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

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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 (TTS4 and TTSP10) and Fuselloviridae (STSV1) infecting Thermus spp. and Sulfolobus

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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.

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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 × 10¹⁸ J; Liao and Guo, 1986), and hottest subterranean reservoir (>200 °C; Zhang et al., 1987). Rehai lies within a circular depression in the Zaotanghe 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 Zaotanghe 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,
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2. Thermophilic bacteria

Many bacterial isolates from Rehai belong to the phylum Firmicutes, representing the orders Bacillales, Clostridiales, and Thermococcales (Fig. 2; Table 2). These organisms are incredibly diverse, including acidiphilic, neutrophilic, and alkaliphilic; thermophiles and mesophiles; aerobes, facultative anaerobes, and strict anaerobes; and chemooorganotrophs 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 Sulfolobus in the phylum Firmicutes. Acidiphilic mesophiles are more diverse, including ferrous iron-and sulfur-oxidizing strains of Acidiphilium, Acidithiobacillus, Alicyclobacillus, Leptospirillum, and Sulfolobus (Jiang et al., 2009; Liu et al., 2007), 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 Dagunungo, Huaitaijing, and Zhenzhuquan (Jiang et al., 2009). Acidiphilic 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_{opt} 70 °C), to Alicyclobacillus sendaiensis (T_{opt} 55 °C), to Alicyclobacillus ferrooxidans (T_{opt} 28 °C). Other isolates belonging to A. acidocaldarius and perhaps novel species have optimal growth temperatures of 43–52 °C (Chen et al., 2004) or growth temperature ranges of 17–40 °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 these characteristics explicitly. Related strains can respire Fe^{2+} in schwertmannite or goethite anaerobically (Lu et al., 2010) or grow chemolithotrophically by oxidizing aqueous Fe^{2+}, pyrite, S_{0}O_{3}^{2-}, or S_{0}O_{6}^{2-} (Jiang et al., 2009, 2008).

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 hydrolasers, including amylyase, 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 hydrolasers, 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 mannosne monomers. The enzyme was crystallized and the structure 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 Sulfolobus isolates have been described from Rehai, and both are closely related to the type strain of Sulfolobus thermosulfidooxidans. Strain YN22 is moderately thermophilic (T_{opt} 53 °C) and extremely acidophilic (pH_{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_{range} 30–55 °C) and was able to grow by aerobic oxidation of Fe^{2+}, pyrite, or S_{0}O_{6}^{2-} (Jiang et al., 2009).

