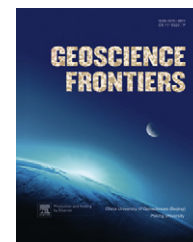


available at www.sciencedirect.com

China University of Geosciences (Beijing)

GEOSCIENCE FRONTIERSjournal homepage: www.elsevier.com/locate/gsf

RESEARCH PAPER

A review of the microbiology of the Rehai geothermal field in Tengchong, Yunnan Province, China

Brian P. Hedlund ^{a,*}, Jessica K. Cole ^{a,b,c,d}, Amanda J. Williams ^a,
Weiguo Hou ^b, Enmin Zhou ^c, Wenjun Li ^c, Hailiang Dong ^{b,d}

^a School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154-4004, USA

^b Geomicrobiology Laboratory, State Key Laboratory of Geological Processes and Mineral Resources, China University of Geosciences, Beijing 100083, China

^c Yunnan Institute of Microbiology, Yunnan University, Kunming 650011, China

^d Department of Geology, Miami University, Oxford, OH 45056, USA

Received 9 October 2011; accepted 6 December 2011

Available online 17 December 2011

KEYWORDS

Geomicrobiology;
Thermophile;
Yunnan;
Rehai;
Tengchong;
PIRE

Abstract The Rehai Geothermal Field, located in Tengchong County, in central-western Yunnan Province, is the largest and most intensively studied geothermal field in China. A wide physicochemical diversity of springs (ambient to ~97 °C; pH from ≤1.8 to ≥9.3) provides a multitude of niches for extremophilic microorganisms. A variety of studies have focused on the cultivation, identification, basic physiology, taxonomy, and biotechnological potential of thermophilic microorganisms from Rehai. Thermophilic bacteria isolated from Rehai belong to the phyla *Firmicutes* and *Deinococcus-Thermus*. *Firmicutes* include neutrophilic or alkaliphilic *Anoxybacillus*, *Bacillus*, *Caldalkalibacillus*, *Caldanaerobacter*, *Laceyella*, and *Geobacillus*, as well as thermoacidophilic *Alicyclobacillus* and *Sulfobacillus*. Isolates from the *Deinococcus-Thermus* phylum include several *Meiothermus* and *Thermus* species. Many of these bacteria synthesize thermostable polymer-degrading enzymes that may be useful for biotechnology. The thermoacidophilic archaea *Acidianus*, *Metallosphaera*, and *Sulfolobus* have also been isolated and studied. A few studies have reported the isolation of thermophilic viruses belonging to *Siphoviridae* (TTSP4 and TTSP10) and *Fuselloviridae* (STSV1) infecting *Thermus* spp. and *Sulfolobus*

* Corresponding author. Tel.: +702 895 0809; fax: 702 895 3950.

E-mail address: brian.hedlund@unlv.edu (B.P. Hedlund).

1674-9871 © 2011, China University of Geosciences (Beijing) and Peking University. Production and hosting by Elsevier B.V. All rights reserved.

Peer-review under responsibility of China University of Geosciences (Beijing).

doi:10.1016/j.gsf.2011.12.006



Production and hosting by Elsevier

spp., respectively. More recently, cultivation-independent studies using 16S rRNA gene sequences, shotgun metagenomics, or “functional gene” sequences have revealed a much broader diversity of microorganisms than represented in culture. Studies of the gene and mRNA encoding the large subunit of the ammonia monooxygenase (*amoA*) of ammonia-oxidizing Archaea (AOA) and the tetraether lipid crenarchaeol, a potential biomarker for AOA, suggest a wide diversity, but possibly low abundance, of thermophilic AOA in Rehai. Finally, we introduce the Tengchong Partnerships in International Research and Education (PIRE) project, an international collaboration between Chinese and U.S. scientists with the goal of promoting international and interdisciplinary cooperation to gain a more holistic and global view of life in terrestrial geothermal springs.

© 2011, China University of Geosciences (Beijing) and Peking University. Production and hosting by Elsevier B.V. All rights reserved.

1. Geological setting and geochemistry

The Rehai (“Hot Sea”) Geothermal Field is located within the Indo-Burma Range near the border between China and Myanmar within Tengchong County (Fig. 1). Geothermal activity is widespread throughout the Indo-Burma Range, resulting from the complex geological history of the region. Tengchong was a microcontinent that separated from the Southern Continent during the Triassic period. It then adhered to the Eurasian Plate during the Jurassic and was captured in the continent-to-continent collision between the Eurasian and Indian Plates during the Late Cretaceous (Liao and Guo, 1986; Wang et al., 2008). During this period, subduction of Tethys oceanic lithosphere drove intrusion of granites into the Tengchong area (Liao and Guo, 1986). Continued plate movement and subduction of oceanic crust lead to extensive volcanism beginning in the Pleiocene or Miocene and reaching maximal activity ~1 Ma during the Late Pliocene (Nakai et al., 1993; Liao, 1995). Current geothermal activity throughout the region is due to latent heat associated with this historic igneous activity and is typically located along arched fault structures or within circular depressions (Liao and Guo, 1986).

Of the >150 km² geothermal areas in and around Tengchong County, Rehai is the site of the most concentrated hydrothermal activity, highest thermal energy (9.21×10^{18} J; Liao and Guo, 1986), and hottest subterranean reservoir (>200 °C; Zhang et al., 1987). Rehai lies within a circular depression in the Zao-tanghe River Valley. The high thermal energy and high Cl[−] discharge are consistent with a local magma source (Liao and Guo, 1986; Zhang et al., 1987). The site is divided by the Zao-tanghe River, which flows from west to east, and most of the geothermal activity and hydrothermally altered rocks and soils are located along a north-south trending fault (Fig. 1), which provides a conduit for geothermal fluids (Zhu and Tong, 1987). Rehai springs display a very wide diversity in source temperature and chemistry, despite their close proximity to each other, which likely results from phase separation in the subsurface and different degrees of mixing with shallow waters. Alkaline springs such as Gumingquan and Zimeiquan, which have source water of ~96 °C and pH ~9.3, represent water-dominated end-members (Zhang et al., 2008b). These springs are characterized by high total dissolved solids (TDS, >1 g/L), high Cl[−], high silica, and are classified as Na-Cl-HCO₃ systems (Zhang et al., 2008b). By contrast,

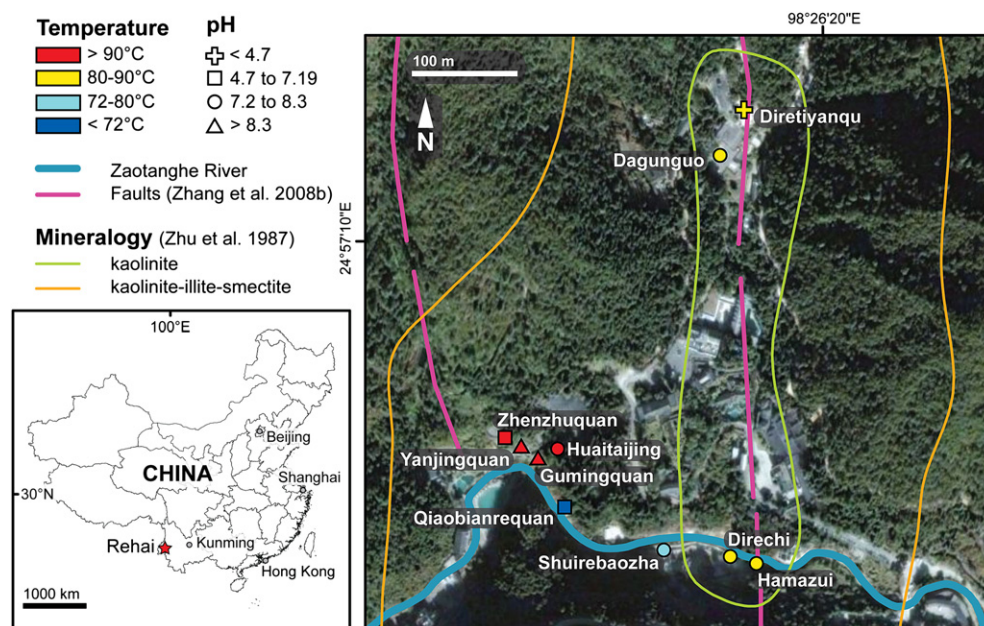


Figure 1 Location and physicochemical properties of geothermal features in the Rehai Geothermal Field, Yunnan, China. Fault locations and delineations of bulk mineralogy are approximate and based upon work from Zhang et al. (2008b) and Zhu and Tong, 1987.

Diretiyanqu, an area with sulfur-depositing fumaroles (Zhu and Tong, 1987), small acidic pools, and extensively altered clays (Zhu and Tong, 1987), represents a vapor-dominated end-member (Zhang et al., 2008b). Other springs, such as Zhenzhuquan may be temporally variable and influenced by shallow sources that are affected by seasonal precipitation. Table 1 summarizes the names and a general description of some of the geothermal features in Rehai from which thermophilic microorganisms have been isolated.

The wide diversity of physical and chemical conditions that exist within Rehai provides niches for diverse microorganisms. The goals of this review include: (1) to summarize the literature on the biology of thermophilic bacteria, archaea, and viruses inhabiting Rehai hot springs; (2) to provide information and citations, in English, of some of the relevant Chinese literature to promote its use by the international community; (3) to provide recommendations for future work; and (4) to introduce the Tengchong PIRE project, which intends to address some of the recommendations.

This review is focused on thermophiles and hyperthermophiles, which, by definition, have optimal growth temperatures of $>55^{\circ}\text{C}$ and $>80^{\circ}\text{C}$, respectively (Gottschal and Prins, 1991; Stetter, 2006); therefore, we do not discuss mesophiles isolated from Rehai in detail. Similarly, we emphasize that this review is focused on the Rehai system and therefore does not generally cover studies conducted in other hot springs. In some cases, the literature is not clear on the precise sampling locations within Yunnan Province and so it is uncertain whether studies were conducted at Rehai. In most of these cases, we have included those studies in this review since those organisms are likely to inhabit Rehai springs, even if isolated elsewhere. Finally, we concede that the literature on the physiology of model organisms isolated from Rehai is extensive and as a result we are unable to summarize all studies on these model organisms, particularly *Caldanaerobacter subterraneus* subsp. *tengcongensis* MB4 and “*Acidianus tengchongensis*”. We have reviewed some studies on the catabolic activities of these organisms, yet we refer the reader to the literature for more detailed information on their molecular biology and anabolic properties.

2. Thermophilic bacteria

Many bacterial isolates from Rehai belong to the phylum *Firmicutes*, representing the orders *Bacillales*, *Clostridiales*, and *Thermoanaerobacteriales* (Fig. 2; Table 2). These organisms are incredibly diverse, including acidophiles, neutrophiles, and alkaliphiles; thermophiles and mesophiles; aerobes, facultative anaerobes, and strict anaerobes; and chemoorganotrophs and chemolithotrophs. Other isolates belong to the *Deinococcus-Thermus* phylum in the genera *Meiothermus* or *Thermus* (Fig. 3; Table 2).

2.1. Thermoacidophilic Firmicutes

Thermoacidophilic bacteria from Rehai belong to the genera *Alicyclobacillus* or *Sulfobacillus* in the phylum *Firmicutes*. Acidophilic mesophiles are more diverse, including ferrous iron- and sulfur-oxidizing strains of *Acidiphilum*, *Acidothiobacillus*, *Alicyclobacillus*, *Leptospirillum*, and *Sulfobacillus* (Liu et al., 2007; Jiang et al., 2009), though these mesophiles are not discussed in detail here. Although the location of sampling within Rehai is generally not reported for thermoacidophilic bacteria,

locations reported for mesophilic or moderately thermophilic acidophiles include heated soils near Dagunguo, Huaitaijing, and Zhenzhuquan (Jiang et al., 2009). Acidophilic bacteria are also inhabitants of Diretiyanqu, which has a number of small pools that vary in pH from 1.8 to 2.9 and in temperature from 40°C to 88°C (Table 1). Zhenzhuquan is also acidic, though cultivation-independent studies show that the source pool is dominated by archaea, specifically *Sulfolobales* (Song et al., 2010).

