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ORIGINAL PAPER

Bacterial and archaeal diversity in two hot spring microbial mats from the geothermal region of Tengchong, China

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Abstract We investigated the bacterial and archaeal diversity in two hot spring microbial mats from the geothermal region of Tengchong in the Yunnan Province, China, using direct molecular analyses. The Langpu (LP) laminated mat was found by the side of a boiling pool with temperature of 60–65 °C and a pH of 8.5, while the Tengchong (TC) streamer mat consisted of white streamers in a slightly acidic (pH 6.5) hot pool outflow with a temperature of 72 °C. Four 16S rRNA gene clone libraries were constructed and restriction enzyme analysis of the inserts was used to identify unique sequences and clone frequencies. From almost 200 clones screened, 55 unique sequences were retrieved. Phylogenetic analysis showed that the LP mat consisted of a diverse bacterial population [Cyanobacteria, *Chloroflexi*, *Chlorobia*, *Nitrospirae*, '*Deinococcus-Thermus*', *Proteobacteria* (alpha, beta and delta subdivisions), *Firmicutes*, *Bacteroidetes* and *Actinobacteria*], while the archaeal population was dominated by methanogenic *Euryarchaeota* and *Crenarchaeota*. In contrast, the TC streamer mat consisted of a bacterial population dominated by *Aquificae*, while the archaeal population also contained *Korarchaeota* as well as *Crenarchaeota* and methanogenic *Euryarchaeota*. These mats harboured clone sequences affiliated to unidentified lineages, suggesting that they are a potential source for discovering novel bacteria and archaea.

Keywords Biodiversity · Cloning · Thermophile ecology · Culture-independent · Microbial mats · Streamers · Tengchong · Hot springs

Introduction

Tengchong is part of the Rehai (Hot Sea) geothermal field, located in the Yunnan Province in southwestern China. The field covers an area of about 10 km^2, at the eastern end of

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the Tibet–Yunnan geothermal zone (Kearey and Wei 1993). It is abundant in thermal surface manifestations such as boiling springs, hot springs, fumaroles, eruption vents, and areas of steaming ground and is prone to hazardous hydrothermal explosions (Shangguan et al. 2005). Geochemical analysis of the Rehai alkaline springs showed high levels of K, Na, F, Cl and SiO_2, whereas acidic springs contained high levels of SO_4^{2-}, Mn and Fe (Zhang et al. 2008a, b). The Rehai geothermal field sits in a circular structure with Langpu Hot Pool at its western-most border (Zhijie and Guoying 1986), containing boiling springs surrounded by agricultural land.

When a surface is in contact with water, microorganisms can attach and aggregate, forming complicated networks that develop into microbial mats. Many mats were observed in the hot springs at the Tengchong National Geological Park and Langpu, in the Rehai geothermal field. One mat from each area was chosen for study because of their distinct morphology and differences in environmental settings (pH, salinity—as indicated by different conductivity readings—and temperature). The Langpu (LP) laminated microbial mat was found in hot alkaline water (pH 8.5) by a wall surrounding a boiling spring and consisted of distinct layers, while the Tengchong (TC) microbial mat was found in a slightly acidic (pH 6.5) hot spring effluent and consisted of white macroscopic filaments called ‘streamers’.

Laminated mats (also called ‘cyanobacterial mats’) tend to develop in neutral to alkaline hot springs and are composed of distinct layers of microorganisms. Many examples are found in the hot springs in Yellowstone National Park (YNP), USA (Boomer et al. 2002; Doemel and Brock 1977; Ramsing et al. 2000; Ward 1978; Ward et al. 1998), Tibet (Lau et al. 2009; Lau and Pointing 2009), Japan (Hanada 2003), China, the Philippines and Thailand (Hongmei et al. 2005) at temperatures ranging from 30 to 74 °C, with pH values ranging from 7 to 9.5. Laminated mats predominantly contain bacteria, with cyanobacteria in the uppermost layer, filamentous anoxic phototrophs in the red layer beneath and then anaerobic heterotrophs in the deeper brown/black layers. These layers are highly ordered and the microorganisms within them cooperate to gain light energy and recycle nutrients (Doemel and Brock 1977; Hanada 2003; Lau and Pointing 2009; Nold and Ward 1996; Nübel et al. 2002; Ramsing et al. 2000; van der Meer et al. 2005; Ward et al. 1998). Methanogenic *Euryarchaeota* were first detected in laminated mats from YNP in the 1970s (Ward 1978), and to date no other archaea have been reported in hot spring laminated mats.

Streamer mats have been found in flowing streams and effluents of hot springs around the world, including Japan (Nakagawa and Fukui 2002; Yamamoto et al. 1998), Iceland (Reigstad et al. 2010; Skirnisdottir et al. 2000; Takacs et al. 2001), Thailand (Purcell et al. 2006), Russia (Perevalova et al. 2008; Reigstad et al. 2010) and YNP, USA (Jahnke et al. 2001; Reysenbach et al. 1994; Reysenbach et al. 2000) at temperatures ranging between 52 and 97 °C and a pH range of <5 to 9. They can appear in a variety of colours including white, blue, pink, grey, green, orange, yellow and black. Despite the variation in colours, they are all predominantly made up of chemolithotrophic bacteria belonging to the *Aquificales* associated with sulphur particulates that aggregate to form distinctive filaments or turf-like structures. Studies have shown that members of the *Korarchaeota* and *Crenarchaeota* can be associated with streamers and that no streamer community is identical (Perevalova et al. 2008; Reysenbach et al. 2000). It has been suggested that development of streamer communities depends on abiotic factors such as temperature, water mineral chemistry, dissolved oxygen concentrations, stream flow and pH (Maki 1986; Purcell et al. 2006; Skirnisdottir et al. 2000), but recently Meyer-Dombard et al. (2011) showed that inter-species interactions can determine whether streamer mats can develop.