2.2. Neutrophilic and alkalophilic Firmicutes

Neutrophilic and alkalophilic thermophiles belonging to the Firmicutes from Rehai include the genera Anaobacillus, Bacillus, Caldikyalbacillus, Geobacillus, Laceyella, or Caldanaerobacter.
<table>
<thead>
<tr>
<th>Pinyin name (synonyms)</th>
<th>Chinese name</th>
<th>English name (synonyms)</th>
<th>GPS location</th>
<th>Source temp. (°C)</th>
<th>Source pH</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dagunguo</td>
<td>大滚锅</td>
<td>Great Boiling Pot (Big Boiling Pan)</td>
<td>N24.95352°, E98.43795°</td>
<td>84.5°</td>
<td>7.20°</td>
<td>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.</td>
</tr>
<tr>
<td>Diretiyanqu</td>
<td>地热体验区</td>
<td>Experimental Site (Science Investigation Area)</td>
<td>N24.95390°, E98.43819°</td>
<td>85.1°, 87.6°</td>
<td>2.58°, 4.93°</td>
<td>Shallow pools and fumaroles. Acidic, with high sulfate and ammonium content. Temporally variable. Sediment minerals: amorphous mineral, kaolinite, quartz, microcline, chlorite-vermiculite-montmorillonite.</td>
</tr>
<tr>
<td>Huaitaijing</td>
<td>怀胎井</td>
<td>Pregnancy Spring</td>
<td>N24.95102°, E98.43645°</td>
<td>90 (left), 92.3° (right)</td>
<td>8.11 (left), 8.05° (right)</td>
<td>Two wells, ~1 m in diameter, with cobble bottoms.</td>
</tr>
<tr>
<td>Gumingquan</td>
<td>鼓鸣泉</td>
<td>Drum-Beating Spring</td>
<td>N24.95093°, E98.43626°</td>
<td>93.0</td>
<td>9.35°</td>
<td>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.</td>
</tr>
<tr>
<td>Yanjingquan (Zimeiquan)</td>
<td>姊妹泉（眼镜泉）</td>
<td>Sister Spring (Spectacles Spring)</td>
<td>N24.95102°, E98.43613°</td>
<td>93.6 (left), 83.2 (right)</td>
<td>9.25 (left), 9.39 (right)</td>
<td>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.</td>
</tr>
<tr>
<td>Zhenzhuquan</td>
<td>珍珠泉</td>
<td>Pearl Spring</td>
<td>N24.95110°, E98.43597°</td>
<td>93.3°, 91.0°</td>
<td>4.70°, 6.14°</td>
<td>Shallow, acidic spring with many vigorous degassing sources. Sediment minerals: amorphous mineral, albite, microcline, quartz.</td>
</tr>
<tr>
<td>Hamazui</td>
<td>蛤蟆嘴</td>
<td>Frog Mouth</td>
<td>N24.95006°, E98.43830°</td>
<td>84°</td>
<td>8.0°</td>
<td>Large geyser next to waterfall along Zaotanghe River.</td>
</tr>
<tr>
<td>Shuirebaozha</td>
<td>水热爆炸</td>
<td>Hydrothermal Explosion</td>
<td>N24.95014°, E98.43743°</td>
<td>72.1°</td>
<td>8.27°</td>
<td>Shallow feature with many geothermal water and gas sources.</td>
</tr>
<tr>
<td>Direchi</td>
<td>地热池</td>
<td>Geothermal Pond</td>
<td>N24.95009°, E98.43807°</td>
<td>83°</td>
<td>8.29°</td>
<td>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. Geothermal water coming from a pipe located next to a small Pagoda on the north side of the Zaotanghe River at the bridge.</td>
</tr>
<tr>
<td>Qiaobianrquan</td>
<td>桥边热泉</td>
<td>Bridge Spring</td>
<td>N24.95044°, E98.43650°</td>
<td>69°</td>
<td>7.0°</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) 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.
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 stea-thermophilus* 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.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Temp. range and [optimum (°C)]</th>
<th>pH range and (optimum)</th>
<th>Comments</th>
<th>Isolation/description reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phylum Firmicutes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Order Bacillales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alicyclobacillus</em> (A. acidocaldarius, A. sendaiensis, A. ferrooxydans, others)</td>
<td>A. ac.: (70)</td>
<td>A. ac.: (3.0)</td>
<td>Characterized strains are aerobic and saccharolytic; degrade soluble starch</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>A. se.: 45–70 (55)</td>
<td>A. se.: 1.5–4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. fe.: 17–40 (28)</td>
<td>A. fe.: 2.0–6.0 (3.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others: 17–52</td>
<td>Others: 3.5–5.5 or NR[c]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anoxybacillus tengchongensis</em></td>
<td>30–75 (50)</td>
<td>7–11 (8.5)</td>
<td>Facultative anaerobe; capable of NO₃⁻ reduction; saccharolytic; degrades starch</td>
<td>Zhang et al., 2011a</td>
</tr>
<tr>
<td><em>Bacillus</em> (B. pumilus, B. halodurans)</td>
<td>B. pu.: 37–70 (55)</td>
<td>NR[c]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. ha.: NR[c]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Caldalkalibacillus thermarum</em></td>
<td>45–65 (60)</td>
<td>7.5–10 (8.5)</td>
<td>Strict aerobe; alkaliphilic; chemoorganotrophic; isolated from Gumingquan</td>
<td>Xue et al., 2001; Fardeau et al., 2004; Lu et al., 2009</td>
</tr>
<tr>
<td><em>Geobacillus</em> spp.</td>
<td>(60)</td>
<td>NR[c]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Laceyella sediminis</em></td>
<td>L. se.: 28–65 (55)</td>
<td>L. se.: 5.0–9.0 (7.0)</td>
<td>Strict aerobe; chemoorganotrophic; oxidizes sugars, amino acids, and starch</td>
<td>Wang et al., 2011; Liao et al., 2010; Chen et al., 2011a</td>
</tr>
<tr>
<td><strong>Order Thermoanaerobacteriales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Caldanaerobacter</em> (C. subterraneus subsp. tengcongensis³, others)</td>
<td>C. su.: 50–80 (75)</td>
<td>C. su.: 5.5–9.0 (7.0–7.5)</td>
<td>C. su.: strict anaerobe capable of fermentation or S⁰ or S₂O₃²⁻ reduction; degrades soluble starch; genome sequenced</td>
<td>Xue et al., 2001; Fardeau et al., 2004; Lu et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Others: 60–80 (70)</td>
<td>Others: 5.5–8.5 (7.0)</td>
<td>Others: strict anaerobe; chemoorganotrophic; saccharolytic, degrades soluble starch</td>
<td></td>
</tr>
<tr>
<td><strong>Order Clostridiales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sulfobacillus thermosulfidoxidans</em></td>
<td>25–60 (53) or 30–55</td>
<td>1.0–5.0 (1.5)</td>
<td>Chemoorganotrophic; oxidize S⁰ or Fe²⁺ (strain YN22) or Fe²⁺, pyrite, S₂O₃²⁻ (strain TCS-5-6); TCS-5-6 isolated from soils near Huaiaitajing</td>
<td>Ding et al., 2007a; Ding et al., 2007b; Jiang et al., 2009</td>
</tr>
<tr>
<td><em>Thermosyntropha tengcongensis</em></td>
<td>55–70 (60)</td>
<td>7.0–9.3 (8.2)</td>
<td>Syntrophic oxidation of fatty acids with a hydrogenotrophic methanogen</td>
<td>Zhang et al., 2011b</td>
</tr>
<tr>
<td><strong>Phylum Deinococcus-Thermus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Meiothermus</em> (M. rosaceus, others)</td>
<td>37–70 (55)</td>
<td>(8)</td>
<td>Many contain plasmids; functions unknown</td>
<td>Chen et al., 2002</td>
</tr>
<tr>
<td></td>
<td>T. re.: 40–80 (65–70)</td>
<td>T. re.: 4.5–10 (7.5–8.5)</td>
<td>T. re.: aerobic chemoorganotroph; reduces NO₃⁻</td>
<td></td>
</tr>
</tbody>
</table>
forming. Only *A. tengchongensis* was reported to reduce NO3 to NO2. 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*<sub>opt</sub> 55°C) and neutrophilic (*pH*<sub>opt</sub> 7.0) and, like other members of the family *Thermoaerobiomycetaceae*, 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*<sub>opt</sub> 60°C) and alkaliphilic (*pH*<sub>opt</sub> 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 NO3 reduction, and chemooorganotrophic, 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). The new species is moderately thermophilic (*T*<sub>opt</sub> 60°C) and alkaliphilic (*pH*<sub>opt</sub> 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.<sup>3</sup>

