a total of 64 clones were sequenced. All the obtained sequences could be grouped into 47 OTUs with the cutoff of 98% similarity (Table 2).

Most if not all retrieved OTUs in this study were closely related (90–99%) to those from thermal environments (Table 3). Fourteen OTUs obtained in this study were grouped into the class *Thermoprotei*, and were closely related (90–99% identity) to hyperthermophilic or thermophilic strains, and other OTUs were affiliated with clone sequences retrieved from thermal environments.

Among the investigated hot springs, the crenarchaeotal communities in the three moderate-temperature (59–77°C) hot springs (WMS, HGS and BSA) were most diverse, containing 15, 10 and 11 OTUs, respectively. In contrast, the numbers of OTUs in other investigated hot springs were apparently lower than in these hot springs (Table 2).

Statistical analysis

Most F_{st} values were higher than 0.2, indicating that community structures were significantly different (Lau et al. 2009). It also was found that there were lower F_{st} values between hot springs having similar temperatures. Furthermore, to show the relationships between temperature/pH and crenarchaeotal community structures, linear regression analysis was performed based on the F_{st} and temperature/pH distances (Fig. 2). It is clear that the F_{st} values were linearly correlated with the temperature distances ($R^2 = 0.55$) among Tengchong hot springs (Fig. 2); pH values, however, do not have an obvious linear correlation with F_{st}.

Discussion

Crenarchaeotal diversity in eight hot springs (temperature 44–96°C; pH 2.8–7.7) was investigated in this study. So far, most of the cultured Crenarchaeota are hyperthermophilic and acidophilic microorganisms with an optimal growth temperature of 80–90 and 65–70°C, respectively (Huber et al. 2006). To our best knowledge, with the