Total genomic DNA was extracted from the microbial mat samples and 16S ribosomal RNA (rRNA) genes were amplified using universal archaeal primers and universal bacterial primers to construct four libraries. About 200 clones were picked and screened and a total of 55 unique sequences were retrieved. Here we present a description of the microbial diversity within the two hot spring microbial mats and show that both mats contained an archaeal population consisting of predominantly *Crenarchaeota* and methanogenic *Euryarchaeota*, but the bacterial population in the TC mat was dominated by *Aquificales*, while the LP mat contained a diverse bacterial community. Both mats also contained sequences affiliated to unknown lineages. Currently, only a handful of culture-independent studies on the microbial diversity in Tengchong's hot springs exist in the literature (reviewed in Hedlund et al. 2012). Therefore, this study further increases our knowledge of microbial diversity and ecology in this geothermal region.

Materials and methods

Sample collection and measuring temperature, pH and conductivity

Samples of the Tengchong (TC) and Langpu (LP) microbial mats were collected aseptically and stored in sterile vials or plastic bags (Whirlpaks). All samples were transported in a cooled container for no more than 4 h until they could be processed (see below). Temperature, pH and conductivity were measured as previously described (Pagaling et al. 2009).

Community DNA extraction, PCR amplification of 16S rRNA genes and construction of 16S rRNA gene clone libraries

Community DNA was extracted using the GenomicPrep cell and tissue DNA isolation kit (Amersham Biosciences). The initial stages of protein precipitation were carried out on site, and samples were stored at −20 °C until DNA purification could be carried out in the laboratory at the University of Leicester, UK. 16S rRNA genes were PCR amplified using universal archaeal primers and universal bacterial primers as previously described (Grant et al. 1999). 16S rRNA gene clone libraries were constructed as previously described (Pagaling et al. 2009).

Screening clones for unique inserts

Up to 40 clones from each library were picked and screened as previously described (Pagaling et al. 2009). Briefly, inserts were PCR amplified from clones using flanking vector primers M13F (5′-GTTTTCCCAGTCACGAC-3′) and M13R (5′-CAGGAAACAGCTATGAC-3′). To look at the restriction fragment length polymorphism (RFLP) patterns of the inserts, the PCR products were digested with *Hae*III (NEB). Fragments were visualised by electrophoresis in Tris–acetate–EDTA (TAE) using 2 % agarose. Identical RFLPs were identified by eye and representative sequences from each RFLP group were sent for sequencing to Lark Technologies, Ltd (Essex, UK) (see Fig. 1).

Computer analysis and library coverage

The CHIMERA_CHECK program available from the ribosomal database project (RDP) (Cole et al. 2003) and Pintail (Ashelford et al. 2005) were used to check for chimeric sequences. Those that were found were removed from further analysis. Rarefaction curves were calculated from RFLP groupings using Analytical Rarefaction version 1.3 available at UGA Stratigraphy Lab (http://www.uga.edu/strata/software/index.html). Homologies of the 16S rRNA gene sequences to known sequences were retrieved using BlastN in August 2011 (Altschul et al. 1990). Sequences were aligned using MEGA version 4 (Tamura et al. 2007). Richness estimator Chao1 was calculated using RFLP groupings using EstimateS (Version 8.2, R. K. Colwell, http://purl.oclc.org/estimates). Library coverage was calculated using the equation $C = [1 - (n_1/N)]\,100$, where n_1 represents the number of species (using RFLP groupings) and N represents the total number of clones in the library (Good 1953).

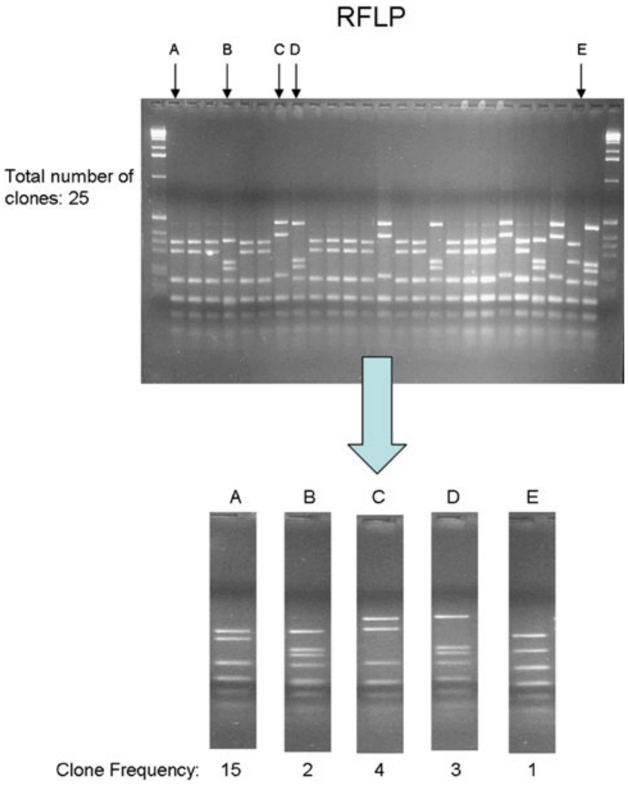

Fig. 1 Schematic diagram for screening clones by restriction fragment length polymorphism (RFLP) patterns

Construction of phylogenetic trees

Phylogenetic analysis was done using MEGA version 4 (Tamura et al. 2007), using the maximum-likelihood method of tree inference and the Jukes and Cantor nucleotide substitution model for sequence alignment. The support for each node was determined by assembling a consensus tree of 500 bootstrap replicates.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences retrieved from the clone libraries were deposited into the EMBL Nucleotide Sequence Database under accession numbers FR668273 to FR668293 and HE657236 to HE657269.

Results

Descriptions of sampling sites

An expedition to the Yunnan province was conducted in March 2003. The Tengchong sampling area (98°26′E, 24°57′N) has a mean elevation of 1,520 m and is located in the Tengchong National Geological Park. Many of the thermal sites had been modified by the addition of stone and concrete paving and containment walls. The TC sampling site was located in a 'Hydrothermal Outbreak Pool, Scientific Research area,' which was at the base of a cliff face above the Zaotang river. The main source for this site was a 50-cm pool with a discharge of <0.11 L s^{-1} located on an upper slope. Water drained from this main source into several secondary pools on lower slopes in the area. Our streamer microbial mat sample was located in a small hot stream running down a slope, which consisted of white filamentous material (sample TC; Fig. 2a). These streamers were firmly attached to the rocks and sediment. At the time of sampling, the site had a temperature of 72 °C, a pH of 6.5 and a conductivity of 2.2 μS cm^{-1}.