### 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 *Thermoaerobacter*, *Thermoaerobacter tengcongensis* (Xue et al., 2001). However, the isolate was later transferred to a new genus and species in the *Thermoaerobiaceae*, *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*<sub>opt</sub> 75°C) and neutrophilic (*pH*<sub>opt</sub> 7.0–7.5) and, like all members of the family *Thermoaerobiaceae*, strictly anaerobic. *C. subterraneus* subsp. *tengcongensis* MB4 is capable of fermentation, with acetate, CO2, H2, ethanol, and alanine as the dominant products of glucose fermentation, or anaerobic respiration with S<sup>-2</sup> or S<sup>2-</sup> as electron acceptors (Fardeau et al., 2004). MB4 is capable of starch utilization and is saccharolytic when growing chemooorganotrophically, 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 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, cytoplasmic Fe-only hydrogenase (Soboh et al., 2009) and the phylogeny was constructed using the maximum-likelihood program DNAML (Felsenstein, 1993). The outgroup was *Meiothermus ruber* ATCC 35948T. Symbols represent bootstrap values for 100 reconstructions. Bar, 0.01 changes per nucleotide.

A number of depolymerizing enzymes from MB4 have been functionally characterized, including glycosyl hydrodrolases and a diversity of esterases. One γ-amylase (glucan 1,4-α-glucosidase; TlGluA) 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., 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 thermostability; however, thermostability gains were moderate (Zhou et al., 2010). A second γ-amylase, *Thermus* sp. RH-0404, 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) (Royer 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 (Abokite 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 (Guo et al., 2003; Lin et al., 2002, 2005). Some isolates belong to the known species *Thermus oshimai* and *Thermus aquaticus*, both 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-glucosidase; 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) (Royer 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 (Abokite et al., 2010; Grosse et al., 2010).
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 ($T_{\text{opt}}$ 65–70°C; $T_{\text{max}}$ < 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$_2$O (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 ($T_{\text{opt}}$ 70°C) and acidophilic (pH$_{\text{opt}}$ 2.5), capable of anaerobic growth with H$_2$ as electron donor and S$^0$ or S$_2$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 oxidize sulfur compounds.