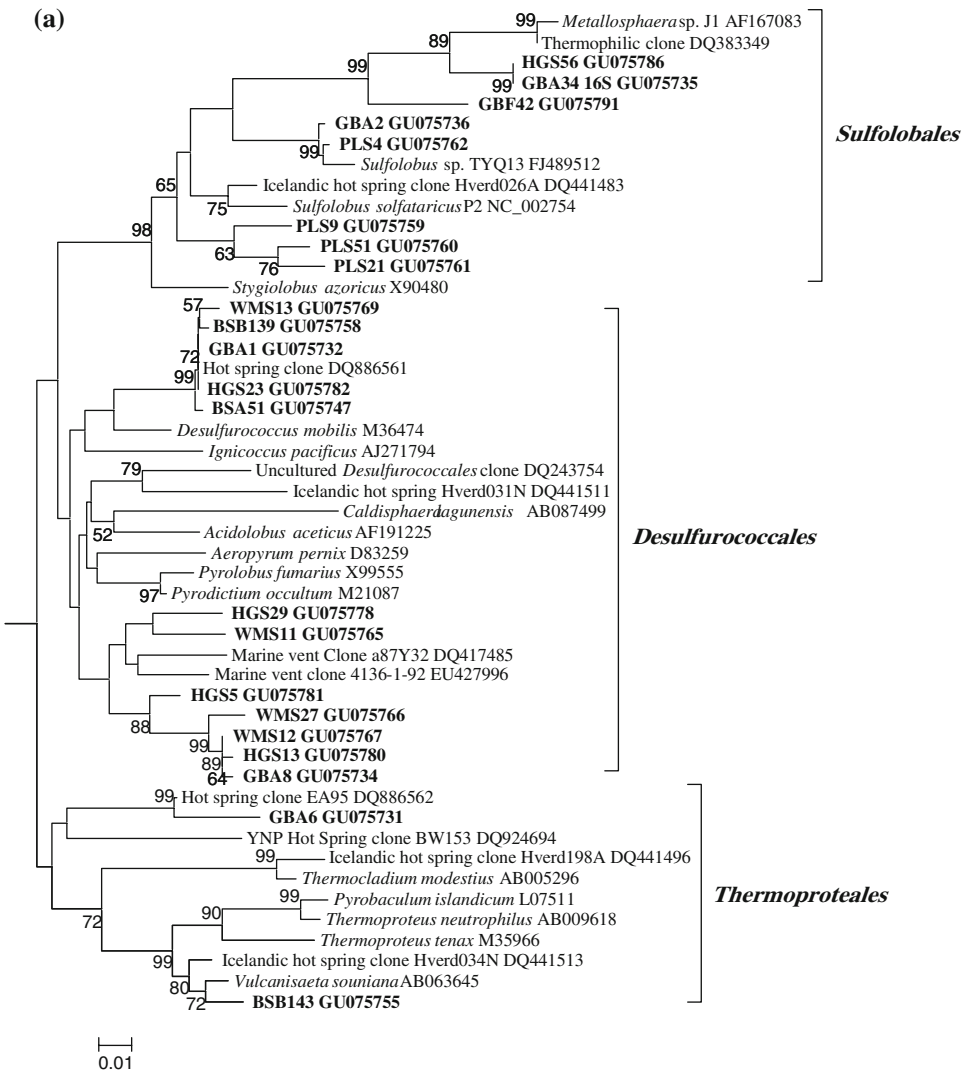

Fig. 1 Neighbor-joining tree showing the phylogenetic relationships of partial crenarchaeotal 16S rRNA gene sequences cloned from studied hot springs (**a** *Thermoprotei*, **b** uncultured groups) to closely related sequences from the GenBank database. *Scale bars* indicate the Jukes-Cantor distances. Bootstrap values of >50% (for 1000 iterations) are shown

exception of ammonia-oxidizing archaea, most cultured Crenarchaeota up-to-date fall into the three orders of *Sulfolobales*, *Desulfurococcales* and *Thermoproteales* in the *Thermoprotei* Class. In the present study, 14 OTUs were affiliated with cultivated organisms of the three orders. All four OTUs retrieved from the PLS hot spring that had the highest temperature (96°C) in this study were grouped into the *Sulfolobales* order (Fig. 1a), among which three OTUs (PLS9, PLS21 and PLS51) formed one unique phylogenetic lineage, showing an average of 93% identity with the closest relatives in the GenBank database. PLS9, PLS21 and PLS51 represent 41% of total clones in the PLS clone library, indicating that the PLS sample contained a new phylogenetic lineage unknown in the *Sulfolobales* order. Previously, a molecular study showed that all OTUs retrieved from a 90°C Icelandic hot spring were grouped into the class *Thermoprotei* (Kvist et al. 2007). Our study and that of Kvist et al. (2007) suggest that the temperature predominates over other environmental parameters in controlling the distribution of *Thermoprotei* taxa in hot spring of high temperatures (90°C or higher).

Beyond the *Thermoprotei* Class, OTU46 [representing a total of 47 clones in GBA, SRQ, GBF and BSB (temperature 44–84°C; pH 3.4–7.5)] was affiliated with environmental clone pJP41 (Table 3; Fig. 1b), which was firstly observed in Obsidian Pool, Yellowstone National Park (Barns et al. 1994). The pJP41 clone formed a typical deep-branching uncultured crenarchaeotal group, and its closest relatives were subsequently obtained in other hot springs globally (Kvist et al. 2007; Perevalova et al. 2008). The temperature of those hot springs ranged from 60 to 81°C. So Perevalova et al. (2008) proposed that the pJP41-related Crenarchaeota could be moderately thermophilic, neutrophilic, or moderately alkaliphilic anaerobic organotrophs independent of the presence of elemental sulfur. In the present study, pJP41-related crenarchaeotal sequences were retrieved from a higher-temperature hot spring (GBA, 84°C) and a lower-temperature hot spring (BSB, 44°C),

Fig. 1 continued

suggesting that the pJP41-related Crenarchaeota may have a wider adaptability and could be globally distributed in terrestrial geothermal features.

OTU41 [representing a total of 25 clones in WMS (77°C; pH 7.7) and HGS (74°C; pH 2.8)] was affiliated with environmental clone pSL12 (Table 3; Fig. 1b), which represents another typical deep-branching uncultured crenarchaeotal group and is adjacent to the group of non-thermophilic marine and soil Crenarchaeota. pSL12-related organisms have been shown to possess ammonia

monooxygenase genes (Francis et al. 2005; Leininger et al. 2006), so they may have the function of nitrification. PS12-related organisms were recently proved to be widely distributed in terrestrial hot springs (de la Torre et al. 2008; Hatzenpichler et al. 2008). Recent studies have shown that ammonia-oxidizing archaea can be isolated from hot springs (de la Torre et al. 2008; Hatzenpichler et al. 2008) and archaeal *amoA* genes were ubiquitous in global geothermal features (Weidler et al. 2007, 2008; Reigstad et al. 2008; Zhang et al. 2008). In addition, one of our previous studies did show that *amoA* genes were present in WMS hot spring (Zhang et al. 2008). Thus the pSL12-related Crenarchaeota may function as nitrifiers in the hot springs of WMS and HGQ.