Langpu is a village approximately 20 km south west of Tengchong in a broad valley (98°23′20″E, 24°54′30″N) at an elevation of 1,119 m. The area had been previously developed for human use as a thermal spa complex, but the project was subsequently abandoned. The site remained covered with derelict buildings. Our sampling site, a large concrete lined basin of hot water (10 m × 8 m, depth 1–2 m), was located at the upper end of the complex. Three sides of the basin were surrounded by a 3-m-high wall, from one of which protruded a pipe that issued a hot water stream feeding a microbial mat. The laminated mat was approximately 3 cm thick with green, red and brown striations (sample LP; Fig. 2b). The site had a temperature of 60–65 °C, a pH of 8.5 and a conductivity reading of 3.2 μS cm^{-1}.

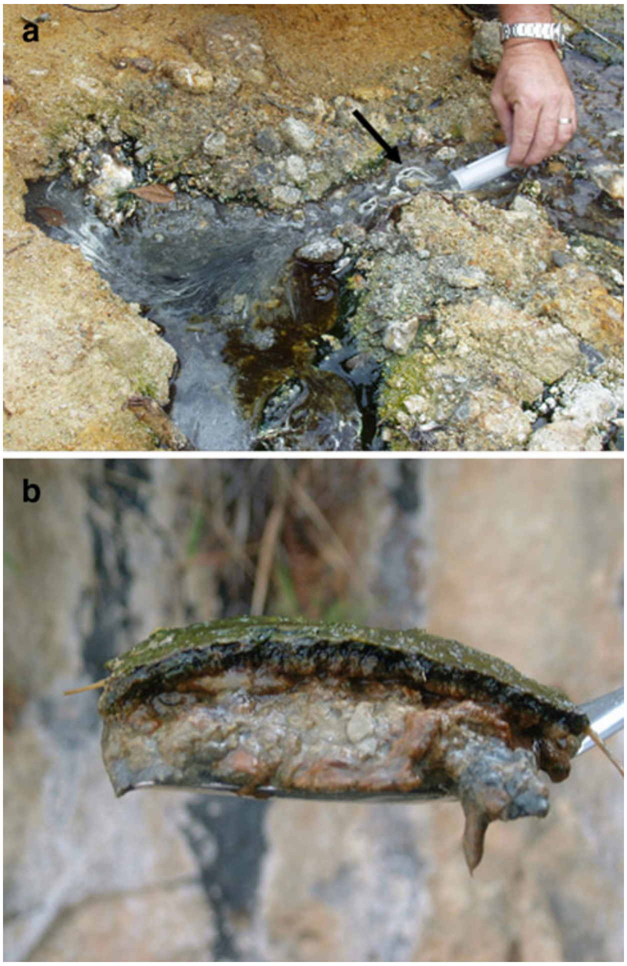

Fig. 2 White streamers (indicated by the *arrow*) sampled at Tengchong Geological Park (**a**). Laminated mat sampled at Langpu (**b**)

Library coverage

Up to 40 clones were picked for each library and the clones were grouped according to RFLP of the plasmid inserts. A representative clone from each RFLP group was chosen for sequencing (see Fig. 1). Hence clone frequencies based on RFLP groupings were used to construct rarefaction curves by plotting the number of unique RFLP groups (and therefore number of species) against the number of clones screened (Fig. 3). Neither of the plots for bacteria approached an asymptotic state, suggesting that there were more species that were not sampled. This was supported by the richness estimator (Chao1) that extrapolated there to be 46 and 43 bacterial species in the LP and TC microbial mats, respectively (and 33 and 29 archaeal species in the LP and TC microbial mats). However, calculated coverage values were 67.5 % for the LP archaeal library, 67 % for the TC archaeal library and 58 % for the TC bacterial library, suggesting that the dominant species in these libraries were sampled. The coverage value for the LP bacterial library was 41.2 %, suggesting that some dominant species remain to be sampled.

Bacterial diversity

One clone sequence (LP15) was most closely related to a thermophilic cyanobacterium, *Leptolyngbya* sp. (Table S1). The other clone sequences were closely related to uncultured bacteria detected in hot springs in the Tengchong region, Western Thailand, Tibet, YNP (USA), Russia, Southwestern Taiwan and Japan as well as other thermal environments such as subsurface gold mines in South Africa and Japan. However, a few were related to clones detected in temperate environments such as waste waters and lakes (Table S1).

A phylogenetic tree was constructed as outlined in "Materials and methods", with *Methanospirillum hungatei* as the out-group (Figs. 4, 5). Known representatives were included in the tree to find the relatedness of the clone sequences to described species. Phylogenetic analysis showed the distribution of the clone sequences into several monophyletic groups: the *Proteobacteria* (alpha, beta and delta groups), *Chlorobia*, *Firmicutes*, Cyanobacteria,

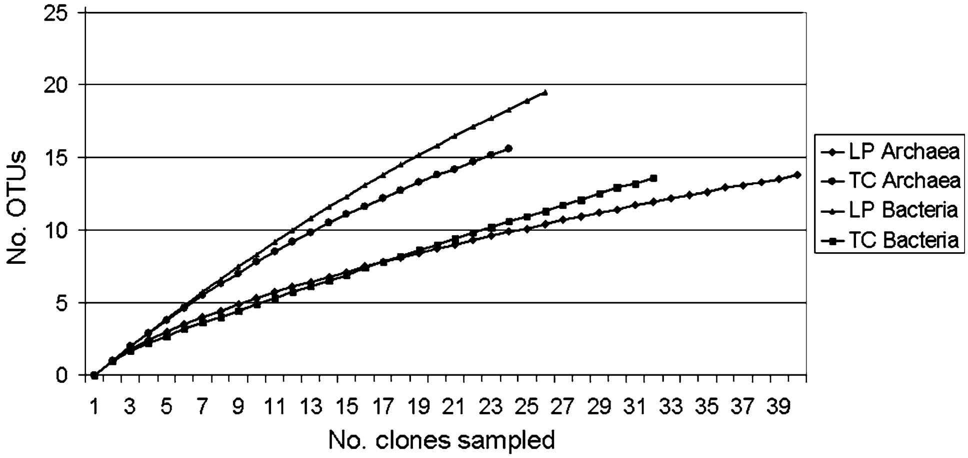

Fig. 3 Rarefaction curves showing the sampling effort for the TC and LP 16S rRNA gene libraries