Alicyclobacillus has been isolated by several research groups (Chen et al., 2004; Ding et al., 2008; Jiang et al., 2008, 2009; Wu et al., 2008). All strains are acidophilic, with pH optima around 3.0. Temperature optima vary considerably, ranging from *Alicyclobacillus acidocaldarius* ($T_{\text{opt}} 70^{\circ}\text{C}$), to *Alicyclobacillus sendaiensis* ($T_{\text{opt}} 55^{\circ}\text{C}$), to *Alicyclobacillus ferrooxydans* ($T_{\text{opt}} 28^{\circ}\text{C}$). Other isolates belonging to *A. acidocaldarius* and perhaps novel species have optimal growth temperatures of $43\text{--}52^{\circ}\text{C}$ (Chen et al., 2004) or growth temperature ranges of $17\text{--}40^{\circ}\text{C}$ (Jiang et al., 2009). All thermophilic strains are aerobic, saccharolytic, endospore-forming chemoorganotrophs capable of starch hydrolysis. None of the thermophilic strains were shown to grow anaerobically or chemolithotrophically, although careful experimentation would be necessary to test those characteristics explicitly. Related strains can respire Fe^{3+} in schwertmannite or goethite anaerobically (Lu et al., 2010) or grow chemolithotrophically by oxidizing aqueous Fe^{2+} , pyrite, $\text{S}_4\text{O}_6^{2-}$, or $\text{S}_2\text{O}_3^{2-}$ (Jiang et al., 2008, 2009).

The genome of *A. acidocaldarius* strain Tc-4-1 was recently reported and it, along with *A. acidocaldarius* strain DSM446, is the only genome sequenced from this genus (Chen et al., 2011b). The 3,124,048 bp genome encodes 44 ABC transporters and several glycoside hydrolases, including amylase, xylanase, mannanase, β -glucosidase, and β -galactosidase. At least one of these enzymes, mannanase AaManA, has been studied biochemically and the function of two active site glutamic acid residues involved in acid-base catalysis and nucleophile catalyst, respectively, were verified (Xu et al., 2011). AaManA was originally identified as the first member of a novel glycoside hydrolase family, GH113, in a different strain, which was also isolated from Rehai, *A. acidocaldarius* Tc-12-31 (Zhang et al., 2008c). AaManA has no significant primary sequence similarity to known glycoside hydrolases, so the enzyme was discovered in a mannanase-positive clone from a clone library from strain Tc-12-31. The purified enzyme is an endo- β -1,4-mannanase with transglycosylase activity and with optimal activity on polymers with five or more mannose monomers. The enzyme was crystallized and the structure was determined by X-ray diffraction, showing that AaManA forms a GH-A (β/α)₈ barrel, with related putative GH113 members in other *Firmicutes* (Zhang et al., 2008c,d).

Two *Sulfobacillus* isolates have been described from Rehai, and both are closely related to the type strain of *Sulfobacillus thermosulfidooxidans*. Strain YN22 is moderately thermophilic ($T_{\text{opt}} 53^{\circ}\text{C}$) and extremely acidophilic ($\text{pH}_{\text{opt}} 1.5$). It was reported to couple Fe^{2+} or S^0 oxidation to growth but was unable to oxidize sulfides (Ding et al., 2007a,b). Strain TCS-5-6, isolated from heated soils near Huaitaijing, was also moderately thermophilic ($T_{\text{range}} 30\text{--}55^{\circ}\text{C}$) and was able to grow by aerobic oxidation of Fe^{2+} , pyrite, or $\text{S}_4\text{O}_6^{2-}$ (Jiang et al., 2009).

2.2. Neutrophilic and alkaliphilic Firmicutes

Neutrophilic and alkaliphilic thermophiles belonging to the *Firmicutes* from Rehai include the genera *Anoxybacillus*, *Bacillus*, *Caldalkalibacillus*, *Geobacillus*, *Laceyella*, or *Caldanaerobacter*.

Table 1 Names and basic descriptions of geothermal features discussed in this review.

Pinyin name (synonyms)	Chinese name	English name (synonyms)	GPS location	Source temp. (°C)	Source pH	Description
Dagunguo	大滚锅	Great Boiling Pot (Big Boiling Pan)	N24.95352°, E98.43795°	84.5 ^a	7.20 ^a	Largest and most prominent spring at Rehai; roughly cylindrical with diameter 5–6 m and depth ~1.5 m, with two vigorous degassing sources. Sediment minerals: amorphous silicate mineral.
Diretiyanqu	地热体验区	Experimental Site (Science Investigation Area)	N24.95390°, E98.43819°	85.1 ^a , 87.6 ^b	2.58 ^a , 4.93 ^b	Shallow pools and fumaroles. Acidic, with high sulfate and ammonium content. ^a Turbid. Temporally variable. Sediment minerals: amorphous mineral, kaolinite, quartz, microcline, chlorite-vermiculite-montmorillonite. ^a
Huaitaijing	怀胎井	Pregnancy Spring	N24.95102°, E98.43645°	90 (left), 92.3 ^a (right)	8.11 (left), 8.05 ^a (right)	Two wells, ~1 m in diameter, with cobble bottoms.
Gumingquan	鼓鸣泉	Drum-Beating Spring	N24.95093°, E98.43626°	93.0	9.35 ^a	Small source pool with a high flow rate (10.4 L/S); lots of pink streamers in pool near bridge at ~80–83 °C. Sediment minerals: amorphous mineral, microcline, albite, quartz. ^a
Yanjingquan (Zimeiquan)	姊妹泉 (眼镜泉)	Sister Spring (Spectacles Spring)	N24.95102°, E98.43613°	93.6 (left), 83.2 (right)	9.25 (left), 9.39 (right)	Pair of small (~1 m diameter) shallow (depth 5–7 cm) springs. Lots of pink streamers in right pool at ~80–83 °C. Sediment minerals: amorphous mineral, microcline, quartz, calcite. ^a
Zhenzhuquan	珍珠泉	Pearl Spring	N24.95110°, E98.43597°	93.3 ^a , 91.0 ^b	4.70 ^a , 6.14 ^b	Shallow, acidic spring with many vigorous degassing sources. Sediment minerals: amorphous mineral, albite, microcline, quartz. ^a
Hamazui	蛤蟆嘴	Frog Mouth	N24.95006°, E98.43830°	84 ^c	8.0 ^c	Large geyser next to waterfall along Zaotanghe River.
Shuirebaozha	水热爆炸	Hydrothermal Explosion	N24.95014°, E98.43743°	72.1 ^d	8.27 ^d	Shallow feature with many geothermal water and gas sources.
Direchi	地热池	Geothermal Pond	N24.95009°, E98.43807°	83 ^d	8.29 ^d	Artificial canal system near Hamazui view point with several geothermal sources in the east end of the canals. Canals flow east to west. Cooler sections have extensive phototrophic mats.
Qiaobianrequan	桥边热泉	Bridge Spring	N24.95044°, E98.43650°	69 ^a	7.0 ^a	Geothermal water coming from a pipe located next to a small Pagoda on the north side of the Zaotanghe River at the bridge.

^a Parameter measured during January, 2011 by PIRE team.^b Parameter measured during August, 2011 by PIRE team.^c Parameter reported in Cai et al., 2006.^d Parameter measured during June, 2011 by PIRE team.

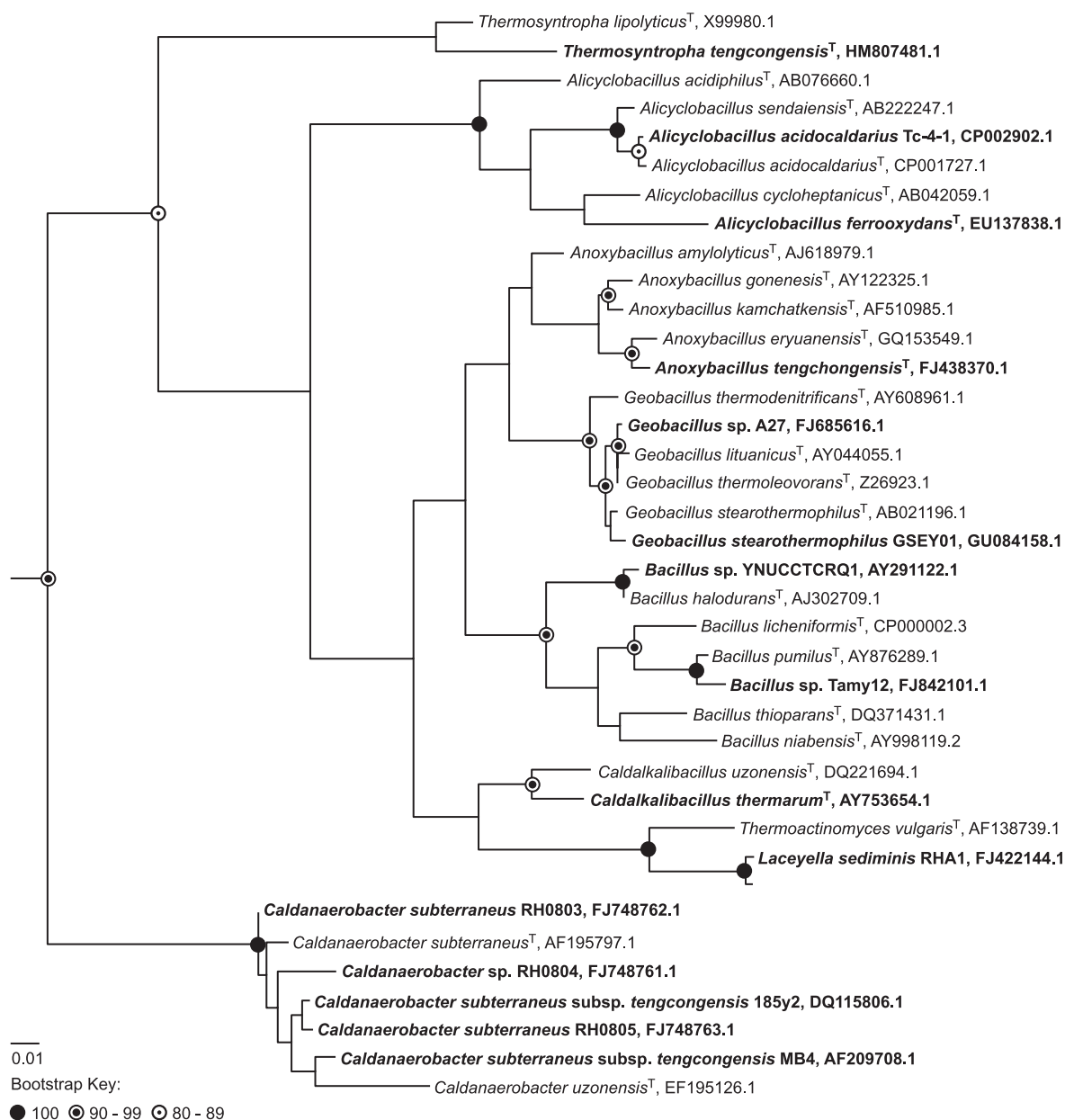


Figure 2 Phylogenetic reconstruction of representative *Firmicutes* isolated from Rehai springs (bold) and related validly published species. The genus *Sulfobacillus* was not included. Nearly complete 16S rRNA gene sequences were aligned to the Silva archaea reference alignment using mothur (Schloss et al., 2009) and the phylogeny was constructed using the maximum-likelihood program DNAML (Felsenstein, 1993). The outgroup was *Streptomyces coelicolor* ATCC 23899^T. Symbols represent bootstrap values for 100 reconstructions. Bar, 0.01 changes per nucleotide.

A few reports have focused on isolates that secrete depolymerizing enzymes with potential for industrial application, whereas others have focused more on identification, characterization, and taxonomy.