Seven OTUs representing 11 percentages of total 826 clones was affiliated with non-thermophilic Crenarchaeota clusters which related with rhizosphere, soil, fresh water and mangrove environments (Table 3; Fig. 1b). Similar results were also found in Icelandic hot spring (Kvist et al. 2005). This indicating it maybe is not a individual phenomenon in terrestrial hot springs. In this study, these OTUs just originated from four hot springs (BSA, SRQ, GBF and BSB) ranging in temperature from 44 to 59°C. So, it seemed that moderate-temperature and lower-temperature hot springs maybe a transitional environment between thermophilic and non-thermophilic Crenarchaeota.

Of the greatest interest is that the moderate-temperature samples (WMS, HGQ, and BSA: 59.2–77°C) possessed the highest crenarchaeotal diversity. Previous studies have shown that microbial diversity decreases as environmental stress (e.g. temperature) increases (Atlas and Bartha 1997; Ferris and Ward 1997; Ferris et al. 1996; Ward et al. 1998; Norris et al. 2002). However, Lau and colleagues found that prokaryotic diversity did not vary in a monotonic fashion to thermal stress in hot springs on the Tibetan Plateau, and the highest taxonomic diversity occurred in streamers of 65–70°C (Lau et al. 2006). In present study, when the temperature is above or below the moderate-temperature samples, the crenarchaeotal diversity in Tengchong hot springs always became less (Table 2). Generally speaking, our result is in agreement with Lau et al. (2006). However, the temperature range for the greatest taxonomic diversity in our study was much wider than theirs (59.2–77 vs. 65–70°C).

Temperature and pH are two major environmental factors that affect the prokaryotic community structures in hot springs. In this research, we described the difference of pair-wise crenarchaeotal community based on *F* statistical analysis, and found that most crenarchaeotal communities in Tengchong hot springs are markedly different from each other (Table 3). Furthermore, the linear regression analysis showed a positive correlation between temperature and F_{st}, suggesting that larger F_{st} values correspond with larger

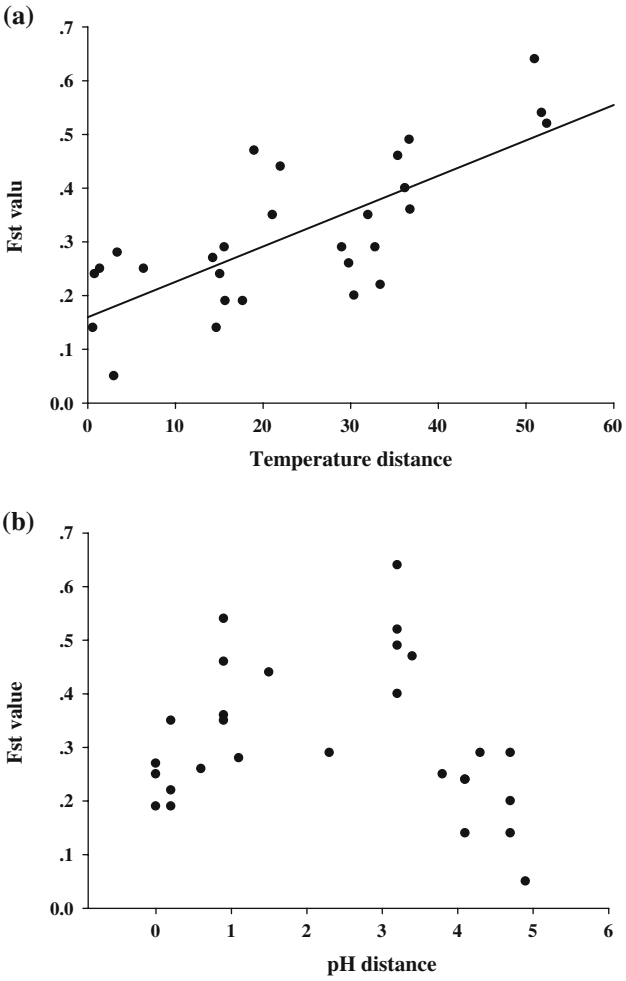

Fig. 2 **a** Relationship between temperature distance and the F_{st} value. Temperature distance was not obviously correlated with pH ($R^2 = 0.55$). **b** Relationship between pH distance and the F_{st} value. pH distance was not obviously correlated with pH ($R^2 = 0.20$)

temperature distances. However, pH does not show such significant correlation with F_{st} (Fig. 2). So it appears that temperature predominates over pH in controlling the crenarchaeotal community structures in Tengchong hot springs.