Bacteroidetes, *Elusimicrobia*, *Nitrospirae*, '*Deinococcus-Thermus*', *Actinobacteria*, *Chloroflexi*, *Aquificae* and *Thermotogae*. In addition to the known lineages, several clone sequences were affiliated to unknown distinct lineages, designated clusters 1 and 2. Cluster 1 (containing clone sequence TC22) is related to, but distinct from the *Thermotogales*, while cluster 2 (containing clone sequences LP14, 18, 20, 21 and 22 and TC19), is related to, but distinct from the *Chloroflexi*. Other phylogenetic trees were constructed using different methods, but all gave similar results (data not shown).

Skirnisdottir and colleagues showed that *Aquificales* from hot spring streamer communities could be classified into J, S, H and P groups (Skirnisdottir et al. 2000). Sequences from these groups were included in the tree (Fig. 4), but none of the *Aquificales* clones from the TC library affiliated with these defined groups, representing a novel branch within the *Aquificae*.

The clone library for LP contained an even distribution of clone sequences within the RFLP groupings so that there was not a single bacterial sequence that dominated. LP clones were also affiliated with almost all the monophyletic groups in the tree (except for *Elusimicrobia* and the deeply branching *Aquificae* and *Thermotogae*), indicating a higher genetic diversity of the species detected. In contrast, the library for TC was dominated by clones affiliated with the *Aquificae*, showing a combined clone frequency of 25 out of 33 clones screened; the remaining TC clones affiliated with the *Deltaproteobacteria*, *Elusimicrobia*, '*Deinococcus-Thermus*' and *Thermotogae*.

Archaeal diversity

The majority of the clone sequences (71 %) were most closely related to uncultured archaea detected in other thermal environments such as volcanic mud, high-temperature petroleum reservoir and a subsurface gold mine (Table S1). Some were related to sequences detected in temperate environments such as the Sea of Okhotsk in Russia and Honghu Lake. The remaining 29 % of sequences were related to methanogenic *Euryarchaeota Methanobacterium* and *Methanothermobacter*.

A phylogenetic tree was constructed as outlined in "Materials and methods" (Fig. 6). *Escherichia coli* was chosen as the out-group. Known representatives within the *Crenarchaeota* and methanogenic *Euryarchaeota* were included in the tree to find the relatedness of the clone sequences to known species. A candidate sequence from the *Korarchaeota* was also included. Phylogenetic analysis showed the distribution of the clone sequences into three monophyletic groups: the *Korarchaeota*, *Crenarchaeota* and *Euryarchaeota*. In addition to the known lineages, several clone sequences belonged to distinct lineages, designated clusters 1, 2 and 3. Cluster 1 (containing clone sequence TC9) is related to, but distinct from all known *Euryarchaeota*; cluster 2 (containing clone sequences LP5, 6 and 13 and TC8) and cluster 3 (containing clone sequences LP10 and TC1) are related to, but distinct from the *Crenarchaeota*. These clusters might represent novel divisions within these clades. Other phylogenetic trees were constructed using different methods, but they all gave similar results.

The clone library for LP was dominated by clone sequence LP1 since this was the most frequently observed RFLP with a clone frequency of 18 out of 40 clones screened (Table S1). This was closely related to a clone sequence that affiliated with the *Crenarchaeota*. Clone sequences LP3, 7, 8, 9 and 11 were all affiliated with the *Methanobacteriales*, giving a combined clone frequency of 12, making this the second most frequently observed group in the LP library. In contrast, the clone library for TC showed a more even distribution within the RFLP groupings and was not dominated by a single clone sequence. The TC clone sequences were also more evenly interspersed throughout the tree, indicating a higher (genetic) diversity of species detected.