Thermophilic strains of the genera *Bacillus* and *Geobacillus* isolated from Rehai have been used for studies of potentially useful depolymerizing enzymes. Strains of *Bacillus pumilus* and a *Geobacillus* spp. produce α -amylases with optimal activity on soluble starch at 80 °C and pH 5.0 and 70 °C and pH 5.6, respectively (Ding et al., 2010; Wang et al., 2011). A strain related to *Bacillus halodurans* produces a xylanase with optimal activity at 80 °C and an extremely wide pH range of activity (pH 2–12)

(Li et al., 2004). A strain related to *Geobacillus stearothermophilus* produces a thermostable protease with optimal activity at 80 °C and pH 7.5 (Liao et al., 2010). Beyond the study of these extracellular enzymes, not much is known about the *Bacillus* and *Geobacillus* isolates from Rehai, though they likely have characteristics typical of these two genera.

A pair of novel species of *Anoxybacillus*, *Anoxybacillus tengchongensis* and *Anoxybacillus eryuanensis*, was recently isolated from hot springs in Tengchong and Eryuan counties in Yunnan Province (Zhang et al., 2011a). Both new species are moderately thermophilic (T_{opt} 50 °C and T_{opt} 55 °C), moderately alkaliphilic (pH_{opt} 8.5 and pH_{opt} 8.0), aerobic, and endospore-

Table 2 Summary of thermophilic bacteria and bacteriophage isolated from hot springs in the Rehai Geothermal Field.

Taxon ^a	Temp. range and [optimum (°C)]	pH range and (optimum)	Comments	Isolation/description reference
Phylum <i>Firmicutes</i>				
Order <i>Bacillales</i>				
<i>Alicyclobacillus</i> (<i>A. acidocaldarius</i> , <i>A. sendaiensis</i> , <i>A. ferrooxydans</i> , others)	<i>A. ac.</i> : (70) <i>A. se.</i> : 45–70 (55) <i>A. fe.</i> : 17–40 (28) Others: 17–52	<i>A. ac.</i> : (3.0) <i>A. se.</i> : 1.5–4.0 <i>A. fe.</i> : 2.0–6.0 (3.0) Others: 3.5–5.5 or NR ^c	Characterized strains are aerobic and saccharolytic; degrade soluble starch <i>A. ac.</i> : genome sequenced and contains glycoside hydrolases <i>A. fe.</i> : Fe ²⁺ , pyrite, S ₄ O ₆ ²⁻ , S ₂ O ₃ ²⁻ oxidizer Others: Fe ²⁺ , pyrite, S ₄ O ₆ ²⁻ oxidizers; isolated from soils near Huaitaiquan or Dagunguo	Chen et al., 2011b; Chen et al., 2004; Ding et al., 2008; Jiang et al., 2008; Jiang et al., 2009; Wu et al., 2008; Zhang et al., 2008c
<i>Anoxybacillus tengchongensis</i>	30–75 (50)	7–11 (8.5)	Facultative anaerobe; capable of NO ₃ ⁻ reduction; saccharolytic; degrades starch	Zhang et al., 2011a
<i>Bacillus</i> (<i>B. pumilus</i> , <i>B. halodurans</i>)	<i>B. pu.</i> : 37–70 (55) <i>B. ha.</i> : NR ^c	NR ^c	<i>B. pu.</i> : used for studies of amylase; <i>B. ha.</i> : used for study of xylanase	Ding et al., 2010; Li et al., 2004
<i>Caldalkalibacillus thermarum</i>	45–65 (60)	7.5–10 (8.5)	Strict aerobic; alkaliphilic; chemoorganotrophic; isolated from Gumingquan	Xue et al., 2006
<i>Geobacillus</i> spp.	(60)	NR ^c	Used for studies of extracellular amylase or protease	Wang et al., 2011; Liao et al., 2010
<i>Laceyella sediminis</i>	<i>L. se.</i> : 28–65 (55)	<i>L. se.</i> : 5.0–9.0 (7.0)	Strict aerobic; chemoorganotrophic; oxidizes sugars, amino acids, and starch	Chen et al., 2011a
Order <i>Thermoanaerobacterales</i>				
<i>Caldanaerobacter</i> (<i>C. subterraneus</i> subsp. <i>tengcongensis</i> ^b , others)	<i>C. su.</i> : 50–80 (75) Others: 60–80 (70)	<i>C. su.</i> : 5.5–9.0 (7.0–7.5) Others: 5.5–8.5 (7.0)	<i>C. su.</i> : strict anaerobe capable of fermentation or S ⁰ or S ₂ O ₃ ²⁻ reduction; degrades soluble starch; genome sequenced Others: strict anaerobe; chemoorganotrophic; saccharolytic, degrades soluble starch	Xue et al., 2001; Fardeau et al., 2004; Lu et al., 2009
Order <i>Clostridiales</i>				
<i>Sulfobacillus thermosulfidoxidans</i>	25–60 (53) or 30–55	1.0–5.0 (1.5)	Chemoorganotrophic; oxidize S ⁰ or Fe ²⁺ (strain YN22) or Fe ²⁺ , pyrite, S ₄ O ₆ ²⁻ (strain TCS-5-6); TCS-5-6 isolated from soils near Huaitaijing	Ding et al., 2007a; Ding et al., 2007b; Jiang et al., 2009
<i>Thermosyntrophia tengcongensis</i>	55–70 (60)	7.0–9.3 (8.2)	Syntrophic oxidation of fatty acids with a hydrogenotrophic methanogen	Zhang et al., 2011b
Phylum <i>Deinococcus-Thermus</i> ^d				
<i>Meiothermus</i> (<i>M. rosaceus</i> , others)	37–70 (55)	(8)	Many contain plasmids; functions unknown	Chen et al., 2002
<i>Thermus</i> (<i>T. Brockianus</i> , <i>T. oshimai</i> , “ <i>Thermus rehai</i> ”, others)	<i>T. br</i> , <i>T. os.</i> : NR ^c <i>T. re.</i> : 40–80 (65–70)	<i>T. br</i> , <i>T. os.</i> : NR ^c <i>T. re.</i> : 4.5–10 (7.5–8.5)	<i>T. br</i> , <i>T. os.</i> : aerobic chemoorganotrophs <i>T. re.</i> : aerobic chemoorganotroph; reduces NO ₃ ⁻	Lin et al., 2002; Lin et al., 2005

Bacteriophage ^d	Taxonomic groups below phylum that were not included in the list of prokaryotic names with standing in nomenclature (http://www.bacterio.cict.fr/index.html) as of 09/27/11 are included in quotations to clarify that these groups have no formal taxonomic affiliation.	50–78 (65)	7.0	Extremely long, flexible tail fibers reaching 785 nm in length	Lin et al., 2010
Tengchong <i>Thermus</i> Siphoviridae phage TTSP4	<i>Thermoanaerobacter tengcongensis</i> was reassigned to a new genus and species, <i>Caldanaerobacter subterraneus</i> , based on phylogenetic and physiological differences from the genus <i>Thermoanaerobacter sensu stricto</i> (Fardeau et al., 2004); however, most of the literature since 2004 refers to the former name.	65	7.6	Extremely long, flexible tail fibers reaching 837 nm in length	Hong et al., 2010

^a Taxonomic groups below phylum that were not included in the list of prokaryotic names with standing in nomenclature (<http://www.bacterio.cict.fr/index.html>) as of 09/27/11 are included in quotations to clarify that these groups have no formal taxonomic affiliation.

^b *Thermoanaerobacter tengcongensis* was reassigned to a new genus and species, *Caldanaerobacter subterraneus*, based on phylogenetic and physiological differences from the genus *Thermoanaerobacter sensu stricto* (Fardeau et al., 2004); however, most of the literature since 2004 refers to the former name.

^c NR, not reported.

^d Another lineage of *Thermus* was isolated by Lin and not ascribed to a species (Lin et al., 2005). A much higher diversity of *Meiothermus* and *Thermus*, including novel species-level groups, have been described recently at an international meeting (Zhou, E., Tang, X., Zhang, J., Yu, T., Song, Z., Tang, S., and Li, W. 2011. Diversity of culturable thermophilic bacteria in geothermal area of Tengchong, China. 11th International Conference on Thermophiles Research. September 11–16, 2011 in Big Sky, MT, U.S.A.).

forming. Only *A. tengchongensis* was reported to reduce NO_3^- to NO_2^- . Both organisms are saccharolytic and degrade soluble starch.

A new species of *Laceyella*, *Laceyella sediminis*, was isolated from a geothermal spring in Rehai (Chen et al., 2011a). *L. sediminis* is a strict aerobe that oxidizes a variety of organic substrates, including sugars, amino acids, and starch. The organism is moderately thermophilic (T_{opt} 55 °C) and neutrophilic (pH_{opt} 7.0) and, like other members of the family *Thermoactinomycetaceae*, produces aerial and substrate mycelia bearing endospores.

A novel genus and species of *Firmicutes*, *Caldalkalibacillus thermarum*, was isolated from Gumingquan. *C. thermarum* is moderately thermophilic (T_{opt} 60 °C) and alkaliphilic (pH_{opt} 8.5), which is consistent with the high pH of the spring from which it was isolated (Xue et al., 2006). It is a strict aerobe, incapable of NO_3^- reduction, and chemoorganotrophic, oxidizing sugars and organic acids. However, in contrast to many thermophilic *Firmicutes* isolated from Rehai, it does not degrade polymers effectively.

A new species, *Thermosyntropha tengcongensis*, and only the second species in this genus, was isolated from a Rehai spring (Zhang et al., 2011b). This new species is moderately thermophilic (T_{opt} 60 °C) and alkaliphilic (pH_{opt} 8.2) and appears to be an obligate organic acid fermenter. The organism was able to grow by fermenting crotonate in pure culture or by fermenting saturated, straight-chain fatty acids with 4–18 carbon atoms or unsaturated fatty acids such as oleate in co-culture with *Methanothermobacter thermoautotrophicus* DSM1053^T.

2.3. *Caldanaerobacter*

The genus *Caldanaerobacter* in the *Firmicutes* is the best-studied group of bacteria isolated from Rehai, with >90 publications on the biology of *Caldanaerobacter* strains isolated from Rehai, most of them focusing on strain MB4. Strain MB4 was isolated from an unidentified spring in Rehai and originally ascribed to a new species in the genus *Thermoanaerobacter*, *Thermoanaerobacter tengcongensis* (Xue et al., 2001). However, the isolate was later transferred to a new genus and species in the *Thermoanaerobiaceae*, *C. subterraneus* (Fardeau et al., 2004). To distinguish it from related isolates from oil reservoirs, the Rehai isolate was given its own subspecies, *C. subterraneus* subsp. *tengcongensis*. MB4 is moderately thermophilic (T_{opt} 75 °C) and neutrophilic (pH_{opt} 7.0–7.5) and, like all members of the family *Thermoanaerobiaceae*, strictly anaerobic. *C. subterraneus* subsp. *tengcongensis* MB4 is capable of fermentation, with acetate, CO_2 , H_2 , ethanol, and alanine as the dominant products of glucose fermentation, or anaerobic respiration with S^0 or $\text{S}_2\text{O}_3^{2-}$ as electron acceptors (Fardeau et al., 2004). MB4 is capable of starch utilization and is saccharolytic when growing chemoorganotrophically, but can also couple CO oxidation to growth (Fardeau et al., 2004). The strain does not produce endospores and the peptidoglycan layer is thin, resulting in a Gram-negative stain, despite its position in the *Firmicutes*. Since the isolation of MB4, other research groups have reported isolation of *Caldanaerobacter* strains from Rehai springs. These include very close relatives of *C. subterraneus* subsp. *tengcongensis*, including one containing an amylase with optimal activity at 70 °C and pH 7.0 (Zhou et al., 2007), and others that likely represent novel species (Lu et al., 2009).