In summary, 16S rRNA gene phylogenetic analysis showed that crenarchaeotal diversity was significantly correlated with temperature in Tengchong hot springs, which is consistent with the observation that hot springs with moderate temperature harbored the highest crenarchaeotal diversity. The upper limit of the moderate temperature range for such hot springs could reach up to 77°C. This work enhances our understanding of crenarchaeotal diversity in global hot springs.

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References

Atlas RM, Bartha R (1997) Microbial ecology: fundamentals and applications. Longman, Menlo Park

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

Boone DR, Castenholz RW (2001) Bergey's Manual of Systematic Bacteriology: Vol. One: the archaea and the deeply branching and phototrophic bacteria. Springer, Berlin

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

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

Dawson S, DeLong EF, Pace NR (2006) Phylogenetic and ecological perspectives on uncultured Crenarchaeota and Korarchaeota. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes, vol 3, 3rd edn. Springer, New York

de la Torre JR, Walker CB, Ingalls AE, Könneke M, Stahl DA (2008) Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. Environ Microbiol 10:810–818

DeLong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89:5685–5689

Ferris MJ, Ward DM (1997) Seasonal distribution of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. Appl Environ Microbiol 63(4):1375–1381

Ferris MJ, Muyzer G, Ward DM (1996) Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. Appl Environ Microbiol 62:340–346

Francis CA, Roberts KJ, Beman JM (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc Natl Acad Sci USA 102:14683–14688

Frontier S (1985) Diversity and structure in aquatic ecosystems. Oceanogr Marine Biol 23:253–312

Fuhrman JA, McCallum K, Davis AA (1992) Novel major archaebacterial group from marine plankton. Nature 356:148–149

Hacine H, Rafa F, Chebhouni N, Boutaiba S, Bhatnagar T, Barratti JC, Ollivier B (2004) Biodiversity of prokaryotic microflora in E1 Golea salt lake, Algerian Sahara. J Arid Environ 58:273–284

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

Hofacker IL (2003) Vienna RNA secondary structure server. Nucleic Acids Res 31:3429–3431

Huang Z, Wiegel J, Zhou J, Hedlund B, Zhang CL (2007) Molecular phylogeny of uncultivated crenarchaeota in Great Basin hot springs of moderately elevated temperature. Geomicrobiology 24:535–542

Huber T, Faulkner G, Hugenholtz P (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20:2317–2319

Huber H, Huber R, Stetter KO (2006) Thermoproteales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes, vol 3, 3rd edn. Springer, New York

Hugenholtz P, Pitulle C, Hershberger KL, Pace NR (1999) Novel division level bacterial diversity in a Yellowstone hot spring. J Bacteriol 180:366–376

Jackson CR, Langner HW, Donahoe-Christiansen J, Inskeep WP, McDermott TR (2001) Molecular analysis of microbial community structure in an arsenite-oxidizing acidic thermal spring. Environ Microbiol 3:532–542

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

Kearey P, Wei H (1993) Geothermal fields of China. J Volcanol Geotherm Res 56:415–428

Könneke 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

Kvist T, Mengewein A, Manzei S, Westermann BK, Ahring P (2005) Diversity of thermophilic and non-thermophilic crenarchaeota at 80°C. FEMS Microbiol Lett 244:61–68

Kvist T, Ahring BK, Westermann P (2007) Archaeal diversity in Icelandic hot springs. FEMS Microbiol Ecol 59:71–80

Lau CY, Jing HM, Jonathan CA, Stephen BP (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 MC, Pointing JC, Aitchison SB (2009) Bacterial community composition in thermophilic microbial mats from five hot springs in central Tibet. Extremophiles 13:139–149

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809

Marteinsson VT, Hobel S, Hauksdottir CF (2001) Phylogenetic diversity analysis of subterranean hot springs in Iceland. Appl Environ Microbiol 67:4242–4248