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 furcii DSM 11204T. 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.

<table>
<thead>
<tr>
<th>Taxona</th>
<th>Temperature range and [optimum (°C)]</th>
<th>pH range and (optimum)</th>
<th>Comments</th>
<th>Isolation/description reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crenarchaeota (Sulfolobales)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acidianus</em> (“<em>A. tengchongensis</em>”, “<em>A. manzaensis</em>”)</td>
<td>A. te.: 55–80 (70)</td>
<td>A. te.: 1.0–5.5 (2.5)</td>
<td><em>A. te.</em>: facultative anaerobe; obligate chemolithoautotroph; anaerobic H₂ oxidation coupled to S⁰ reduction; aerobic growth coupled to S⁰ or S₂O₃⁻ oxidation</td>
<td>Ding et al., 2011; He et al., 2000; He et al., 2004; He et al., 2008</td>
</tr>
<tr>
<td></td>
<td>A. ma.: 50–85 (65)</td>
<td>A. ma.: 1.0–6.0 (1.5–2.5)</td>
<td><em>A. ma.</em>: same as above, but able to grow chemoorganotrophically on sugars, amino acids; can oxidize Fe²⁺</td>
<td></td>
</tr>
<tr>
<td><strong>Metallosphaera cuprina</strong></td>
<td>55–75 (65)</td>
<td>2.5–5.5 (3.5)</td>
<td>Strict aerobe; facultative chemolithotroph; saccharolytic; capable of chemolithotrophic growth on S⁰, S₂O₃⁻, FeSO₄, pyrite or chalcopyrite; genome sequenced</td>
<td>Liu et al., 2011b</td>
</tr>
<tr>
<td><strong>“Sulfolobus tengchongensis”</strong></td>
<td>S. te.: 65–95 (85–90)</td>
<td>S. te.: 1.7–6.5 (3.5)</td>
<td>Strict aerobe; facultative chemolithotroph; saccharolytic; capable chemolithotrophic growth on S⁰</td>
<td>Chen et al., 2008; Han et al., 2010; Xiang et al., 2003</td>
</tr>
<tr>
<td>Other <em>Sulfolobus</em> strainsb</td>
<td>Others: 55–93 (75)</td>
<td>Others: 1.5–6.0 (3.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sulfolobus tengchongensis</em> Spindle-shaped virus (STSV1)c</td>
<td>80</td>
<td>3.3</td>
<td>Largest known member of <em>Fuselloviridae</em>; genome sequenced (75 kb)</td>
<td>Xiang et al., 2005</td>
</tr>
</tbody>
</table>

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*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 O2-dependent disproportionation of S0 into HSO3- and S2-, which are each oxidized to SO42- 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 Acididus from Rehai, strain YN25, was recently identified as “Acididus 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 chemorganotrophically and by chemolithotrophic oxidation of Fe2+ (He et al., 2008; Ding et al., 2011). Strain YN25 was shown to efficiently leach Cu2+ from chalcopyrite, which was enhanced in the presence of activated carbon (Li 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 (Tmax 65 °C), and acidophilic (pHopt 3.5). Unlike other Metallosphaera species, M. cuprina can grow chemooorganotrophically as well as chemolithoautotrophically by oxidizing sugars as well as chemolithoautotrophically by oxidizing S0, S03-, FeSO4, 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 chemooautotrophic 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 S0. “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 Zaotanghe 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 Thermodeudsolobacteraceae in samples from Dagunguo (Wang et al., 2003a; Wang et al., 2003b). 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 (Huangguajing, 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 archael community in Zhenzhuquan was exclusively *Sulfolobales*, composed of *M. curpina* and three novel species-or genus-level groups most closely related to *M. curpina* and *Sulfolobus tokodaii*. The other two acidic samples contained higher diversity, including novel species- to genus-level groups of *Sulfolobales* and *Desulfoarcococcales* related to *Metallosphera*, *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 *Frankiniae*, relatives of "*Candidatus Microthrix spp.*" mesophilic, filamentous *Actinobacteria* yet to 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 monoxygenase 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$_3^-$ and NO$_2^-$ 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 habitats 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: maat het milieu selecteer” (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” to the level of the bacterial or archael 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 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 Natural National 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.

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