The genome sequence of *C. subterraneus* subsp. *tengcongensis* MB4 was determined almost 10 years ago (Bao et al., 2002), leading to a large number of studies on the molecular biology of

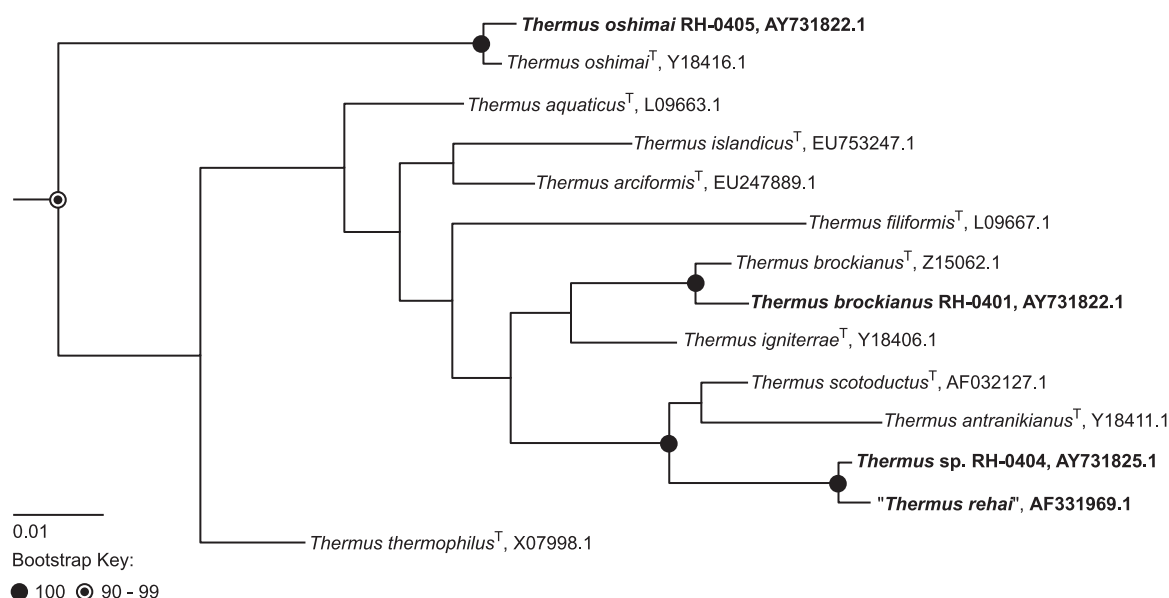


Figure 3 Phylogenetic reconstruction of representative *Thermus* strains isolated from Rehai springs (bold) and all validly published species in the genus *Thermus*. Nearly complete 16S rRNA gene sequences were aligned to the Silva bacteria reference alignment using mothur (Schloss et al., 2009) and the phylogeny was constructed using the maximum-likelihood program DNAML (Felsenstein, 1993). The outgroup was *Meiothermus ruber* ATCC 35948^T. Symbols represent bootstrap values for 100 reconstructions. Bar, 0.01 changes per nucleotide.

the organism. The 2,689,554 bp genome encodes 2588 predicted coding sequences, of which the vast majority (86.7%) are located on the leading strand of DNA replication. Strain MB4 has two hydrogenases that have been verified biochemically, a ferredoxin-dependent, membrane-bound NiFe hydrogenase and an NADH-dependent, cytoplasmic Fe-only hydrogenase (Soboh et al., 2004). Phylogenetic analysis has shown that the NiFe hydrogenase was transferred horizontally from a relative of the *Methanosarcinales* (Calteau et al., 2005). Hydrogen production was sensitive to [H₂], with higher partial pressures of H₂ leading to a shift toward lower H₂ production and a concomitant increase in ethanol production (Soboh et al., 2004).

A number of depolymerizing enzymes from MB4 have been functionally characterized, including glycosyl hydrolases and a diversity of esterases. One γ -amylase (glucan 1,4- α -glucosidase; TtGluA) is relatively unstable at high temperature and therefore used for site-directed mutagenesis efforts to increase thermostability; however, thermostability gains were moderate (Zhou et al., 2010). A second γ -amylase (TtcGA) from strain MB4 is the most thermostable bacterial γ -amylase known, with optimal activity at 75 °C and pH 5.0 and with no activity loss after incubation at 75 °C for 6 h (Zheng et al., 2010). The enzyme hydrolyzes both 1,4- α - and 1,6- α -glycosidic linkages with preference for malto-oligosaccharides over polysaccharides. A β -1,4-endoglucanase, Cel5A, from MB4 was similarly heat stable, with optimal activity at 75–80 °C on carboxymethyl cellulose. It is also exceptionally tolerant of high salt concentration, with half maximal activity in 3 mol/L NaCl or 4 mol/L KCl, and highly resistant to ionic solvents used for cellulose pre-treatment during biofuels processing (Liang et al., 2011a). Subsequently, error-prone polymerase chain reaction (PCR) was used to mutate Cel5 and mutant forms with wider temperature ranges and higher specific activity were recovered (Liang et al., 2011b).

Several esterases from MB4 have been studied biochemically. The *lipA* gene of MB4 has amino acid similarity to true lipases as

well as carboxylesterases; however, studies of LipA expressed in *Escherichia coli* showed high activity on short-chain *p*-nitrophenyl esters (C3–C4), moderate activity on middle-chain *p*-nitrophenyl esters (C6–C12), but very little activity on long-chain *p*-nitrophenyl esters (C16), meaning that it is not a true lipase (Zhang et al., 2003). The enzyme was optimally active at 70 °C and at pH 9, and extremely thermostable, with >80% activity after 20 h of incubation at 50 °C. A true lipase from MB4, LipCst, was shown to have optimal activity on *p*-nitrophenyl caprate (C10) and 30% activity on *p*-nitrophenyl palmitate (C16) (Royter et al., 2009). LipCst was optimally active at 75 °C and pH 8 and had a half-life of 48 h at 75 °C. The enzyme was remarkably resistant to organic solvents and detergents and was highly stereoselective. A third esterase from MB4, LipA3, with maximal activity at 70 °C and pH 9.5 was shown to have optimal activity on *p*-nitrophenyl caprate (C10) and significant activity on larger substrates (Rao et al., 2011). LipA3 was shown to be phylogenetically distinct from other characterized lipases and has a modified consensus catalytic domain for bacterial lipases, leading to the proposal of a novel family of lipases, Family XIV, with LipA3 as the first characterized member. Two other esterases with activity on aromatic esters have also recently been described from MB4, one of which is a thermostable feruloyl esterase (Abokitse et al., 2010; Grosse et al., 2010).

2.4. *Deinococcus-Thermus* phylum

A few studies have reported the isolation and study of isolates of the genera *Meiothermus* or *Thermus* from Rehai. Over a hundred strains identified as "*Meiothermus rosaceus*" have been isolated from hot springs in Tengchong and Eryuan counties in Yunnan Province (Chen et al., 2002); however, these strains have not been described in detail and "*M. rosaceus*" is not a validly described species. Over 50 strains identified as *Thermus* have been isolated from Rehai alone (Lin et al., 2002, 2005; Guo et al., 2003). Some isolates belong to the known species *Thermus oshimai* and

Thermus brockianus, but others appear to represent at least two novel species (Lin et al., 2005). All described strains have the common characteristics of the genus *Thermus*, described as non-sporeforming moderate thermophiles with optimum growth near neutral pH and capable of growth by aerobic oxidation of sugars, sugar alcohols, and some polymers. One novel group has been named “*Thermus rehai*”, but that name is not validly published (Lin et al., 2002, 2005). “*T. rehai*” is moderately thermophilic (T_{opt} 65–70 °C; T_{max} < 80 °C) and is capable of starch depolymerization and reduction of NO_3^- to NO_2^- . However, the NO_3^- reduction activities of *Thermus* are diverse, including incomplete denitrification to N_2O (Hedlund et al., 2011), and further experimentation is necessary to better understand the activities of the Rehai isolates. No *Thermus* strains from Rehai are reported to grow anaerobically by metal reduction; however, some *Thermus* strains are known to reduce metals (Balkwill et al., 2004), and therefore explicit testing would be required to determine whether the strains from Rehai can reduce metals.

3. Thermophilic archaea

3.1. Thermoacidophilic archaea

In comparison with bacteria, the studies reporting cultivation and characterization of archaea from Rehai are rather limited, and all

reports focus on the order *Sulfolobales* in the phylum *Crenarchaeota* (Fig. 4; Table 3). All known members of the *Sulfolobales* are thermoacidophiles and those described from Rehai are no exception. The genera isolated from Rehai are *Acidianus*, *Metallosphaera*, and *Sulfolobus*. Two different strains of *Acidianus* isolated from Rehai have been reported. *Acidianus* strain S5 was isolated from an unidentified spring at Rehai and proposed to represent a novel species, “*A. tengchongensis*” (He et al., 2000, 2004). “*A. tengchongensis*” differs from its closest relative, *Acidianus brierleyi*, by its inability to grow chemolithotrophically by Fe^{2+} oxidation, its inability to grow chemoorganotrophically, and by low DNA-DNA hybridization values (44%). However, strain S5 has a nearly identical 16S rRNA gene sequence to *A. brierleyi* (99.8% nucleotide identity) and the new species has never been validly published. Strain S5 is moderately thermophilic (T_{opt} 70 °C) and acidophilic (pH_{opt} 2.5), capable of anaerobic growth with H_2 as electron donor and S^0 or $\text{S}_2\text{O}_3^{2-}$ as terminal electron acceptors, or aerobic growth by S^0 oxidation to sulfuric acid.

A few studies into the physiology and molecular biology of “*A. tengchongensis*” have been conducted. Strain S5 has been used as a model system for the study of the biochemistry of chemolithotrophic oxidation of S^0 and other inorganic sulfur species, along with the better-studied species *Acidianus ambivalens*. Both organisms lack the multi-subunit Sox complex common in bacterial sulfur-oxidizing chemolithotrophs and, instead, use a cytoplasmic sulfur oxidoreductase (SOR) enzyme to

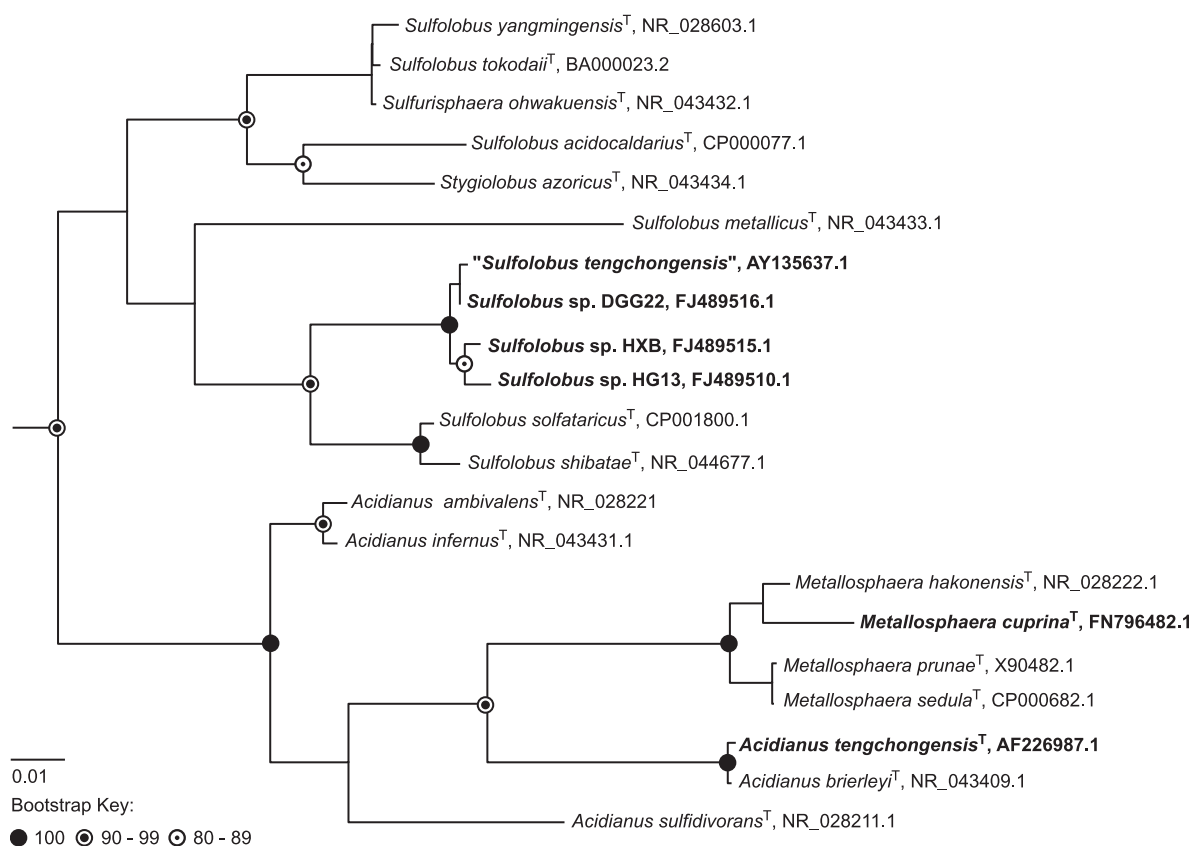


Figure 4 Phylogenetic reconstruction of representative archaea isolated from Rehai springs (bold) and all validly published species in the order *Sulfolobales*. “*Acidianus manzaensis*” isolates from Rehai were not included. Nearly complete 16S rRNA gene sequences were aligned to the Silva archaea reference alignment using mothur (Schloss et al., 2009) and the phylogeny was constructed using the maximum-likelihood program DNAML (Felsenstein, 1993). The outgroup was *Pyrolobus fumarii* DSM 11204^T. Symbols represent bootstrap values for 100 reconstructions. Bar, 0.01 changes per nucleotide.