Martin AP (2002) Phylogenetic approaches for describing and comparing the diversity of microbial communities. Appl Environ Microbiol 68:3673–3682

Mathur J, Bizzoco RW, Ellis DG, Lipson DA, Poole AW, Levine R, Kelley ST (2007) Effects of abiotic factors on the phylogenetic diversity of bacterial communities in acidic thermal spring. Appl Environ Microbiol 73:2612–2623

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

Norris TB, McDermott TR, Castenholz RW (2002) The longterm effects of UV exclusion on the microbial composition and photosynthetic competence of bacteria in hot-spring microbial mats. FEMS Microbiol Ecol 39:193–209

Ochsenreiter T, Selezi D, Quaiser A, Bonch-Osmolovskaya L, Schleper C (2003) Diversity and abundance of *Crenarchaeota* in terrestrial habitats studied by 16S RNA surveys and real time PCR. Environ Microbiol 5:787–797

Perevalova A, Tatiana A, Kolganova V, Birkeland NK, Christa S, Bonch-Osmolovskaya EA, Alexander VL (2008) Distribution of *Crenarchaeota* representatives in terrestrial hot springs of Russia and Iceland. Appl Environ Microbiol 74:7620–7628

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

Schleper C, Holben W, Klenk HP (1997) Recovery of crenarchaeotal ribosomal DNA sequences from freshwater lake sediments. Appl Environ Microbiol 63:321–323

Schleper C, Jurgens G, Jonuscheit M (2005) Genomic studies of uncultivated archaea. Nature Rev Microbiol 3:470–488

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

Skirnisdottir S, Hreggvidsson GO, Rleifsdottir SH, Marteinsson VT, Petursdottir SK, Holst O, Kristiansson JK (2000) Influence of sulfide and temperature on species composition community structure of hot spring microbial mats. Appl Environ Microbiol 66:2835–2841

Song ZQ, Zhi XY, Jiang HC, Zhang CL, Dong HL, Li WJ (2009) Actinobacterial diversity in hot springs in Tengchong (China), Kamchatka (Russia), and Nevada (USA). Geomicrobiol J 26(4):256–263

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

Stetter KO (1996) Hyperthermophilic procaryotes. FEMS Microbiol Rev 18:149–158

Takai K, Sako Y (1999) A molecular view of archaeal diversity in marine and terrestrial hot water environments. FEMS Microbiol Ecol 28:177–188

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Vick TJ, Dodsworth JA, Costa KC, Shock EL, Hedlund BP (2010) Microbiology and geochemistry of Little Hot Creek, a hyperthermophilic hot spring environment in the Long Valley Caldera. Geobiology 8:1–14

Ward DM, Ferris MJ, Nold SC, Bateson MM, Kopczynski ED, Ruff-Roberts AL (1998) Species diversity in hot spring microbial mats as revealed by both molecular and enrichment culture approaches—relationship between diversity and community structure. In: Stal LJ, Caumette P (eds) Microbial mats: structure, development and environmental significance. Springer, Heidelberg, pp 33–44

Weidler GW, Dornmayr-Pfaffenhuemer M, Gerbl FW, Heinen W, Stan-Lotter H (2007) Communities of Archaea and Bacteria in a subsurface radioactive thermal spring in the Austrian central alps, and evidence of ammonia-oxidizing Crenarchaeota. Appl Environ Microbiol 73:259–270

Weidler GW, Gerbl FW, Stan-Lotter H (2008) Crenarchaeota and their role in the nitrogen cycle in a subsurface radioactive thermal spring in the Austrian central alps. Appl Environ Microbiol 74:5934–5942

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

Winker S, Woese CR (1991) A definition of the domains *Archaea*, *Bacteria* and *Eucarya* in terms of small subunit ribosomal RNA characteristics. Syst Appl Microbiol 14:305–310

Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains *Archaea*, *Bacteria*, and *Eucarya*. Proc Natl Acad Sci USA 87:4576–4579

Zhang CL, Qi Y, Huang ZY, Li WJ, Chen JQ, Song ZQ, Zhao WD, 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

Zhang CL, Hedlund BP, Meng J (2010) Diversity of archaea in terrestrial hot springs and role in ammonia oxidation. In: de Bruijn FJ (ed) Handbook of molecular microbial ecology II: metagenomics in different habitats. Wiley, Hoboken (in press)