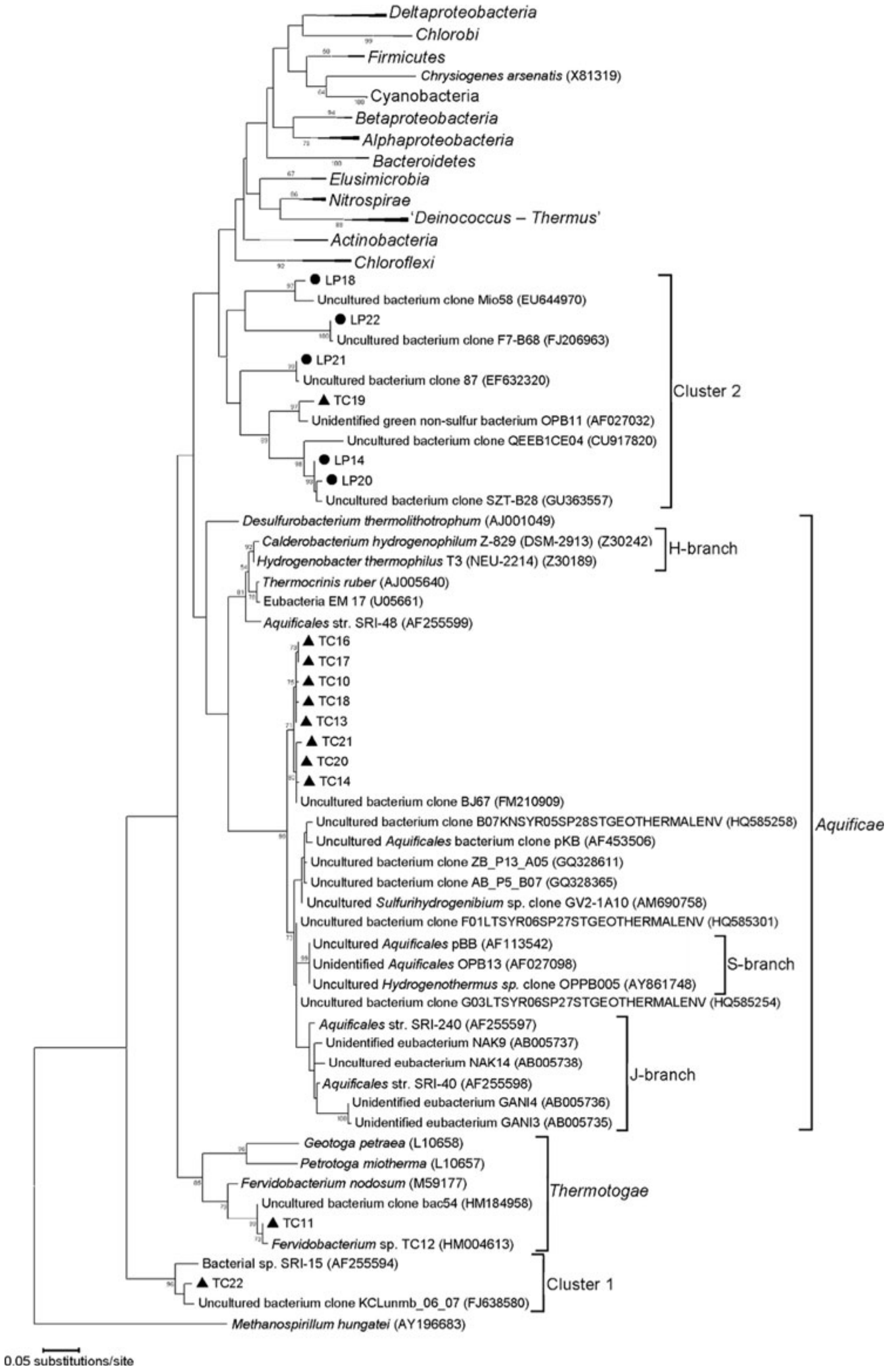

Fig. 4 Phylogenetic tree of the bacterial population in the hot spring mats. *Solid circles* indicate sequences from the LP mat library and *solid triangles* indicate sequences from the TC mat library. Branches for the *Aquificae*, *Thermotogae* and clusters 1 and 2 have been expanded

Discussion

Bacterial community composition in the Langpu mat

The Langpu laminated mat was detected in 65°C hot waters—the uppermost limit for cyanobacterial mat development (Papke et al. 2003). Laminated mats consist of horizontal layers (in descending order) of cyanobacteria (including *Synechococcus*), *Chloroflexus*, *Roseiflexus* and anoxic sediment (Doemel and Brock 1977; Hanada 2003; Ward et al. 1998). Likewise, the LP laminated mat contained very clear striations. The uppermost green layer was