Table 3 Summary of thermophilic archaea and archaeal viruses isolated from hot springs in the Rehai Geothermal field.

Taxon ^a	Temperature range and [optimum (°C)]	pH range and (optimum)	Comments	Isolation/description reference
<i>Crenarchaeota</i> (<i>Sulfolobales</i>)				
<i>Acidianus</i> (“ <i>A. tengchongensis</i> ”, “ <i>A. manzaensis</i> ”)	<i>A. te.</i> : 55–80 (70) <i>A. ma.</i> : 50–85 (65)	<i>A. te.</i> : 1.0–5.5 (2.5) <i>A. ma.</i> : 1.0–6.0 (1.5–2.5)	<i>A. te.</i> : facultative anaerobe; obligate chemolithoautotroph; anaerobic H ₂ oxidation coupled to S ⁰ reduction; aerobic growth coupled to S ⁰ or S ₂ O ₃ ²⁻ oxidation <i>A. ma.</i> : same as above, but able to grow chemoorganotrophically on sugars, amino acids; can oxidize Fe ²⁺	Ding et al., 2011; He et al., 2000; He et al., 2004; He et al., 2008
<i>Metallosphaera cuprina</i>	55–75 (65)	2.5–5.5 (3.5)	Strict aerobe; facultative chemolithotroph; saccharolytic; capable of chemolithotrophic growth on S ⁰ , S ₄ O ₆ ⁴⁻ , FeSO ₄ , pyrite or chalcopyrite; genome sequenced	Liu et al., 2011b
“ <i>Sulfolobus tengchongensis</i> ” Other <i>Sulfolobus</i> strains ^b	<i>S. te.</i> : 65–95 (85–90) Others: 55–93 (75)	<i>S. te.</i> : 1.7–6.5 (3.5) Others: 1.5–6.0 (3.5)	Strict aerobe; facultative chemolithotroph; saccharolytic; capable chemolithotrophic growth on S ⁰	Chen et al., 2008; Han et al., 2010; Xiang et al., 2003
Viruses				
<i>Sulfolobus tengchongensis</i> Spindle-shaped virus (STSV1) ^c	80	3.3	Largest known member of <i>Fuselloviridae</i> ; genome sequenced (75 kb)	Xiang et al., 2005

^a Taxonomic groups below phylum that were not included in the List of Prokaryotic Names with Standing in Nomenclature (<http://www.bacterio.cict.fr/index.html>) as of 09/27/11 are included in quotations to clarify that these groups have no formal taxonomic affiliation.

^b The other *Sulfolobus* strains isolated from Rehai are phylogenetically and physiologically very similar to “*S. tengchongensis*” and they likely represent a single, well-defined species.

^c Temperature and pH reported here were those used for growth. These conditions were not optimized.

catalyze the O_2 -dependent disproportionation of S^0 into HSO_3^- and S^{2-} , which are each oxidized to SO_4^{2-} via the combined action of other oxidoreductases (Sun et al., 2003; He et al., 2004; Kletzin, 2008). Site-directed mutagenesis showed that conserved cysteines in the “*A. tengchongensis*” SOR are involved in SOR function (Chen et al., 2005). Electron micrographs of immunogold-labeled SOR suggested an association with the cytoplasmic membrane (Chen et al., 2005), where SOR forms a 24-subunit hollow sphere (Li et al., 2008).

A second strain of *Acidianus* from Rehai, strain YN25, was recently identified as “*Acidianus manzaensis*” due to its close affiliation with a strain from a Japanese fumarole. However, “*A. manzaensis*” is not validly described. Strain YN25 has similar properties to “*A. tengchongensis*”, but is able to grow chemoorganotrophically and by chemolithotrophic oxidation of Fe^{2+} (He et al., 2008; Ding et al., 2011). Strain YN25 was shown to efficiently leach Cu^{2+} from chalcopyrite, which was enhanced in the presence of activated carbon (Liang et al., 2010).

A single strain of *Metallosphaera* has been reported from Rehai, representing the novel species *Metallosphaera cuprina* (Liu et al., 2011b). Like other members of the genus, *M. cuprina* is strictly aerobic, moderately thermophilic (T_{opt} 65 °C), and acidophilic (pH_{opt} 3.5). Unlike other *Metallosphaera* species, *M. cuprina* can grow chemoorganotrophically by oxidizing sugars as well as chemolithoautotrophically by oxidizing S^0 , $S_4O_6^{4-}$, $FeSO_4$, pyrite or chalcopyrite. The genome of *M. cuprina* was recently reported (Liu et al., 2011a). The genome is 1,840,348 bp, 16% smaller than its relative *Metallosphaera sedula*, and analysis of the genome was consistent with the facultative chemoautotrophic lifestyle of *M. cuprina*. The genome contains a 2-hydroxypropionate/4-hydroxybutyrate cycle for carbon fixation. In addition, it has an Entner-Doudoroff pathway, a complete tricarboxylic acid cycle, and an incomplete pentose phosphate pathway for transport and oxidation of organic compounds.

A few members of the genus *Sulfolobus* have been isolated from Rehai, including “*Sulfolobus tengchongensis*” RT8-4 and closely related strains (Xiang et al., 2003; Chen et al., 2008; Han et al., 2010). “*S. tengchongensis*” RT8-4 has characteristics common to the genus *Sulfolobus* (Xiang et al., 2003). It is a strict aerobe capable of growth by oxidation of sugars or S^0 . “*S. tengchongensis*” is a true hyperthermophile, with optimal growth at 85 °C and maximal growth at 95 °C, which is the highest described for the genus. RT8-4 has low DNA-DNA hybridization values with other *Sulfolobus* species and is phylogenetically distinct; however, “*S. tengchongensis*” has not been validly published.

Twelve strains closely related to “*S. tengchongensis*” RT8-4 were subsequently isolated from a variety of geothermal features at Rehai, including Dagunguo (though the acidic pH and temperature reported indicates these samples were not from the source pool), Diretiyanqu, Huangguajing, and heated acidic soils near Hamazui and the Zaoatanghe River (Chen et al., 2008; Han et al., 2010). Together, all strains form a coherent phylogenetic group.

4. Thermophilic viruses

Viruses are the only known predators of microorganisms living above ~60 °C, with an incredible diversity of thermophilic viruses known to infect thermophilic bacteria and archaea (Rachel et al., 2002; Prangishvili and Garrett, 2004; Prangishvili et al.,

2006). A few studies have reported isolation of thermophilic viruses from Rehai infecting either *Thermus* spp. or *Sulfolobus* spp. Two phage infecting *Thermus* host strains have been described in the literature, with both belonging to the family *Siphoviridae*. *Thermus Siphoviridae* phage 4 (TSP4) and TSP10 are both lytic phage isolated on the basis of plaque formation on lawns of uncharacterized *Thermus* strains (Hong et al., 2010; Lin et al., 2010). TSP4 and TSP10 have naked (no envelope) icosahedral heads of 73 nm and 67 nm diameter, respectively, and exceptionally long tails reaching 785 nm and 837 nm in length and 10 nm in diameter.

A single virus infecting *S. tengchongensis* has been isolated by selecting plaques from filtered spring water on a lawn of the host strain (Xiang et al., 2005). *S. tengchongensis* spindle-shaped virus 1 (STSV1) is the largest known member of the *Fuselloviridae*, with a spindle-like morphology (230 by 107 nm) and a tail of variable length (68 nm average). STSV1 is enveloped and not lytic; plaque formation is attributed to a retardation of growth of *Sulfolobus*. STSV1 does not integrate into the host genome and its DNA is heavily modified. The genome of STSV1 is 75,294 bp, encoding 74 predicted coding sequences, including genes predicted or known to code for structural proteins, polysaccharide synthesis, nucleotide metabolism, and DNA modification. STSV1 is similar in structure to viruses infecting *Sulfolobus* spp. in Yellowstone National Park (Rachel et al., 2002; Rice et al., 2004); however, the host range of STSV1 is distinct from those strains and the genomes share little similarity.

5. Cultivation-independent studies

5.1. Phylogenetic censuses and metagenomics

A small number of studies have reported microbial communities in hot springs in and near Tengchong, including Rehai, by analysis of 16S rRNA genes that were amplified by the polymerase chain reaction (PCR) from DNA isolated directly from environmental samples. Two of these studies relied on density-gradient gel electrophoresis (DGGE) instead of DNA sequencing (Liu et al., 2006; Li, 2009; Ren et al., 2009). DGGE can be difficult to interpret and, in these cases, was not used to identify the organisms. A few other studies performed sequencing of up to 23 16S rRNA genes, which may allow for the identification of a few dominant organisms, but is far from exhaustive, even in extreme environments. Altogether, these studies identified *Hydrogenobacter*, *Thermus*, *Pseudomonas*, *Bacillus*, and a member of the *Thermodesulfobacteriaceae* in samples from Dagunguo (Wang et al., 2003a, b). Most of the 16S rRNA gene sequences were related to mesophilic *Pseudomonas* and *Bacillus* species, so these samples may not have been collected or stored properly. Another study reported 16S rRNA gene sequences representing *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Deinococcus-Thermus*, and *Aquificae* from a pink streamer community from Zimeiquan (Zhang et al., 2004).

A couple of recent cultivation-independent studies were significantly more comprehensive. One focused on archaea in eight Tengchong spring samples, including sites in Rehai and other locations in Tengchong, resulting in a total of 826 16S rRNA gene fragments, representing 47 species-level operational taxonomic units (OTUs) (Song et al., 2010). Three samples were from low pH habitats, which ranged in temperature from 96 °C (Zhenzhuquan, pH 4.3), to 74 °C (Huangguanqing, pH 2.8), to

44 °C (Dagunguo, pH 3.4; this sample is from a photosynthetic mat adjacent to the source pool and not from the source pool itself). The archaeal community in Zhenzhuquan was exclusively *Sulfolobales*, composed of *M. cuprina* and three novel species- or genus-level groups most closely related to *M. cuprina* and *Sulfolobus tokodaii*. The other two acidic samples contained higher diversity, including novel species- to genus-level groups of *Sulfolobales* and *Desulfurococcales* related to *Metallosphaera*, *Thermosphaera*, *Ignisphaera*, and *Staphylothermus*, and members of uncultivated lineages related to *Crenarchaeota* such as the pJP41 group and the pSL12 group.

Five samples from circumneutral habitats ranged in temperature from 84 °C (Dagunguo source pool, pH 6.6), to 77 °C (Wuming, pH 7.7), to 59 °C (Bridge Spring site A, pH 7.5), to 45 °C (Shuirebaozhaqu, pH 7.5), to 44 °C (Bridge Spring site B, pH 7.5). The highest temperature site, Dagunguo, was comprised almost entirely of *Thermoprotei*, with representatives of all three orders. In contrast, the lowest temperature sites, Shuirebaozhaqu and Bridge Spring site B, were composed almost entirely of novel archaea related to *Crenarchaeota* or *Thaumarchaeota*. The highest diversity was found in the 77 °C and 59 °C springs.

Another phylogenetic census using *Actinobacteria*-specific 16S rRNA gene primers resulted in sequencing of 126 16S rRNA gene fragments from a 81 °C, pH 7.5 spring sample near Hamazui (Song et al., 2009). The study revealed organisms closely related to *Sporichthya polymorpha*, a mesophile in the *Frankinae*, relatives of “*Candidatus Microthrix* spp.”, mesophilic, filamentous *Actinobacteria* yet to be cultivated axenically, and a high number and diversity of novel *Actinobacteria* related to 16S rRNA gene fragments from geographically and physicochemically diverse habitats such as freshwater lakes and soils. Since none of the related organisms grow at high temperature, the role of these organisms in the natural habitat from which they were sampled is uncertain.

A single report has described a metagenomic study on a single sample from a spring in Rehai near Hamazui (Cai et al., 2006; 84 °C, pH 8.0). A small insert library (~3–8 kb) was created and thirty clones were randomly sequenced, with related sequences then identified by BLAST. Most sequences were related to genes from thermophilic bacteria or archaea and are predicted to encode a variety of cellular functions.

5.2. Ammonia-oxidizing archaea

A few studies have focused on biomarkers that may be specific for AOA from hot springs in Tengchong, including Rehai (Dodsworth et al., 2011). One study examined the relative abundance of glycerol dialkyl glycerol tetraether lipids (GDGTs) in hot spring samples; however, crenarchaeol, a possible biomarker for AOA, was not a significant component of the two samples from Tengchong springs, both of which were unidentified 80 °C, pH 6.6 sites (Pearson et al., 2008). A second study used PCR primers specific to the gene encoding the ammonia monooxygenase catalytic subunit (*amoA*), revealing 10 OTUs at 2% nucleic acid identity from five samples from Tengchong hot springs, including two samples from Bridge Spring in Rehai (Zhang et al., 2008a). Most of these *amoA* genes belonged to Cluster A, which is typically found in aquatic environments, including geothermal springs. These samples were all circumneutral and ranged from 43.6 °C to 77 °C. The geochemistry of two samples from Bridge Spring suggested that NH_3 in the source water was oxidized to NO_2^- and NO_3^- in the outflow, suggesting these populations may be active.

Two recent studies have addressed the potential activity of AOA in Tengchong springs by quantifying and sequencing *amoA* transcripts amplified from natural samples by reverse-transcriptase PCR (Huang et al., 2010; Jiang et al., 2010). Interestingly, the transcripts belonged predominantly to Cluster B, which is typically found in soils and sediments, and was a minor component of the *amoA* genes amplified from Tengchong springs. This difference may suggest that AOA with Cluster B-type *AmoA* are less abundant but more active than those with Cluster A-type *AmoA*; however, different springs were studied in the two reports so direct comparisons cannot be made. The studies reported a very low abundance of *amoA* transcripts in the Tengchong hot spring samples, ranging from 4.50×10^4 to 5.62×10^5 mRNA copies per gram of sediment. The abundance of *amoA* transcripts was three to five orders of magnitude lower than the number of 16S rRNA gene copies suggesting low activity and/or low abundance of AOA in these habitats.

6. Conclusion and comparison of microbiology of Rehai and Yellowstone National Park

A variety of microbiology studies have focused on Rehai and other geothermal springs in Yunnan Province. The strength of these studies is rooted in the cultivation, identification, and physiology of microorganisms in pure culture. A few isolates, particularly *C. subterraneus* subsp. *tengcongensis* MB4, have been studied in great detail in terms of genomics, physiology, and biochemistry. More recent work has begun to take advantage of cultivation-independent approaches such as the study of nucleic acid sequences retrieved from natural samples. In most cases, these studies revealed that pure cultures are a poor representation of the natural microbial communities; however, in the case of the high temperature acidic site Zhenzhuquan, there is reasonably good agreement between the low diversity of *Sulfolobales* in cultivation-independent studies and microorganisms in pure culture.

The microbiology work that has been done at Rehai allows some comparison to microbial communities at other locations, such as Yellowstone National Park. Comparisons can be made of microbial isolates as well as cultivation-independent studies. However, it's important to note that cultivation-independent studies, to date, are generally insufficient to make robust comparisons, particularly when viewed within the context of the incredible diversity of physicochemical habits at both locations.

It is worth emphasizing that high temperature acidic sites are clearly the best understood habitats in Rehai from the perspective that they host a very low diversity of organisms belonging to the *Sulfolobales* and their viruses, several of which are available in pure culture. Deep phylogenetic censuses of high temperature acidic sites in Rehai (>85 °C) show that bacteria do not make up a significant part of those communities (unpublished data). The microbial community in Zhenzhuquan is at least superficially similar with those in comparable vapor-dominated springs in Yellowstone National Park, such as Alice Spring (Crater Hills; pH 2.6; 70–74 °C) and Beowulf Spring (Norris Geyser Basin; pH 3.1; 64–70 °C), both of which are dominated by *Sulfolobales*, as evidenced by both 16S rRNA gene censuses and metagenomics (Inskeep et al., 2010). Although communities in both Yellowstone springs are more diverse than that in Zhenzhuquan, this may be due to the significantly higher temperature of Zhenzhuquan. Although cultivation-independent studies of other types of springs

in Rehai are insufficient to allow comparison to Yellowstone springs, the work that has been done shows that a few of the same genera are located in springs in the two locations, such as *Hydrogenobacter* and *Thermus*. Certainly, more cultivation-independent work is needed to support more robust comparisons, particularly taxon-independent and deep 16S rRNA gene censuses, metagenomics, and hybridization-based studies such as PhyloChip (Brodie et al., 2006) and GeoChip (He et al., 2007).

Direct comparisons of microbial isolates from Rehai and Yellowstone or other geothermal systems can also lead to interesting insights. To date, no genera or higher level taxa in axenic culture are unique to Rehai, and in general, we feel it is unlikely that any genus-level or higher groups will be unique to Rehai or Yellowstone. However, the data that exist do suggest that certain species might be endemic to Rehai, or at least present in Rehai but absent from Yellowstone geothermal features. An example of this is “*T. rehai*”, which is composed of a cluster of Rehai isolates with nearly identical 16S rRNA gene sequences (>99% nucleic acid identity), with relatively distant relatives from other geographic regions (<96% nucleic acid identity). If this biogeographic structure for some thermophiles can be confirmed by more detailed study (i.e. some endemism at the species level but cosmopolitanism at the genus level and above), this would conflict with the strict interpretation of Baas-Becking’s dictum “*alles is overal: maar het milieu selecteert*” (everything is everywhere, but the environment selects) (Baas-Becking, 1934; de Wit and Bouvier 2006) and support the proposal to elevate the interpretation of “*alles*” (everything) to the level of the bacterial or archaeal genus, at least in some cases (Hedlund and Staley, 2003). Whether this species-level endemism applies to thermophiles at Rehai and whether it is functionally important remains a topic of future study.

7. Future directions: the Tengchong PIRE project

In the future we anticipate continued progress using this combination of cultivation-dependent and -independent approaches, particularly if new technologies are used within a strong intellectual framework. We recognize the logistical and intellectual limitations inherent to small research groups. To address these limitations, and to work toward a more holistic understanding of the geobiology of Rehai, we advocate a collaborative, interdisciplinary framework. Recognizing the enormous potential of a collaborative framework, one of the authors, Dong, has worked for many years to bring Chinese and U.S. scientists together to increase communication and cooperation in geobiology through a series of international meetings generously funded by both Chinese and American agencies, including the National Natural Science Foundation (China), the Ministry of Science and Technology (China), the Ministry of Education (China), and the National Science Foundation (U.S.). The crowning achievement of these meetings, thus far, is the funding of a China-U.S. collaboration focused on the geobiology of Rehai and other Tengchong hot springs, which is supported by the U.S. National Science Foundation’s Partnerships in International Research and Education (PIRE) program. This partnership, called the Tengchong PIRE project, includes six Chinese universities and eight U.S. universities and it offers research opportunities in China for U.S. students, post-docs, professors, and even high-school teachers (<http://faculty.unlv.edu/pire/>; Stone, 2011). The Tengchong PIRE project has diverse goals, however, unifying scientific goals

include: (1) increasing coordination between collection, analysis, and synthesis of complex datasets, including geochemistry, community composition, community function, and community genomics; (2) integration of data, particularly carbon- and nitrogen-cycle activities to enhance our understanding of the functional consequences of thermophily and the relationship between microbial community structure, composition, and function; (3) integration of data from Rehai with similar data from geographically and biogeochemically diverse settings to work toward global models of biodiversity within terrestrial geothermal habitats and determine whether any functional consequences of endemism exist; and (4) increasing our understanding of the functions of major groups (i.e. novel phyla and classes) of microorganisms that have never been cultivated in the laboratory.

Acknowledgments

The authors thank the entire Tengchong PIRE team for stimulating discussions and strong support. We also thank Chaolei Jiang, Jianxin Fang, Jinchuan Yin, Shaochuan Gong, Jiafu Sheng, and Mr. Ma and the entire staff from Yunnan Tengchong Volcano and Spa Tourist Attraction Development Corporation for strong support. This work was supported generously by the U.S. National Science Foundation (Grant Nos. MCB-0546865 and OISE-0968421 & OISE-0836450) and National Natural Science Foundation of China (Grant No. 31070007).

References

- Abokitse, K., Wu, M., Bergeron, H., Grosse, S., Lau, P.C., 2010. Thermotable feruloyl esterase for the bioproduction of ferulic acid from triticale bran. *Applied Microbiology and Biotechnology* 87, 195–203.
- Balkwill, D.L., Kieft, T.L., Tsukuda, T., Kostandarithes, H.M., Onstott, T.C., Macnaughton, S., Bownas, J., Fredrickson, J.K., 2004. Identification of iron-reducing *Thermus* strains as *Thermus scotoductus*. *Extremophiles* 8, 37–44.
- Bao, Q., Tian, Y., Li, W., Xu, Z., Xuan, Z., Hu, S., Dong, W., Yang, J., Chen, Y., Xue, Y., Xu, Y., Lai, X., Huang, L., Dong, X., Ma, Y., Ling, L., Tan, H., Chen, R., Wang, J., Yu, J., Yang, H., 2002. A complete sequence of the *T. tengcongensis* genome. *Genome Research* 12, 689–700.
- Baas-Becking, L.G.M., 1934. *Geobiologie of Inleiding tot de Milieukunde*. Van Stockum & Zon, The Hague.
- Brodie, E.L., DeSantis, T.Z., Joyner, D.C., Baek, S.M., Larsen, J.T., Andersen, G.L., Hazen, T.C., Richardson, P.M., Herman, D.J., Tokunaga, T.K., Wan, J.M., Firestone, M.K., 2006. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. *Applied and Environmental Microbiology* 72, 6288–6298.
- Cai, Y., Chen, X.Z., Yang, K.Q., Huang, L., Dong, Z.Y., 2006. Construction and analysis of a metagenomic library from Tengchong hot spring soil in Yunnan Province. *Acta Microbiologica Sinica* 46, 427–431 (in Chinese with English abstract).
- Calteau, A., Gouy, M., Perriere, G., 2005. Horizontal transfer of two operons coding for hydrogenases between bacteria and archaea. *Journal of Molecular Evolution* 60, 557–565.
- Chen, B., Wei, Y.L., Jing, S.R., Ji, X.L., Lu, Y.Q., Lin, L.B., 2008. Identification of a thermoacidophilic *Sulfolobus* sp. isolated from a hot spring in Tengchong Rehai. *Microbiology* 35, 1868–1872.
- Chen, C., Lin, L., Peng, Q., Ben, K., Zhou, Z., 2002. *Meiothermus roseaceus* sp. nov. isolated from Tengchong hot spring in Yunnan, China. *FEMS Microbiology Letters* 216, 263–268.
- Chen, J.J., Lin, L.B., Zhang, L.L., Zhang, J., Tang, S.K., Wei, Y.L., Li, W.J., 2011a. *Laceyella sediminis* sp. nov., a thermophilic bacterium