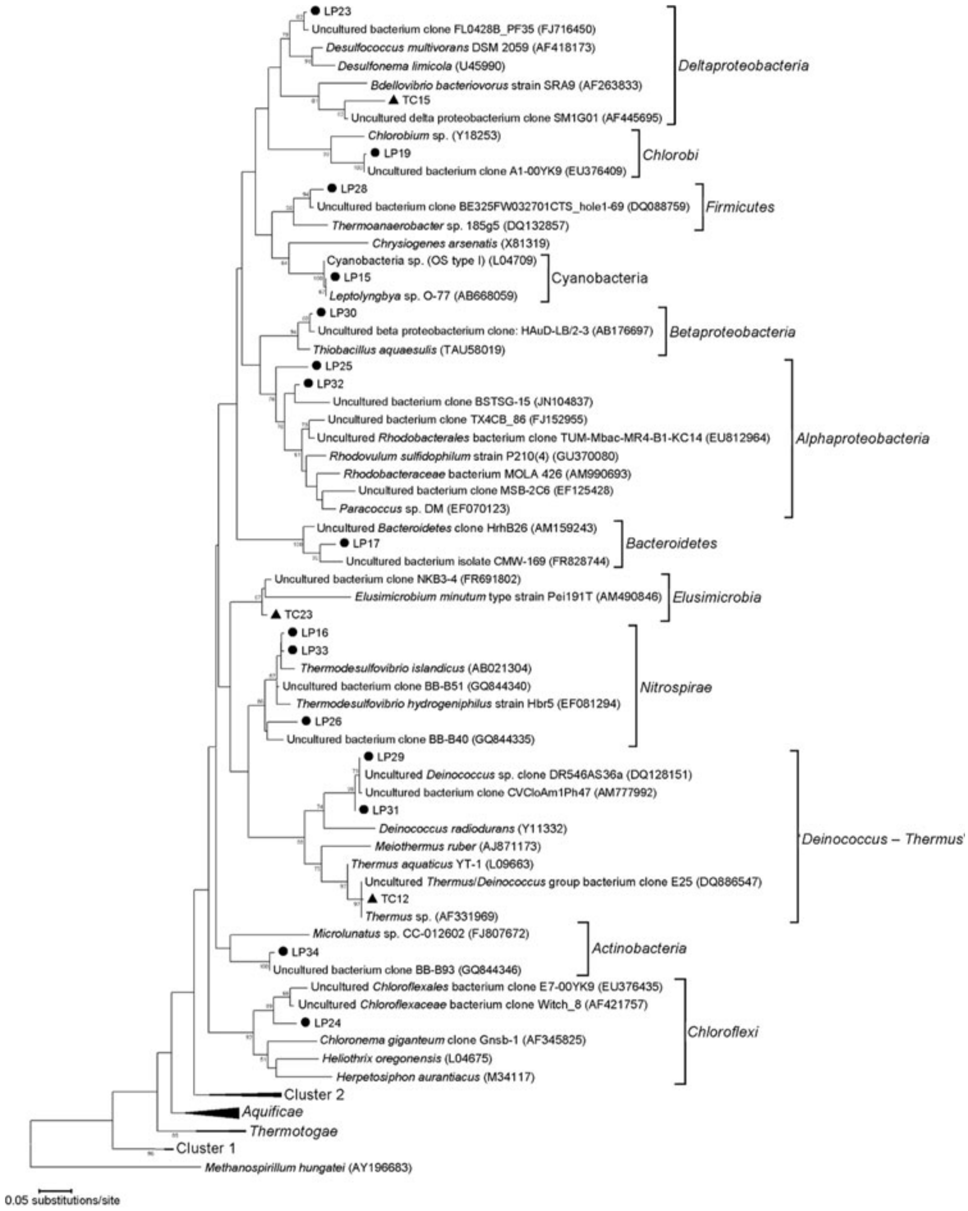

Fig. 5 Phylogenetic tree of the bacterial population in the hot spring mats. *Solid circles* indicate sequences from the LP mat library and *solid triangles* indicate sequences from the TC mat library. Branches for the *Proteobacteria*, *Chlorobi*, *Firmicutes*, cyanobacteria, *Bacteroidetes*, *Elusimicrobia*, *Nitrospirae*, '*Deinococcus-Thermus*,' *Actinobacteria* and *Chloroflexi* have been expanded

only a few millimetres thick and was likely to be composed of cyanobacteria. One clone was related to *Leptolyngbya* sp. O-77 (99 %) which are known thermophilic cyanobacteria (Roeselers et al. 2007) and were likely responsible for primary production in the laminated mat community. *Synechococcus* are often found associated with filamentous cyanobacteria in laminated mats found in North America (Ramsing et al. 2000), but no *Synechococcus* sequences were detected in the library. This may be consistent with studies that demonstrate that *Synechococcus* were absent from hot springs in Iceland, Alaska and Azores, indicating endemism of thermophilic cyanobacteria (Papke et al. 2003). However, there appears to be a general lack of clones relating to cyanobacteria in the library. This may be because the DNA extraction methods used in this study were ineffective for cyanobacteria, which are known for being difficult to extract DNA from (Morin et al. 2010). Additionally, it is possible that the cyanobacterial 16S rRNA gene sequences may have evaded PCR amplification by the bacterial primers used in this study.

Chlorobi have not been recorded as components of laminated hot spring mat biomass, but their ability to photosynthesise anaerobically suggests that they could be found in the photic zone. Clone LP19, affiliated with the *Chlorobi* was most closely related to a clone that was detected in hot spring unlaminated mats in Thailand (Portillo et al. 2009). Likewise, *Chlorobium* species have been isolated from unlaminated mats in New Zealand hot

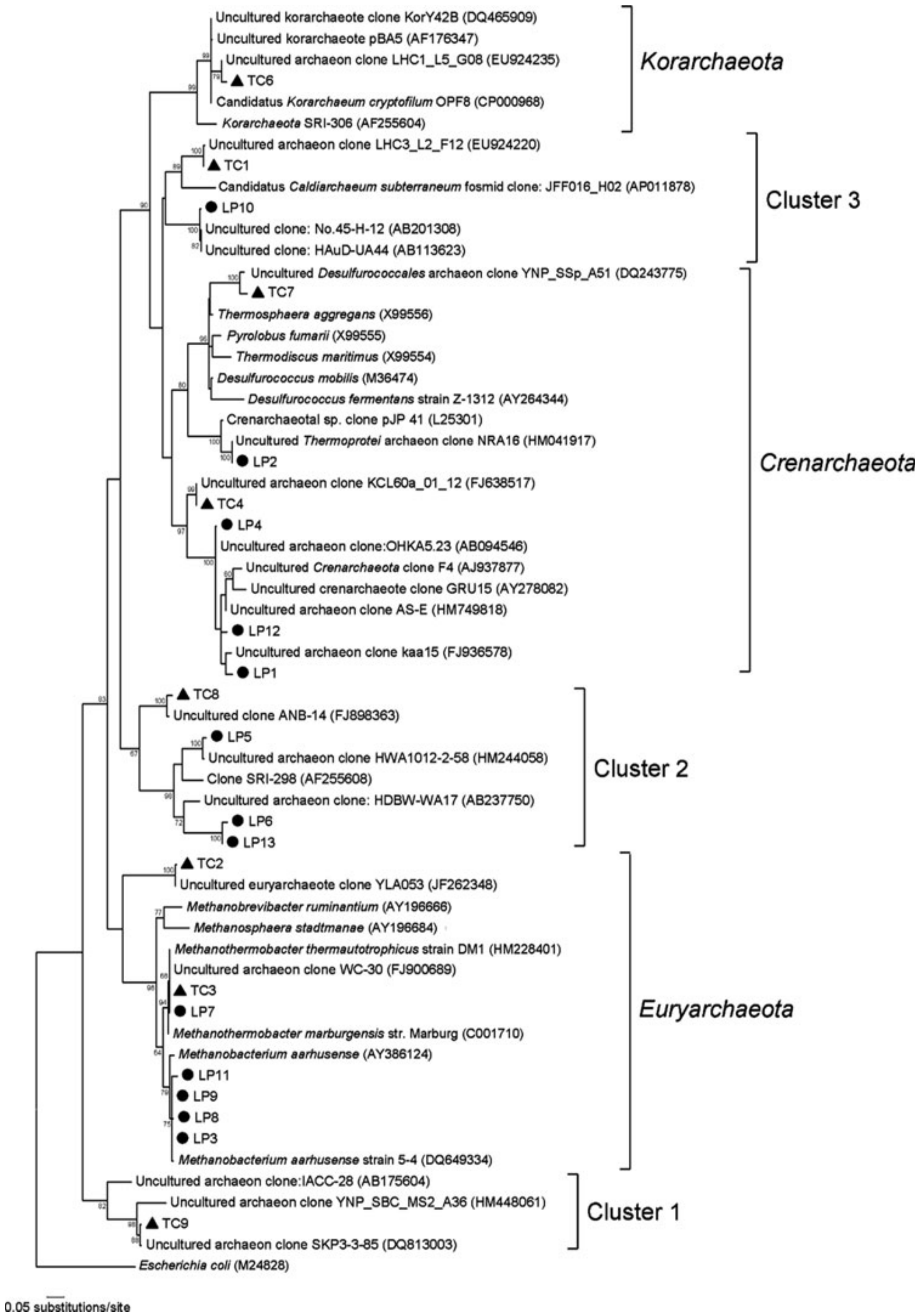

Fig. 6 Phylogenetic tree of the archaeal population in the hot spring mats. *Solid circles* indicate sequences from the LP mat library and *solid triangles* indicate sequences from the TC mat library

springs (Wahlund et al. 1991) and detected in unlaminated hot spring mats in Greenland (Roeselers et al. 2007). However, green sulphur bacterium-like sequences have been retrieved from cyanobacterial mats at Octopus Spring but further investigation was required to confirm that these affiliated with the *Chlorobi* (Ward et al. 1998). Similarly, *Chlorobi* were detected ubiquitously in several Tibetan hot spring mats (Lau et al. 2009). However, it is not known to what extend *Chlorobi* contribute to primary production and structural integrity to the laminated mat community.

Below this green photic zone usually lie layers of filamentous anoxygenic phototrophs (FAPS) (Hanada 2003), which also contribute to primary production. Studies have shown that the underlying brown layer associated with cyanobacteria consists of *Chloroflexus*, while the red layer below this consists of *Roseiflexus* (Boomer et al. 2002; Hanada 2003; Nübel et al. 2002; van der Meer et al. 2005). Similarly, clone LP24 was affiliated with the *Chloroflexi*, and its closest relative was also detected in hot spring mats in Thailand (Table S1). Few clones in the library were affiliated to cyanobacteria, *Chloroflexus* and *Roseiflexus*, which may be because sampling of the clone library did not attain completion according to the rarefaction curves, and so further sampling from the library may have revealed more of these sequences. On the other hand, cluster 2 was closely related to the *Chloroflexi* and contained *Chloroflexi*-like sequences detected in thermal environments (Table S1, Fig. 5). For example, clone LP22 was most closely related to a clone that was detected in the red layer of a microbial mat. This novel lineage may represent novel *Chloroflexi*-like organisms present in the LP mat. Similarly, recent metagenomic analysis of cyanobacterial mats from YNP showed the presence of the first chlorophototrophic member of Kingdom *Chloroflexi* that lies outside the monophyletic group of chlorophototrophs of the Order *Chloroflexales* (Klatt et al. 2011).

This layered mat community lay on top of anoxic sediment. Studies have shown that these sediments contain anaerobic bacteria that ferment organic substrates from the upper layers thereby recycling nutrients within the mat community (Doemel and Brock 1977; Ward et al. 1998). Indeed, some clones affiliated with the *Proteobacteria* and *Firmicutes*, which are groups that have varied metabolism, including anaerobic degradation and sulphate reduction.

Two deeply branching bacterial groups, *Nitrospirae* and *Deinococcus* were also detected in the LP mat. Three clone sequences affiliated within the *Nitrospirae* showed relatedness to the genus *Thermodesulfovibrio*, which is the only thermophilic genus in this phylum. It is a fermentative, sulphate-reducing bacterium that oxidises substrates to acetate (Maki 2001), though the clones from the library represent distant relatives of *Thermodesulfovibrio* and so it is not known if they share the same physiology. Similarly, *Nitrospirae*-like sequences have previously been detected in hot spring mats in Tibet (Lau et al. 2009). Two clones from the library were affiliated to the *Deinococcales*, whose thermophilic representatives have been isolated from hot springs (Ferreira et al. 1997), though to our knowledge they are not often associated with laminated mats.

Other bacteria detected in the clone library belonged to the *Proteobacteria* (delta, beta and alpha divisions), *Firmicutes*, *Bacteroidetes* and *Actinobacteria*. Similarly, these groups have been detected in other hot spring mats in YNP (Ward et al. 1998), Thailand (Portillo et al. 2009) and Tibet (Lau et al. 2009). These groups have a varied metabolism, so we can only speculate at what functions these bacteria play in the mat community.

Archaeal community composition in the LP mat

Methanogenesis was first reported in hot spring laminated mats as a major functional process within these environments by Ward in (1978). Methanogenic *Euryarchaeota* has since been detected within many laminated mats (Ward et al. 1998). This is entirely consistent with the molecular analysis in this study where the *Methanobacteriales* was one of the most frequently observed groups. Heterotrophic microorganisms in the lower layers decompose organic matter from the primary producers and synthesise organic acids (Doemel and Brock 1977). Methanogenic archaea within the deeper anoxic layers contributes to the carbon cycling by converting these organic acids into methane.

The other archaeal clones from the library that affiliated with the *Crenarchaeota* and other *Euryarchaeota* showed little homology to sequences from known species. The recently described *Thaumarchaeota* play an important role in the nitrogen cycle by oxidising ammonia to nitrite in the marine environment. Surveys have shown the occurrence of archaeal genes involved in oxidising ammonia, most likely occurring from hyperthermophilic *Crenarchaetoa* in hot spring (unlaminated) mats in Iceland, Russia, the Great Basin and YNP (USA) and China (Reigstad et al. 2008; Zhang et al. 2008a, b). It is not known if this also occurs in laminated mats, especially since studies on the microbial communities in hot spring laminated mats in Japan (Nakagawa and Fukui 2002) and Thailand (Portillo et al. 2009) failed to detect any archaea.

Bacterial community composition in the Tengchong mat

The TC mat was composed of macroscopic white streamers that were firmly attached to the surrounding rocks. Similar morphological structures have been characterised from a number of different regions (Nakagawa and Fukui 2002; Purcell et al. 2006; Reysenbach et al. 1994; Reysenbach et al. 2000; Skirnisdottir et al. 2000; Takacs et al. 2001; Yamamoto et al. 1998). Although such streamers appear in a range of colours they are all predominantly made up of *Aquificales* which are important in the biological oxidation of sulphur compounds. Similarly, the TC library was dominated by clones affiliated with the *Aquificae*, giving a combined clone frequency of 25 out of 33 clones screened (Table S1) and likely made up the bulk mass of the streamer mat community. Phylogenetic analysis showed that the TC *Aquificae* did not affiliate with any of the known hot spring streamer groups of *Aquificae* according to the groupings outlined by Skirnisdottir et al. (2000). Much research has been conducted into the abiotic factors that influence streamer mat development. However, it has recently been suggested that mere presence of *Aquificae* in a hot spring environment is not the sole reason for the development of streamer mats (since *Aquificae* were ubiquitous in the samples from that study, even where streamer mats were absent), but rather the presence or absence of other bacteria determines whether streamer mats can develop. It was postulated that *Thermotogae* was a group that was required for streamer mat development (Meyer-Dombard et al. 2011), a group that was likewise detected in the TC streamer community.