- isolated from a hot spring in China. *International Journal of Systematic and Evolutionary Microbiology* ijs.0.028282-0.
- Chen, Z.W., Jiang, C.Y., Liu, S.J., 2004. Survey on and phylogeny of *Alicyclobacillus* species in hot springs of southern China's Guangdong and Yunnan Provinces. *Microbiology* 31, 50–54 (in Chinese with English abstract).
- Chen, Y., He, Y., Zhang, B., Yang, J., Li, W., Dong, Z., Hu, S., 2011b. Complete genome sequence of *Alicyclobacillus acidocaldarius* strain Tc-4-1. *Journal of Bacteriology* 193, 5602–5603.
- Chen, Z.W., Jiang, C.Y., She, Q., Liu, S.J., Zhou, P.J., 2005. Key role of cysteine residues in catalysis and subcellular localization of sulfur oxygenase-reductase of *Acidianus tengchongensis*. *Applied and Environmental Microbiology* 71, 621–628.
- de Wit, R., Bouvier, T., 2006. 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? *Environmental Microbiology* 8, 755–758.
- Ding, B., Li, Q., Li, X., Liao, Y., Wei, Y., Lin, L., 2010. Study on a thermophilic *Bacillus pumilus* sp. and its thermostable α -amylase. *Science and Technology of Food Industry* 31, 185–190 (in Chinese with English abstract).
- Ding, J., Gao, J., Wu, X., Zhang, C., Wang, D., Qiu, G., 2007a. Jarosite-type precipitates mediated by YN22, *Sulfobacillus thermosulfidooxidans*, and their influences on strain. *Transactions of Nonferrous Metals Society of China* 17, 1038–1044.
- Ding, J., He, H., Zhang, C., Yu, Y., Qiu, G., 2008. Isolation and characterization of YNTC-1, a novel *Alicyclobacillus sentainesis* strain. *Journal of Central South University of Technology* 15, 508–514.
- Ding, J., Yu, Y., He, H., Yin, H., Zhang, C., Qiu, G., 2007b. Isolation and identification of new strain in *Sulfobacillus thermosulfidooxidans*. *Journal of Natural Science of Hunan Normal University* 30, 104–109 (in Chinese with English abstract).
- Ding, J., Zhang, R., Yu, Y., Jin, D., Liang, C., Yi, Y., Zhu, W., Xia, J., 2011. Novel acidophilic, thermophilic iron and sulfur-oxidizing archaeon isolate from a hot spring in Tengchong, Yunnan, China. *Brazilian Journal of Microbiology* 42, 514–525.
- Dodsworth, J.A., Hungate, B., de la Torre, J.R., Jiang, H., Hedlund, B.P., 2011. Measuring nitrification, denitrification, and related biomarkers in continental geothermal ecosystems. *Methods in Enzymology* 486, 171–203.
- Fardeau, M.L., Bonilla Salinas, M., L'Haridon, S., Jeanthon, C., Verhe, F., Cayol, J.L., Patel, B.K., Garcia, J.L., Ollivier, B., 2004. Isolation from oil reservoirs of novel thermophilic anaerobes phylogenetically related to *Thermoanaerobacter subterraneus*: reassignment of *T. subterraneus*, *Thermoanaerobacter yonseiensis*, *Thermoanaerobacter tengcongensis* and *Carboxydibrachium pacificum* to *Caldanaerobacter subterraneus* gen. nov., sp. nov., comb. nov. as four novel subspecies. *International Journal of Systematic and Evolutionary Microbiology* 54, 467–474.
- Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package), Version 3.5. University of Washington, Department of Genetics, Seattle, WA.
- Gottschal, J.C., Prins, R.A., 1991. Thermophiles. A life at elevated temperatures. *Trends in Ecology and Evolution* 6, 157–162.
- Grosse, S., Bergeron, H., Imura, A., Boyd, J., Wang, S., Kubota, K., Miyadera, A., Sulea, T., Lau, P.C., 2010. Nature versus nurture in two highly enantioselective esterases from *Bacillus cereus* and *Thermoanaerobacter tengcongensis*. *Microbial Biotechnology* 3, 65–73.
- Guo, C., Wang, T., Zhu, W., Zhang, D., Cui, X., Xu, L., Peng, Q., 2003. The phylotype of *Thermus* from the rehai geothermal area, Tengchong China. *Journal of Microbiology* 41, 152–156.
- Han, J., Chen, B., Dai, X., Hong, W., Ji, X., Wei, Y., Lin, L., 2010. Diversity of thermoacidophilic *Sulfolobus* in hot springs in Tengchong of Yunnan, China. *Chinese Journal of Applied and Environmental Biology* 16, 692–696.
- He, H., Yang, Y., Xia, J., Ding, J., Zhao, X., Nie, Z., 2008. Growth and surface properties of new thermoacidophilic Archaea strain *Acidianus manzaensis* YN-25 grown on different substrates. *Transactions of Nonferrous Metals Society of China* 18, 1374–1378 (in Chinese with English abstract).
- He, Z.G., Zhong, H., Li, Y., 2004. *Acidianus tengchongensis* sp. nov., a new species of acidothermophilic archaeon isolated from an acidothermal spring. *Current Microbiology* 48, 159–163.
- He, Z., Gentry, T.J., Schadt, C.W., Wu, L., Liebich, J., Chong, S.C., Huang, Z., Wu, W., Gu, B., Jardine, P., Criddle, C., Zhou, J., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *The ISME Journal* 1, 67–77.
- He, Z., Li, Y., Zhou, P., Liu, S., 2000. Cloning and heterologous expression of a sulfur oxygenase/reductase gene from the thermoacidophilic archaeon *Acidianus* sp. S5 in *Escherichia coli*. *FEMS Microbiology Letters* 193, 217–221.
- Hedlund, B.P., Staley, J.T., 2003. Microbial endemism and biogeography, p. 225–231. In: Bull, A.T. (Ed.), *Microbial Diversity and Bioprospecting*. ASM Press, Washington, DC.
- Hedlund, B.P., McDonald, A.I., Lam, J., Dodsworth, J.A., Brown, J.R., Hungate, B.A., 2011. Potential role of *Thermus thermophilus* and *T. oshimai* in high rates of nitrous oxide (N₂O) production in ~80°C hot springs in the U.S. Great Basin. *Geobiology* 9, 471–480.
- Hong, W., Han, J., Dai, X., Wei, Y., Lin, L., 2010. Isolation and characterization of a *Thermus* bacteriophage lytic from Tengchong Rehai hot spring lytic. *Acta Microbiologica Sinica* 50, 322–327 (in Chinese with English abstract).
- Huang, Q., Jiang, H., Zhang, C., Li, W., Deng, S., Yu, B., Dong, H., 2010. Abundance of ammonia-oxidizing microorganisms in response to environmental variables of hot springs in Yunnan Province, China. *Acta Microbiologica Sinica* 50, 132–136.
- Inskip, W.P., Rusch, D.P., Jay, Z.J., Herrgard, M.J., Kozubal, M.A., Richardson, T.H., Macur, R.E., Hamamura, N., Jennings, R.deM., Fouke, B.W., Reysenbach, A.L., Roberto, F., Young, M., Schwartz, A., Boyd, E.S., Badger, J.H., Mathur, E.J., Ortmann, A.C., Bateson, M., Geesey, G., Frazier, M., 2010. Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. *PLoS One* 5, e9773.
- Jiang, C., Liu, Y., Guo, X., Liu, S.J., 2009. Isolation and characterization of ferrous- and sulfur-oxidizing bacteria from Tengchong solfataric region, China. *Journal of Environmental Sciences (China)* 21, 1247–1252.
- Jiang, C.Y., Liu, Y., Liu, Y.Y., You, X.Y., Guo, X., Liu, S.J., 2008. *Alicyclobacillus ferrooxydans* sp. nov., a ferrous-oxidizing bacterium from solfataric soil. *International Journal of Systematic and Evolutionary Microbiology* 58, 2898–28903.
- 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. *Applied and Environmental Microbiology* 76, 4538–4541.
- Kletzin, A., 2008. Oxidation of sulfur and inorganic sulfur compounds in *Acidianus ambivalens*. In: Dahl, C., Friedrich, C.G. (Eds.), *Microbial Sulfur Metabolism*. Springer, pp. 184–201.
- Li, H., 2009. Comparative analysis of the prokaryote diversities of two hyperthermal springs in the west of Yunnan. *Journal of Anhui Agricultural Sciences* 37, 133–135 (in Chinese with English abstract).
- Li, M., Chen, Z., Zhang, P., Pan, X., Jiang, C., An, X., Liu, S., Chang, W., 2008. Crystal structure studies on sulfur oxygenase reductase from *Acidianus tengchongensis*. *Biochemical and Biophysical Research in Communication* 369, 919–923.
- Li, W., Liao, C., Liu, S., Wang, J., Zhou, J., 2004. Xylanase from alkali-tolerant *Bacillus* sp. strain YNUCCTCRQ1 and its phylogenetic analysis. *Chemistry and Industry of Forest Products* 24, 41–44 (in Chinese with English abstract).
- Liang, C., Xue, Y., Fioroni, M., Rodriguez-Ropero, F., Schwaneberg, U., Ma, Y., 2011a. Cloning and characterization of a thermostable and halo-tolerant endoglucanase from *Thermoanaerobacter tengchongensis* MB4. *Applied Microbiology and Biotechnology* 89, 315–326.
- Liang, C., Fioroni, M., Rodriguez-Ropero, F., Xue, Y., Schwaneberg, U., Ma, Y., 2011b. Directed evolution of a thermophilic endoglucanase (Cel5A) into highly active Cel5A variants with an expanded temperature profile. *Journal of Biotechnology* 154, 46–53.