Other bacteria detected in the TC streamer mat community belonged to the *Deltaproteobacteria*, *Elusimicrobia*, '*Deinococcus-Thermus*', *Thermotogae* and unidentified clusters 1 and 2. However, the frequency that these clones were observed in the library (once each) suggests that they were rarer members of the streamer mat community. The closest relatives of these clones were previously detected in thermal environments: the closest relative to clone TC15 was found in Mammoth Hot Springs in YNP, and clone TC12 was most closely related to a *Thermus* sp. that was also detected Frog Mouth hot spring in the Tengchong region; likewise, clone TC11 was most closely related to clone detected in Eryuan hot spring in the Yunnan region, while clone TC23 was most closely related to a clone that was detected in a sulphur-metabolising microbial mat which was found Nakabusa hot spring in Japan (Table S1). The TC clones affiliated with clusters 1 and 2 represent possible novel taxa whose metabolism and ecological role are yet to be determined. However, these too have relatives that were previously observed in thermal environments: clone TC22 was closely related to a clone detected in a hot spring in Taiwan and clone TC19 was closely related to a clone detected in a hot spring in YNP (Table S1). *Deltaproteobacteria*, '*Deinococcus-Thermus*', *Thermotogae* have all previously been reported in streamer mat communities (Meyer-Dombard et al. 2011; Nakagawa and Fukui 2002; Skirnisdottir et al. 2000).

Archaeal community composition in the TC mat

Clone sequences TC4 and 7 (with a combined clone frequency of 3) were affiliated with the *Crenarchaeota*, which have likewise been previously reported in other streamer mat communities (Meyer-Dombard et al. 2011; Nakagawa and Fukui 2002; Purcell et al. 2006; Skirnisdottir et al. 2000). In particular, TC7 was affiliated with the *Desulfurococcales*, which are hyperthermophilic organisms that can grow organotrophically by sulphur respiration of various organic substrates or by fermentation. Members of the *Desulfurococcales* thereby provide a dual role in carbon fixation and utilising organic substrates from primary production. However, they can also grow chemolithotrophically, though cultivated lithotrophs in this group have only ever been isolated from hydrothermal vents (Huber and Stetter 2001).

One clone (TC3; clone frequency of 3) clustered with the *Methanobacteriales*, and so it is tempting to speculate that methanogenesis occurs within this streamer community. However, methanogenesis in streamer mat communities has not been previously observed, so further studies would be needed to confirm this. A *Methanothermococcus*-like euryarchaeon clone was detected in a streamer community in hot springs in Thailand (Purcell et al. 2006); to our knowledge, this is the only other example of a streamer community containing any *Euryarchaeota*.

Clone TC6 (which occurred only once in the library) is affiliated with the *Korarchaeota*. Similarly, *Korarchaeota* have been detected in black streamer communities in Octopus Spring at YNP (Reysenbach et al. 2000) and the Hengill geothermal areas of Iceland (Skirnisdottir et al. 2000). The major component of the archaeal community (77 %) in the latter streamer mat was the *Korarchaeota* (Skirnisdottir et al. 2000), which is in contrast to our findings where the observed frequency in our library suggests that the *Korarchaeota* were rare in the TC mat community. A more recent study showed *Korarchaeota* to comprise only a minor fraction of the total microbial community in hot springs of Iceland and Russia and that where *Korarchaeota* was present, *Aquificae* was the most dominant organism found (Reigstad et al. 2010). Our study and previous findings confirm the co-existence of *Korarchaeota* with *Aquificales* in hot spring environments.

Clone TC1's (affiliated with cluster 3) closest known relative was Candidatus *Caldiarchaeum subterraneum*, a recently described archaeon from geothermal water that is distinct from all known phyla within the domain *Archaea* (Nunoura et al. 2011). Clone TC1 shows 85 % homology with *Caldiarchaeum subterraneum* and may represent further members of this novel phylum that is yet to be proposed. The ecological significance of this archaeon remains to be seen, though preliminary genome sequence studies indicate that it may be chemolithotrophic (Nunoura et al. 2011).

Clones TC9 and TC8 were affiliated with clusters 1 and 2, respectively indicating the presence of novel archaea within this consortium that are distinct from known organisms. Further cultivation studies are required to determine what roles these potentially novel archaea play in the streamer mat. The community structures of different streamers vary widely. For example, in contrast to our results, blue streamers found hot springs in Iceland (Takacs et al. 2001) and the pink streamers found in YNP (Reysenbach et al. 1994) did not contain any archaeal phylotypic signals. The variations in community composition within different streamers can presumably be explained by the geochemical differences that promote different physiological activities in the environments that they inhabit.

Conclusions

The microbial community compositions of the two microbial mats have been shown to be phylogenetically distinct, a result which is consistent with both the macroscopic morphological characteristics of the communities and the dominant microenvironmental parameters. Since the bacterial communities most likely made up the bulk mass of the microbial mat, it is not clear as to what extent the archaea contribute to the structure of the mats, though they are likely to be functional components of the larger prokaryotic communities. Since many of the 16S rRNA gene sequences were not closely matched to those of known cultured organisms, cultivation followed by physiological studies would be required to determine their precise functional roles within the mat communities. Nevertheless, this culture-independent study has provided a valuable insight into the diversity of potentially novel microorganisms that inhabit these thermal environments. Moreover, this study will contribute to a wider global study of thermophile diversity and ecology of terrestrial geothermal ecosystems (Hedlund et al. 2012).

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