- Liang, C., Xia, J., Zhao, X., Yang, Y., Gong, S., Nie, Z., Ma, C., Zheng, L., Zhao, Y., Qiu, G., 2010. Effect of activated carbon on chalcopyrite bioleaching with extreme thermophile *Acidianus manzaensis*. *Hydrometallurgy* 105, 179–185.
- Liao, Y., Ji, X., Wei, Y., Lin, L., 2010. Study on a thermophilic *Geobacillus* sp. GSEY01 and its thermophilic protease. *Biotechnology Bulletin* 6, 189–194.
- Liao, Z., 1995. A Review of the Rehai (Hot Sea) Geothermal System in Tengchong County, Yunnan Province, China. Geology Department, Peking University, Beijing, China, pp. 1207–1211.
- Liao, Z., Guo, G., 1986. Geology of the Tengchong Geothermal Field and surrounding area, West Yunnan, China. *Geothermics* 15, 339–345.
- Lin, L., Chen, C., Peng, Q., Ben, K., Zhou, Z., 2002. *Thermus rehai* sp. nov., isolated from Rehai of Tengchong, Yunnan Province, China. *Journal of Basic Microbiology* 42, 337–344.
- Lin, L., Hong, W., Ji, X., Han, J., Huang, L., Wei, Y., 2010. Isolation and characterization of an extremely long tail *Thermus* bacteriophage from Tengchong hot springs in China. *Journal of Basic Microbiology* 50, 452–456.
- Lin, L., Zhang, J., Wei, Y., Chen, C., Peng, Q., 2005. Phylogenetic analysis of several *Thermus* strains from Rehai of Tengchong, Yunnan, China. *Canadian Journal of Microbiology* 51, 881–886.
- Liu, H.W., Peng, Q., Li, Q.Y., Xiao, W., Cui, X.L., Lin, L.B., Liu, Z.J., Jiang, C.L., Xu, L.H., Chen, Y.G., Wang, Z.G., Ren, Z., Deng, L., Wen, M.L., 2006. Comparison of direct and indirect environmental DNA extraction methods for samples from a high-temperature environment. *Acta Microbiologica Sinica* 46, 1018–1022 (in Chinese with English abstract).
- Liu, L., You, X.Y., Zheng, H., Wang, S., Jiang, C., Liu, S., 2011a. Complete genome sequence of *Metallosphaera cuprina*, a metal sulfide-oxidizing archaeon from a hot spring. *Journal of Bacteriology* 193, 3387–3388.
- Liu, L.J., You, X.Y., Guo, X., Liu, S.J., Jiang, C.Y., 2011b. *Metallosphaera cuprina* sp. nov., an acidothermophilic, metal-mobilizing archaeon. *International Journal of Systematic and Evolutionary Microbiology* 61, 2395–2400.
- Liu, Y.Y., Chen, Z.W., Jiang, C.Y., Liu, S.J., 2007. Isolation and characterization of *Acidiphilium* strain teng-A and its metabolism of Fe(III) during pure- and mixed cultivation. *Acta Microbiologica Sinica* 47, 350–354 (in Chinese with English abstract).
- Lu, S., Gischkat, S., Reiche, M., Akob, D.M., Hallberg, K.B., Kusel, K., 2010. Ecophysiology of Fe-cycling bacteria in acidic sediments. *Applied and Environmental Microbiology* 76, 8174–8183.
- Lu, Y., Chen, B., Liu, X., Ji, X., Wei, Y., Lin, L., 2009. Isolation and identification of seven thermophilic and anaerobic bacteria from hot springs in Tengchong Rehai. *Acta Microbiologica Sinica* 49, 1234–1239 (in Chinese with English abstract).
- Nakai, S., Xu, S., Wakita, H., Fujii, N., Nagao, K., Orinhashi, Y., Wang, X.B., Chen, J.F., Liao, Z.J., 1993. K-Ar ages of young volcanic rocks from Tengchong Area, western Yunnan, China. *Bulletin Volcanological Society of Japan* 38, 167–171.
- Pearson, A., Pi, Y., Zhao, W., Li, W., Li, Y., Inskeep, W., Perevalova, A., Romanek, C., Li, S., Zhang, C.L., 2008. Factors controlling the distribution of archaeal tetraethers in terrestrial hot springs. *Applied and Environmental Microbiology* 74, 3523–3532.
- Prangishvili, D., Forterre, P., Garrett, R.A., 2006. Viruses of the archaea: a unifying view. *Nature Reviews Microbiology* 4, 837–848.
- Prangishvili, D., Garrett, R.A., 2004. Exceptionally diverse morphotypes and genomes of crenarchaeal hyperthermophilic viruses. *Biochemical Society Transactions* 32, 204–208.
- Rachel, R., Bettstetter, M., Hedlund, B.P., Haring, M., Kessler, A., Stetter, K.O., Prangishvili, D., 2002. Remarkable morphological diversity of viruses and virus-like particles in hot terrestrial environments. *Archives of Virology* 147, 2419–2429.
- Rao, L., Xue, Y., Zhou, C., Tao, J., Li, G., Lu, J.R., Ma, Y., 2011. A thermostable esterase from *Thermoanaerobacter tengcongensis* opening up a new family of bacterial lipolytic enzymes. *Biochimica et Biophysica Acta* 1814, 1695–1702.
- Ren, Z., Wang, Z., Yin, M., Liu, J., Li, Q., Wang, Y., Cui, X., 2009. Comparison of environmental total DNA extraction methods from microbial mat samples in Shizitou hot spring. *Journal of Microbiology* 29, 1–7.
- Rice, G., Tang, L., Stedman, K., Roberto, F., Spuhler, J., Gillitzer, E., Johnson, J.E., Douglas, T., Young, M., 2004. The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. *Proceedings of the National Academy of Sciences of the United States of America* 101, 7716–7720.
- Royter, M., Schmidt, M., Elend, C., Hobenreich, H., Schafer, T., Bornscheuer, U.T., Antranikian, G., 2009. Thermostable lipases from the extreme thermophilic anaerobic bacteria *Thermoanaerobacter thermohydrosulfuricus* SOL1 and *Caldanaerobacter subterraneus* subsp. *tengcongensis*. *Extremophiles* 13, 769–783.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75, 7537–7541.
- Soboh, B., Linder, D., Hedderich, R., 2004. A multisubunit membrane-bound [NiFe] hydrogenase and an NADH-dependent Fe-only hydrogenase in the fermenting bacterium *Thermoanaerobacter tengcongensis*. *Microbiology* 150, 2451–2463.
- Song, Z.Q., Chen, J.Q., Jiang, H.C., Zhou, E.M., Tang, S.K., Zhi, X.Y., Zhang, L.X., Zhang, C.L., Li, W.J., 2010. Diversity of *Crenarchaeota* in terrestrial hot springs in Tengchong, China. *Extremophiles* 14, 287–296.
- Song, Z.Q., Zhi, X.Y., Li, W.J., Jiang, H., Zhang, C.L., Dong, H., 2009. Actinobacterial diversity in hot springs in Tengchong (China), Kamchatka (Russia) and Nevada (USA). *Geomicrobiology Journal* 26, 256–263.
- Stetter, K.O., 2006. History of discovery of the first hyperthermophiles. *Extremophiles* 10, 357–362.
- Stone, R., 2011. Biological dark matter exerts irresistible pull in Yunnan. *Science* 333, 1209.
- Sun, C.W., Chen, Z.W., He, Z.G., Zhou, P.J., Liu, S.J., 2003. Purification and properties of the sulfur oxygenase/reductase from the acidothermophilic archaeon, *Acidianus* strain S5. *Extremophiles* 7, 131–134.
- Wang, C.S., Zhao, X.X., Liu, Z.F., Lippert, P.C., Graham, S.A., Coe, R.S., Yi, H.S., Zhu, L.D., Liu, S., Li, Y.L., 2008. Constraints on the early uplift history of the Tibetan Plateau. *Proceedings of the National Academy of Sciences of the United States of America* 105, 4987–4992.
- Wang, T., Chai, L., Cui, X., Peng, Q., Jiang, C., 2003a. Preliminary study on bacterial diversity in a hot spring by using culture-independent approach. *Acta Microbiologica Sinica* 43, 541–546 (in Chinese with English abstract).
- Wang, T., Chai, L., Guo, C., Cui, X., Xu, L., 2003b. Study on nine 16S rDNA clones came from two hot springs in the west of Yunnan. *Microbiology* 30, 38–42.
- Wang, X., Gao, R., Liao, C., Meng, Y., Tang, X., Li, J., Xu, B., Huang, Z., 2011. Isolation of *Geobacillus* sp. A27 and characterization of its α -amylase. *Science and Technology of Food Industry* 32, 151–155 (in Chinese with English abstract).
- Wu, X., Zhu, R., Ding, J., Wei, J., Li, Y., Qiu, G., 2008. Isolation, characterization of heterotrophic acidothermophile TC-2 and its influence on bioleaching of chalcopyrite. *Chinese Journal of Nonferrous Metals* 18, 2259–2264 (in Chinese with English abstract).
- Xiang, X., Chen, L., Huang, X., Luo, Y., She, Q., Huang, L., 2005. *Sulfolobus tengchongensis* spindle-shaped virus STSV1: virus-host interactions and genomic features. *Journal of Virology* 79, 8677–8686.
- Xiang, X., Dong, X., Huang, L., 2003. *Sulfolobus tengchongensis* sp. nov., a novel thermoacidophilic archaeon isolated from a hot spring in Tengchong, China. *Extremophiles* 7, 493–498.
- Xu, S., Zhang, C., Xue, Z., He, G., Ju, J., Zhao, B., 2011. Identification of the catalytic residues of mannanase from *Alicyclobacillus acidocaldarius*. *Acta Microbiologica Sinica* 51, 66–74 (in Chinese with English abstract).

- Xue, Y., Xu, Y., Liu, Y., Ma, Y., Zhou, P., 2001. *Thermoanaerobacter tengcongensis* sp. nov., a novel anaerobic, saccharolytic, thermophilic bacterium isolated from a hot spring in Tengchong, China. *International Journal of Systematic and Evolutionary Microbiology* 51, 1335–1341.
- Xue, Y., Zhang, X., Zhou, C., Zhao, Y., Cowan, D.A., Heaphy, S., Grant, W.D., Jones, B.E., Ventosa, A., Ma, Y., 2006. *Caldalkalibacillus thermarum* gen. nov., sp. nov., a novel alkalithermophilic bacterium from a hot spring in China. *International Journal of Systematic and Evolutionary Microbiology* 56, 1217–1221.
- Zhang, C.L., Ye, Q., Huang, Z., Li, W., Chen, J., Song, Z., Zhao, W., Bagwell, C., Inskeep, W.P., Ross, C., Gao, L., Wiegel, J., Romanek, C.S., Shock, E.L., Hedlund, B.P., 2008a. Global occurrence of archaeal *amoA* genes in terrestrial hot springs. *Applied Environmental Microbiology* 74, 6417–6426.
- Zhang, C.M., Huang, X.W., Pan, W.Z., Zhang, J., Wei, K.B., Klenk, H.P., Tang, S.K., Li, W.J., Zhang, K.Q., 2011a. *Anoxybacillus tengchongensis* sp. nov. and *Anoxybacillus eryuanensis* sp. nov., facultatively anaerobic, alkalitolerant bacteria from hot springs. *International Journal of Systematic and Evolutionary Microbiology* 61, 118–122.
- Zhang, D.H., Li, Q.Y., Liu, Y., Peng, Q., 2004. Analysis of bacterial composition of the pink mat from spectacles hot spring in Tengchong by culture-independent approach. *Acta Microbiologica Sinica* 44, 820–823 (in Chinese with English abstract).
- Zhang, F., Liu, X., Dong, X., 2011b. *Thermosyntropho tengcongensis* sp. nov., a thermophilic bacterium that syntrophically degrades long-chain fatty acids. *International Journal of Systematic and Evolutionary Microbiology*. doi:10.1099/ijs.0.033456-0.
- Zhang, G., Liu, C.-Q., Liu, H., Jin, Z., Han, G., Li, L., 2008b. Geochemistry of the Rehai and Ruidian geothermal waters, Yunnan Province, China. *Geothermics* 37, 73–83.
- Zhang, Y., Ju, J., Peng, H., Gao, F., Zhou, C., Zeng, Y., Xue, Y., Li, Y., Henrissat, B., Gao, G.F., Ma, Y., 2008c. Biochemical and structural characterization of the intracellular mannanase AaManA of *Alicyclobacillus acidocaldarius* reveal a novel glycoside hydrolase family belonging to clan GH-A. *Journal of Biological Chemistry* 283, 31551–31558.
- Zhang, Y., Gao, F., Xue, Y., Zeng, Y., Peng, H., Qi, J., Ma, Y., 2008d. Crystallization and preliminary X-ray study of the native and selenomethionyl β -1,4-mannanase AaManA of *Alicyclobacillus acidocaldarius* Tc-12-31. *Journal of Biological Chemistry* 283, 31551–31558.
- Zhang, J., Liu, J., Zhou, J., Ren, Y., Dai, X., Xiang, H., 2003. Thermostable esterase from *Thermoanaerobacter tengcongensis*: high-level expression, purification and characterization. *Biotechnology Letters* 25, 1463–1467.
- Zhang, Z., Liu, S., Zhao, F., 1987. Geochemistry of thermal waters in the Tengchong volcanic geothermal area, West Yunnan Province, China. *Geothermics* 16, 169–179.
- Zheng, Y., Xue, Y., Zhang, Y., Zhou, C., Schwaneberg, U., Ma, Y., 2010. Cloning, expression, and characterization of a thermostable glucoamylase from *Thermoanaerobacter tengcongensis* MB4. *Applied Microbiology and Biotechnology* 87, 225–233.
- Zhou, C., Xue, Y., Ma, Y., 2010. Enhancing the thermostability of alpha-glucosidase from *Thermoanaerobacter tengcongensis* MB4 by single proline substitution. *Journal of Bioscience and Bioengineering* 110, 12–17.
- Zhou, Z., Mei, Q., Gao, Y., Yu, F., Shen, B., 2007. Isolation and characterization of *Thermoanaerobacter* sp. 185y2, clone and expression of its thermophilic α -amylase gene. *Journal of Nanjing Agricultural University* 30, 55–60 (in Chinese with English abstract).
- Zhu, M., Tong, W., 1987. Surface hydrothermal minerals and their distribution in the Tengchong geothermal area, China. *Geothermics* 2, 181–195.